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

23 March 2017
[08–17]

Supporting document 1

Risk assessment (at Approval) – Application A1134

Increased Concentration of Plant Sterols in Breakfast Cereals

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Executive summary

This Application seeks permission to extend the addition of total plant sterol equivalents in breakfast cereals up to a maximum of 2 g per serve of breakfast cereal. The serve size has not been explicitly stated by the Applicant.

Foods with added plant sterols have been in the food supply since the 1990s, particularly in Europe and the USA. In Australia and New Zealand, it is permitted to add plant sterols to edible oil spreads, cheese, low fat milk, yoghurt and breakfast cereals meeting the sugar and fibre criteria. In Australia and New Zealand, breakfast cereals are currently permitted to contain total plant sterol equivalents at a content that is no less than 15 g/kg and no more than 19 g/kg.

For the purpose of this report, phytosterols, phytostanols and their esters are collectively referred to as plant sterols. The term total plant sterol equivalents includes phytosterols and phytostanols (i.e. free form) as well as the hydrolysis products of their esters.

Adding plant sterols at higher levels than currently permitted in breakfast cereals has been concluded to be technologically feasible as methods are available to incorporate them into such foods. There are analytical methods available and specifications already in the *Australia New Zealand Food Standards Code* for plant sterols.

A review of the recent literature has not identified evidence to alter the conclusion reached previously by FSANZ, that a specified Acceptable Daily Intake (ADI) is not justified for plant sterols for the general population. FSANZ has no toxicological concerns regarding the addition of plant sterols to breakfast cereals up to the concentrations proposed in the Application, for consumption by the general population. However, appropriate risk management measures are required for individuals with phytosterolaemia (sitosterolaemia).

Consuming total plant sterol equivalents at doses between 0.8 and 2 g/day[1] has been shown to reduce total and low density lipoprotein (LDL) blood cholesterol concentrations without adversely affecting high density lipoprotein (HDL) cholesterol concentration. Dose-response models reliably predicted that a daily dose of 2 g/day of plant sterols reduce LDL blood cholesterol concentrations by 9%.

[1] The draft variation allows a maximum permitted amount of 2.2 g of total plant sterol equivalents per serving rather than 2 g as requested. Refer to section 2.3.2.2. of the Approval report for further explanation.

For daily doses above 3 g/day, the models predict that the reduction in blood LDL concentration will approach an asymptotic value of 12.7%. Pregnant and lactating women and children under 5 years of age do not need to lower their cholesterol levels because growing children and developing embryos have an increased need for cholesterol and, therefore, may not benefit from consuming plant sterol-enriched foods.

There is currently no robust evidence to support the concerns that the consumption of plant sterols will increase the risk of cardiovascular disease or that the oxidation products of dietary plant sterols pose a risk to consumers. Some dietary intervention studies using plant sterols show a reduction in blood concentration of provitamin A carotenoids. These lipid-soluble phytochemicals are transported in blood by low density lipoprotein cholesterol, which is reduced by the intake of plant sterols. Consequently, the decrease in circulating amounts of carotenoids is not unexpected. After adjusting for the change in total blood cholesterol concentration β-carotene is the only carotenoid whose concentration remains significantly different from the control group value. However, it should be noted that the blood concentrations of carotenoids of subjects consuming plant sterols remain within the broad natural range of variation.

Clinical studies in which up to 9 g/day of total plant sterol equivalents were tested in adults did not show statistically significant changes in fat-soluble vitamins. Clinical studies in which up to daily doses of 6 g of total plant sterol equivalents were consumed by children (2-17 years of age) for up to six months demonstrate that total and LDL cholesterol concentrations are significantly decreased without affecting HDL concentrations and show no evidence of a nutritional safety risk. Similarly, consumption of 0.7 g and 0.8 g of total plant sterol equivalents during pregnancy and one-month post-partum, respectively, did not show evidence of a nutritional safety concern for both the women and their infants and did not significantly decrease maternal total or LDL cholesterol concentrations. The clinical evidence indicates that consumption of up to approximately 9 g/day of total plant sterol equivalents is unlikely to pose a nutritional safety concern for children and adults.

The dietary exposure assessment (DEA) used two approaches to estimate plant sterol exposure from breakfast cereals containing added plant sterols. The first approach estimated total dietary exposure based on consumption of foods with existing permissions to add plant sterols (i.e. the baseline exposure, estimated from the consumption of plant sterol-containing foods as reported in recent National Nutrition Surveys for Australian and New Zealand populations) and consumption of a serving of breakfast cereal per day containing 2.2 g of plant sterols per serving. Across all surveys and age groups assessed for Australian (aged 2 years and over) and New Zealand (aged 15 years and over) populations, the total estimated dietary exposures to plant sterols by this approach were 2.7–4.0 g/day and 3.0–5.1 g/day for the mean and 90th percentile (P90) exposures, respectively, expressed as plant sterol equivalents.

The second approach used a scenario model to estimate chronic plant sterol exposure based on baseline exposure and exposure from breakfast cereal consumption. The scenario assumed all breakfast cereals meeting the specified nutrient criteria contained plant sterols at the proposed maximum amount of 2.2 g/serve. This exposure estimate represented a conservative estimate since it assumed that persons who reported consuming breakfast cereal in the survey would consume the same amount of cereal if it contained added plant sterols at the proposed maximum amount. This scenario accounts for the brand loyal consumer. The total estimated dietary exposures to plant sterols for Australian consumers aged 2 years and above were 3.2 g/day and 6.4 g/day for the mean and P90 exposures, respectively, expressed as total plant sterol equivalents. Using this conservative approach, it was predicted that about 2% of the population aged 2 years and up would be exposed to more than 9 g/day of added dietary total plant sterol equivalents, an amount that has been shown in humans to cause no adverse health effects.

Occasional ingestion of plant sterols at these levels is unlikely to pose any safety concerns.

Overall, the available data for plant sterols are considered to provide a high level of confidence in the safety and suitability of plant sterol fortified breakfast cereal products at the proposed maximum concentration, for all population groups.

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1 Introduction

The *Australia New Zealand Food Standards Code* (the Code) currently permits the addition of phytosterols, phytostanols and their esters, which are collectively, termed as plant sterols in this report. The total plant sterol equivalents specifically encompass phytosterols and phytostanols (i.e. free form) as well as the hydrolysis products of their esters. Plant sterols are classified as novel food ingredients and are permitted to be added to a range of different food products, subject to the conditions of use shown in section S25—2.

Food Standards Australia New Zealand (FSANZ) has previously approved permissions arising from Application A433 – Phytosterol Esters derived from Vegetable Oils in Breakfast Cereals so that breakfast cereals are permitted to contain total plant sterol equivalents at a content no less than 15 g/kg and no more than 19 g/kg (FSANZ 2005). Additionally, plant sterols in food have been assessed by FSANZ in other applications (Applications A1019 and A1024 in particular) and are permitted in foods such as edible oil spreads, cheese and low fat milk and yoghurt.

This review forms a part of the assessment of an Application submitted to FSANZ requesting permission to add plant sterols to breakfast cereals at a content of no less than 0.8 g and no more than 2 g per serve.

The objectives of this review are to update plant sterol exposure estimates based on the most recent nutrition survey data and to evaluate relevant information that may have emerged since the last assessment done by FSANZ on any potential adverse effects of plant sterols in the diet arising from the proposed fortification of breakfast cereals.

2 Food technology assessment

2.1 Description of substance

Since plant sterols have been comprehensively assessed in earlier applications (including Applications A1019 and A1024 in particular) and are currently permitted in the Code, the food technology assessment is only intended to provide a summary of the chemistry of these novel food ingredients for background purposes. The assessment is principally concerned with assessing the suitability of incorporating higher concentrations of plant sterols into breakfast cereals.

2.1.1 Identity

All permitted plant sterols need to meet the specifications of section S3—24 (phytosterols, phytostanols and their esters) in Schedule 3 (Identity and Purity) of the Code. There are quite a range of different types of plant sterols, plant stanols and their esters. The chemical properties and structures of them are summarised in section 2.2 below, which also highlights their differences.

2.1.2 Technological justification

The technological justification that is being assessed is whether higher levels of plant sterols than currently permitted can be appropriately incorporated into breakfast cereals. That is, can plant sterols be incorporated into breakfast cereals, are they stable once incorporated and are there any technological challenges identified that could limit this objective.

2.2 Chemical properties

The chemical and physical properties of plant sterols are provided in detail in section 2.2 of SD1 for Application A1024 (FSANZ 2010a). Therefore, this information will not be repeated here except for some pertinent points.

The difference between phytosterols and the corresponding phytostanols is that the double bond between carbon atoms 5 and 6 (Figure 1) in the phytosterol has been hydrogenated so the four ring steroid structure is fully hydrogenated. Chemical structures are provided in the A1024 report: Figure 2 is the steroid skeleton; Figure 3 provides some examples of different plant sterols while an example of a phytostanol ester is provided in Figure 4 of the A1024 assessment report.

Phytosterols and phytostanols can be esterified by reacting with vegetable oil long chain fatty acids to form plant sterol esters which improve their solubility in food products that have fat components.

2.2.1 Chemical names, identification and structure

Chemical name:	**Some common plant sterols**: Sitosterol: (3β)-Stigmast-5-en-3-ol Sitostanol: (3β,5α)-Stigmastan-3-ol Campesterol: (3β)-Ergost-5-en-3-ol Campestanol: (3β,5α)-Ergostan-3-ol Stigmasterol: (3β)-Stigmasta-5,22-dien-3-ol Brassicasterol: (3β)-Ergosta-5,22-dien-3-ol
Common names:	Phytosterols, Phytostanols, Phytosterol esters, Phytostanol esters, Plant sterols, plant sterol esters
CAS registry number:	Sitosterol: 83-46-5 Sitostanol: 83-45-4 Campesterol: 474-62-4 Campestanol: 474-60-2 Stigmasterol: 83-48-7 Brassicasterol: 474-67-9
Chemical formula:	Sitosterol: $C_{29}H_{50}O$ Sitostanol: $C_{29}H_{52}O$ Campesterol: $C_{28}H_{48}O$ Campestanol: $C_{28}H_{50}O$ Stigmasterol: $C_{29}H_{48}O$ Brassicasterol: $C_{28}H_{46}O$ Campesteryl oleate: $C_{46}H_{81}O_2$ Sitostanyl oleate: $C_{47}H_{85}O_2$
Molecular weight (g/mol):	Sitosterol: 414.72 Sitostanol: 416.73 Campesterol: 400.69 Campestanol: 402.70 Stigmasterol: 412.67 Brassicasterol: 398.67 Campesteryl oleate: 683.19 Sitostanyl oleate: 699.19

Chemical name:	**Some common plant sterols**: Sitosterol: (3β)-Stigmast-5-en-3-ol Sitostanol: (3β,5α)-Stigmastan-3-ol Campesterol: (3β)-Ergost-5-en-3-ol Campestanol: (3β,5α)-Ergostan-3-ol Stigmasterol: (3β)-Stigmasta-5,22-dien-3-ol Brassicasterol: (3β)-Ergosta-5,22-dien-3-ol

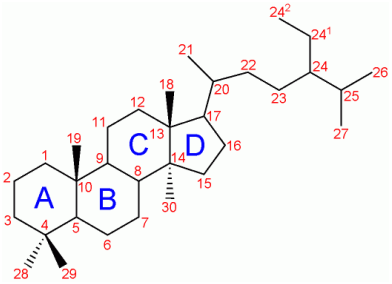


Figure 1: Steroid skeleton structure for plant sterols (taken from JECFA 2008) *(full chemical structures are also provided for sitosterol, sitostanol, campesterol and campestanol, along with sitostanyl oleate in this reference)*

2.3 Analytical method for detection

Plant sterols have been permitted to be added to different types of food in Australia and New Zealand, and many other countries, for many years. The analysis of the presence of, and the amounts of, added plant sterols in different food matrices has also been well established and published in the scientific analytical literature (Laakso 2005). Analytical methods for quantification of plant sterols include the trimethylsilyl (TMS) derivatives, by gas chromatography with a flame ionisation detector (GC-FID).

Laakso (2005) indicates that some food matrices, such as pasta (but which could include breakfast cereals), may require an acid hydrolysis step to release the plant sterols bound to the food matrix, before the saponification step which uses 2M ethanolic potassium hydroxide.

There is an Official Method of Analysis of AOAC International (Official Method 994.10) for cholesterol in food which is a GC-FID method which can be modified to analyse for plant sterols (AOAC International 2011). There is also an ISO method, ISO 12228-1:2014 “Determination of individual and total sterols contents – Gas chromatography method-Part 1: Animal and vegetable fats and oils” which can also be modified as required.

More recent analytical methods have been published which use the same methods as above. The US FDA published work it had done in 2015 (Srigley and Haile 2015). The US FDA also collaborated with the supplier of plant sterols, Cargill, on analytical methods (as reported in the Food Navigator article, 23 January 2017, http://www.foodnavigator-usa.com/content/view/print/1359869). This method is based on an earlier publication from Cargill (Clement et al 2010).

2.4 Manufacturing processes for plant sterols

2.4.1 Production from vegetable oil

Commercially, plant sterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil. These vegetable oils normally undergo a series of refining steps to remove unwanted constituents and to improve their quality and shelf lives.

The last step in the oil purification process is deodorisation which produces a distillate known as the 'vegetable oil deodorised distillate (VOD)' (EFSA 2007).

The VOD is the starting material for the extraction of plant sterols which are subjected to a series of distillation, filtration and crystallisation steps to remove unwanted by-products including fatty acids, di- and tri-glycerides, waxes, and fatty acid esters.

2.4.2 Production from tall oil

Plant sterols can also be isolated from a by-product of wood pulp from pine trees (*Pinus sp.*). Crude tall oil is a by-product of the wood pulping process. Plant sterols are concentrated in the residue after the crude tall oil is distilled into different fractions. The fraction called tall oil pitch contains up to 5-15% plant sterols (JECFA 2008).

The tall oil pitch is saponified with caustic soda to cleave the plant sterol esters. The mixture is then neutralised with mineral acid and the aqueous phase removed. The plant sterol fraction is recovered by distillation of the residual pitch in a number of steps. Finally, the plant sterols are purified via solvent re-crystallisation (JECFA 2008).

2.4.3 Product specification

All plant sterols permitted in the Code to be added to food as listed in section S25—2, including those of this Application, need to comply with the specification in Schedule 3 – Identity and purity.

As noted in section 2.1.1, section S3—24 is the specification for phytosterols, phytostanols and their esters. This specification requires compliance with either a primary source (section S3—2) or secondary source (section S3—3) of specifications, along with some additional requirements. The primary sources of specification do have specifications for plant sterols: JECFA (paragraph S3—2(1)(1)(b)) titled phytosterols, phytostanols and their esters and Food Chemical Codex (paragraph S3—2(1)(c)) titled vegetable oil phytosterol esters.

2.4.4 Product Stability

No specific references have been found addressing the stability of plant sterols incorporated into breakfast cereals. However, there are many references dealing with the stability of plant sterols in different foods (Yanishlieva-Maslarova and Marinova 1985; Albi et al. 1997; Piironen et al. 2000; Johnsson and Dutta 2006; Dutta et al. 2007; JECFA 2008; DFG 2014). Plant sterol degradation due to oxidation depends on temperature, heating time, heating method, as well as the composition of the food matrix. Plant sterols are generally very stable compounds and undergo very limited damage during food-processing (Ferrari et al. 1997; JECFA 2008).

Under specific conditions, such as high temperatures (>100°C) and in the presence of air, some plant sterols can oxidise in the same way as cholesterol (Yanishlieva-Maslrova and Marinova 1985). Based on the stability of the plant sterols during food processing conditions, no significant changes in total sterol contents are likely to take place in most practical situations (Piironen 2000), which is expected to include incorporating into breakfast cereals. However, after prolonged storage, some limited oxidation products may be formed.

Because some consumers may use microwave ovens to heat either the liquid added to the cereal or the cereal with liquid before consumption, an investigation into the use of microwaves on the stability of plant sterols was undertaken. No information was located in the literature on the impact of microwave heating on plant sterols in breakfast cereals themselves.

But some limited information was available on the stability of plant sterols themselves or when added to milk after being heated using a microwave oven (Menéndez-Carreño et al. 2008; Leal-Castañeda et al. 2015).

In summary, plant sterols added to different food matrices are stable to heating, including the usual short microwave heating time consumers use when preparing breakfast cereals for consumption straight after heating.

2.5 Incorporating plant sterols into breakfast cereals

The suitability of adding plant sterols into different food types will depend on their physical properties as well as the properties of the food matrix, which for this Application are breakfast cereals. Plant sterols need to be fully and uniformly distributed throughout the food matrix and not cause any appearance, odour or flavour defects in the final product. It is also important that plant sterols are stably incorporated into the food and does not degrade during processing or during the shelf life of the product.

Plant sterols have high melting points that range between 138-158°C and they exist as solid crystalline structures at room temperatures. To more easily incorporate plant sterols into solid food, they are often esterified (see Section 2.2) to increase their solubility in fats and oils. The solubility of plant sterol esters is 10 to 20 times higher in fats and oils than their non-esterified counterparts (Engel and Schubert 2005). The esters are also soluble in non-polar solvents but not in water. The increased solubility allows the esterified plant sterols and plant stanols to be dissolved into the fat components of spreads or fat-containing food products which have their fat components already homogenously distributed or emulsified. In addition varying the fatty acid composition of plant sterol esters can ‘tailor’ the solubility to improve incorporation into different food matrices. Esterified plant sterols have much lower melting temperatures, between 26 and 40°C. With lowered melting points, the esters can be blended more easily in semi-solid foods such as spreads and yoghurt during processing at a temperature slightly higher than 40°C. At this temperature, the esters behave like liquid oil, for example allowing an even coating of cereal grains for breakfast cereals.

Breakfast cereal manufacturers could use plant sterol esters, either directly as liquid oils at temperatures slightly above room temperature which could then coat the surfaces of the cereal flakes or components that make up the breakfast cereal, or they could be dissolved into a vegetable oil or fat to also coat the cereal ingredients of the breakfast cereal product. However there may be other techniques that breakfast cereal manufacturers could use to incorporate plant sterols into breakfast cereals.

There are commercial breakfast cereals (rolled oats used to produce porridge) sold in different countries that contain similar amounts of plant sterols to that proposed by the Applicant, so techniques and processes are known to achieve this. There is therefore no reason to believe the Applicant or other cereal manufacturers do not already, or could not readily obtain, the expertise, and if needed the equipment, to produce commercial breakfast cereals containing the higher levels of plant sterols requested in the Application which the Applicant has indicated they have investigated. Such methods would need to also meet the requirements of being uniformly distributed to comply with enforcement requirements relating to permissions and not cause organoleptic issues.

2.6 Food technology conclusions

There are very few losses due to oxidation, provided the food containing the added plant sterols is not subject to very high processing temperatures and oxidising conditions.

Breakfast cereals are not subjected to these extreme conditions in their manufacture or use. Plant sterols and plant stanols have relatively low fat solubility and high melting temperatures, so they are often esterified with long chain fatty acids from vegetable oils to form plant sterol esters. These plant sterol esters have much lower melting points and also have greater solubility in fats and oils. These properties can be used to incorporate plant sterols into breakfast cereals, either by using them directly at temperatures around 40°C when they are liquids or dissolving them in fats or oils. Either of these liquids can be used to coat cereal ingredients to ensure the appropriate amount of plant sterol is uniformly incorporated.

There may also be other process methods available to incorporate plant sterols into breakfast cereals. It is known that there are commercial breakfast cereals (rolled oats products) sold in other countries that contain amounts of plant sterols similar to that requested by the Application so methods do exist to achieve this. Adding plant sterols at higher amounts than currently permitted in breakfast cereal products as proposed by the Applicant is therefore concluded to be technologically feasible.

3 Hazard assessment

FSANZ has conducted a number of assessments of plant sterols in the past. The most recent comprehensive assessment was in 2010, as part of A1019 – Exclusive Use of Phytosterol Esters in Lower-fat Cheese Products. The current assessment is therefore limited to a review of the relevant literature since that assessment.

An ADI of 0-40 mg/kg of body weight, expressed as the sum of phytosterols and phytostanols in their free form, was established at the 69th JECFA (JECFA 2008). FSANZ concluded in 2010 that there is no justification for an ADI for plant sterols, because the apparent treatment-related adverse effect is entirely explained by the background incidence of pathology found in historical control data for the strain of rats used in the studies on which JECFA based the ADI.

3.1 Review of the current relevant evidence

No new non-clinical or clinical studies were located in literature searches concerning the toxicity or safety of plant sterols since 2010, with the exception of one study on plant sterol oxidation products (POPs) in rats (Scholz et al. 2015) and one study on formation of POPs in humans (Baumgartner et al. 2013). However, a small number of reviews and meta-analyses related to the safety of dietary plant sterols supplementation were found. Concerns about the safety of dietary plant sterols raised in those reviews may be summarised as the following:

- moderately elevated plasma plant sterol levels have been associated with increased cardiovascular risk
- genetic variants associated with increased serum concentrations of plant sterols are also associated with cardiovascular risk
- POPs may pose a risk to consumers of plant sterols
- no intervention studies have been performed to show that clinically relevant cardiovascular endpoints may be reduced
- plant sterols pose a risk because patients with sitosterolaemia may develop early onset atherosclerosis
- plant sterols supplementation may result in decreased circulating levels of carotenoids, which might in turn lead to a higher incidence of certain cancers and of macular degeneration.

These concerns are addressed in order in this review.

1. Moderately elevated plasma plant sterols levels have been associated with increased cardiovascular risk

There are contradictory reports in the literature concerning correlation between plasma plant sterol concentrations and cardiovascular risk. Some studies have shown a positive correlation, whereas others have shown a negative correlation or no correlation (Weingärtner et al. 2014; Silbernagel et al. 2015; Weingärtner et al. 2015). This variability appears to reflect the confounding effects of individual cholesterol absorption rate on plasma plant sterol concentrations and on cardiovascular risk (Silbernagel et al. 2015). Genser et al. (2012) conducted a systematic review of studies published between January 1950 and April 2010, and identified 17 papers suitable for inclusion in a meta-analysis. The meta-analysis did not identify a consistent association of circulating plant sterols and cardiovascular disease.

While the quantitative analysis of plasma cholesterol is standardised internationally, the measurement of plasma plant sterols is not, which makes it difficult to compare the results from different laboratories (Weingärtner et al. 2014).

2. Genetic variants associated with increased serum concentrations of plant sterols are also associated with cardiovascular risk

Genetic variants in the ATP binding cassette transporter G5 and G8 (ABCG5/8) and ABO genes are associated with cardiovascular disease. People with these variants have higher serum plant sterol concentrations than other people, but they also have increased cholesterol absorption and in particular, high circulating concentrations of low density lipoprotein (LDL) cholesterol. Thus, the increased risk of cardiovascular disease in people with these genetic variants may be mediated by LDL cholesterol rather than by plant sterols (Silbernagel et al. 2015).

3. Plant sterol oxidation products (POPs) may pose a risk to consumers of plant sterols

Plant sterols may become oxidised in food and there is also *in vitro* evidence that plant sterols may be oxidised in the body. In common with cholesterol oxidation products (COPs), POPs are cytotoxic in vitro, but are less potent than COPs. It has been suggested that POPs may be atherogenic, however overall the information on potential adverse effects is not sufficient to support robust risk assessment (Scholz et al. 2015).

The results of a 90-day study in rats identified a NOAEL for POPs of 128 mg/kg/day for males and 144 mg/kg/day for females, although the effects, which were on bodyweight, haematological parameters, some clinical pathology parameters, serum lipids and liver weight, were inconsistent between the sexes (Scholz et al. 2015).

Baumgartner et al. (2013) conducted a randomised double-blind crossover trial in which 43 healthy subjects, ranging in age from 18 to 70 years, consumed margarines with or without enrichment (3.0 g/day) with phytosterols or phytostanols for 4 weeks, separated by wash-out periods of 4 weeks. No increases in plasma POPs as a result of consumption of enriched margarines were found by GC-MS.

4. No intervention studies have been performed to show that clinically relevant cardiovascular endpoints may be reduced

This concern relates to efficacy rather than hazard and is therefore not relevant to this hazard assessment. However, Silbernagel et al. (2015) suggest that a randomised controlled study to test whether regular dietary exposure to plant sterol-containing foods will reduce clinically relevant cardiovascular endpoints, would be extremely difficult and expensive to conduct.

They calculate that it may be necessary to have around 33,000 participants, and to follow them for approximately 10 years.

5. Patients with phytosterolaemia (sitosterolaemia) may develop early onset atherosclerosis

Phytosterolaemia, or sitosterolaemia, is a rare inherited disorder. Findings include elevated serum plant sterol concentrations, xanthomas and the early onset of atherosclerosis. Patients have increased absorption and decreased excretion of plant sterols. The discovery of this disease led to speculation that plant sterols themselves may be atherogenic in normal individuals, if chronically consumed (Weingärtner et al. 2014).

However, serum plant sterol concentrations in individuals with phytosterolaemia are approximately 30 times higher than those of frequent users of plant sterol-containing foods. In addition, patients often also have high plasma concentration of LDL cholesterol, which may be the main risk factor for early atherosclerosis in these patients (Silbernagel et al. 2015).

Overall, there is a lack of convincing evidence that the premature atherosclerosis observed in congenital phytosterolaemia is relevant to an assessment of the likelihood that plants sterols are atherogenic in the general population.

The specific risk to this susceptible subpopulation can be addressed by appropriate risk management measures.

6. Plant sterol supplementation may result in decreased circulating levels of carotenoids, which might in turn lead to a higher incidence of certain cancers and of macular degeneration

Mannarino et al. (2014) reported that there is no evidence of increased incidence of cancer or of macular degeneration associated with consumption of plant sterol-enriched foods.

3.2 Conclusions

Some concerns have been raised in the scientific literature about potential risks associated with chronic consumption of plant sterols-enriched foods. While some epidemiological studies have found a positive association between moderate elevation of plasma plant sterol concentrations and increased cardiovascular risk, other studies have found an inverse association or no association.

There is currently no robust evidence to support concerns that the oxidation products of dietary plant sterols pose a risk to consumers or that any such risk outweighs the cholesterol-lowering benefits of dietary plant sterol consumption. Similarly, there is no evidence that plant sterol supplementation leads to adverse outcomes through the mechanism of decreasing circulating amounts of carotenoids.

While elevations in serum plant sterol concentrations in individuals with certain genetic variants in the ATP binding cassette transporter G5 and G8 (ABCG5/8) or ABO genes show a correlation with risk of cardiovascular disease, these individuals also have increased cholesterol absorption and in particular, high serum concentrations of LDL cholesterol. High serum LDL cholesterol is a well-recognised marker of increased cardiovascular risk.

FSANZ has previously stated that safety data for pregnant women, lactating women, and children under five years of age is relatively limited compared to the extensive data available for the target population.

However, based on knowledge of the mechanisms of phytosterol action, the now extensive experience of use of phytosterol-enriched foods in the general population and the absence of effects in pregnant animals and their offspring, there was no basis for postulating a risk to these population subgroups (FSANZ 2012a). No new data was identified that would change this conclusion.

Occasional consumption of plant sterol-enriched breakfast cereal by young children or pregnant or lactating women is therefore not considered to be of toxicological concern.

4 Nutrition assessment

FSANZ concluded in previous assessments that a reduction in the absorption of β-carotene with consumption of plant sterols is expected, however this was considered to have no nutritional safety implications (FSANZ 2010b; FSANZ 2012a; FSANZ 2012b).

Despite plant sterols and cholesterol having similar chemical structures, they differ markedly in their biosynthesis, intestinal absorption and metabolism (Moghadasian and Frohlich 1999; Moreau et al. 2002; Gleize et al. 2016). The hypocholesterolaemic effect of plant sterols has been consistently confirmed through human clinical studies (Richelle et al. 2004; Lau et al. 2005; Bañuls et al. 2010). Furthermore, meta-analyses of randomised controlled trials in humans have demonstrated a dose-dependent LDL cholesterol lowering effect of plant sterols (Demonty et al. 2008; Musa-Veloso et al. 2011). Based on 141 strata from 84 studies, Demonty et al. (2008) used a regression analysis to derive a continuous dose-response relationship between the consumption of plant sterols and LDL cholesterol reduction. A daily dose of 2 g of free plant sterols (or the equivalent weight of total plant sterols) was predicted to reduce LDL cholesterol by 9%, which closely agrees with the meta-analytic pooled estimate of an 8.8% (95% CI: -9.4, -8.3%) decrease in LDL cholesterol for a mean daily dose of 2.15 g of free plant sterols (or the equivalent weight of total plant sterols). The predicted reduction is also consistent with the mean 8.9% reduction in LDL cholesterol reported by Katan et al. (2003) for studies that tested daily doses of free plant sterols (or the equivalent weight of total plant sterols) that ranged from 2.0 to 2.4 g. At daily doses of 3 g, the predicted change in LDL cholesterol is -11% and at higher daily doses the change approaches the asymptotic value of -12.7% (95% CI: -15.4, -10%).

Statistically significant reductions in LDL cholesterol concentrations have been reported (Ishizaki et al. 2003; Kurokawa et al. 2008) in trials that tested daily doses of around 0.8 g of plant sterols (or the equivalent weight of total plant sterols). However, other studies that tested similar daily doses (0.8–1.0 g) reported confidence intervals for the mean reductions in LDL cholesterol concentrations that either approached or crossed the line of no-effect (Vanhanen et al. 1994; McPherson et al. 2005; Niittynen et al. 2008). Doses of 400 mg plant sterols (or equivalent weight of total plant sterols) per day did not show statistically significant reductions in healthy subjects (Seki et al. 2003; Kurokawa et al. 2008; Racette et al. 2010). However, it should be noted that a four-week study by Saito et al. (2006) testing the effect of plant sterols esters in mildly hypercholesterolaemic males showed statistically significant reductions in LDL cholesterol concentrations with daily doses of 0.3, 0.4, and 0.5 g when compared with the respective baseline values in each of the three groups. However, the 0.3 and 0.4 g/day arms of the study did not significantly differ from the control group that consumed a diet that was not supplemented with plant sterols, but was otherwise identical to that received by the subjects in the treatment arms.

Clinical studies consistently show that the lowering effect of plant sterols on serum LDL cholesterol concentrations does not affect serum HDL cholesterol concentrations (Plat et al. 2000; Mensink et al. 2002; Brufau et al. 2008; Bruckert et al. 2014).

4.1 Absorption of plant sterols and proposed mode of hypocholesterolaemic action

Cholesterol homeostasis in humans has been well-studied with pathways for cholesterol biosynthesis and excretion fully described (Brufau et al. 2011; Van Der Wulp et al. 2013). Cholesterol from both the diet and bile is absorbed in the small intestine and regulated by cellular receptors in the enterocytes (Repa et al. 2002; Masson et al. 2010; Dash et al. 2015).

Current evidence suggests that cholesterol and plant sterol absorption occurs by multiple mechanisms and is possibly a multi-step process regulated by multiple genes at the enterocyte level (Sehayek 2003; Brufau et al. 2008; Calpe-Berdiel et al. 2009). Competition between cholesterol and plant sterols for incorporation into mixed micelles has been proposed as the primary mechanism for the cholesterol reduction effect of these phytochemicals. When simultaneously present in the intestinal lumen, cholesterol and plant sterols compete for inclusion into the micelles (Ikeda et al. 1989; Trautwein et al. 2003). The more hydrophobic plant sterols are preferentially incorporated into the micelle structure, resulting in a decrease in cholesterol absorption and its consequent elimination in the faeces (Sanclemente et al. 2009). However, the formation of poorly absorbable mixed micelles containing plant sterols may not necessarily have a particularly large effect on cholesterol reduction (Mel'nikov et al. 2004). Plant sterols have been also proposed to exert an unknown molecular action within enterocytes or hepatocytes and therefore may not need to be present within the intestinal lumen to inhibit or reduce cholesterol absorption (Moghadasian and Frohlich 1999; Plat and Mensink 2000)(Plat et al. 2000)(Plat et al. 2000).

Consumption of foods fortified with plant sterols has been reported to increase the plasma concentration of these phytochemicals (Clifton et al. 2004). However, the levels remain at less than 1% of total plasma sterols and, generally, do not exceed 25 μmol/L even in diets that have high levels of plant sterols (Windler et al. 2009). For instance, in a non-randomised study in which 35 mildly hypercholesterolaemic subjects consumed 6.6 g of plant sterol esters per day over a period of 12 weeks, the combined plasma concentrations of campesterol and β-sitosterol, i.e. two of the major phytosterols in plants, did not exceed 0.5% of the total plasma cholesterol concentration (Clifton et al. 2004). Furthermore, the reported plasma concentrations of campesterol and β-sitosterol of subjects receiving supplemental phytosterols are within the ranges for the general population (Matvienko et al. 2002; Clifton et al. 2004; Fransen et al. 2007), which were derived by analysing data obtained from population-based studies and clinical trials (Chan et al. 2006). The absorption of dietary plant sterols appears to be similar in both children and adults (Tammi et al. 2001; Amundsen et al. 2002; Chan et al. 2006).

By contrast with adult subjects, there are fewer reported clinical trials of plant sterols in children and these are mainly limited to child subjects with familial hypercholesterolaemia (FH) (Guardamagna et al. 2011). Results from such studies in which up to daily doses of 6 g of the of total plant sterol equivalents were consumed by children (2–17 years of age) for up to six months show that total and LDL cholesterol concentrations are significantly decreased without adversely affecting HDL cholesterol concentrations (Becker et al. 1992; Gylling et al. 1995; de Jongh et al. 2003; Jakulj et al. 2006; Matsuyama et al. 2007; Garoufi et al. 2014). A double-blind, cross-over clinical study (Amundsen et al. 2002) in which thirty-eight children (19 girls, aged 7–13 years of age) all of whom had at least one parent with familial hypercholesterolaemia (FH), but were otherwise healthy, showed that the consumption of 1.6 g/day of plant sterol esters within a diet low in both saturated fatty acids and cholesterol and rich in unsaturated fatty acids, fruits, and vegetables, reduced (8.1%, $p = 0.015$) the mean serum lycopene concentration but not that for the other carotenoids after adjusting for changes in blood lipids.

Furthermore, the lipid-adjusted retinol and α-tocopherol concentrations were both significantly higher (15.6 and 7.1%, respectively) at the end of the eight-week study. It should, however, be noted that the mean serum alanine aminotransferase concentration increased during the intervention phase of the trial, although none of the children had a serum alanine aminotransferase concentration that was outside the normal range. The authors noted the mean serum alanine aminotransferase concentration at the beginning of the intervention phase of the trial was significantly lower than that at the beginning of the control phase. It should also be noted that the mean serum alanine aminotransferase concentration (16.3 ± 5.5 U/L) at the end of the intervention phase was the same as that (16.4 ± 6.0 U/L) at the beginning of the control phase of the trial. Regression to the mean is a reasonable explanation for the increase in the serum alanine aminotransferase concentration – particularly given that the changes in the other two liver enzymes (alkaline phosphatase and aspartate transaminase) that were measured during the trial were not significant. An open-label follow-up study (Amundsen et al. 2004) involving children (n = 37) with FH revealed that the mean serum HDL cholesterol concentration had decreased by 4.8% (p = 0.041) after consuming 1.5 g of plant sterol esters for 26 weeks. However, the total to HDL-cholesterol ratio improved with the dietary exposure to plant sterols. The authors attributed the decrease in the HDL concentration to the design of the open-label study, which, in contrast to the previous cross-over study (Amundsen et al. 2002), did not control the intake of macronutrients such as fats and carbohydrates.

A search for clinical studies of the effects of plant sterols during pregnancy and on lactating women and their infants retrieved two articles from the PubMed database. One article (Mellies et al. 1978) describes a cross-over trial (n = 14) that was designed to study the effect of maternal intake of cholesterol, fatty acids and plant sterols on maternal and infant blood and breast milk concentrations of fatty acids and the two sterols. The other article (Laitinen et al. 2009) describes a study with a parallel design in which pregnant women were randomised to control (n = 10) and intervention (n = 11) arms to evaluate the clinical safety of phytostanol esters.

In the cross-over study by Mellies et al. (1978), mothers had an *ad libitum* diet for 30 days after delivery and then were randomised to one of two diets: i) a diet low in cholesterol (190 mg/day) and high in plant sterol (1200 mg/day) with a polyunsaturated to saturated fatty acid ratio of 1.8; and ii) a diet high in cholesterol (520 mg/day) and low in plant sterol (50 mg/day) with a polyunsaturated to saturated fatty acid ratio of 0.12. After four weeks, the mothers were crossed over to the alternative diet. Cholesterol concentrations in the breast milk did not change after the ad libitum phase or following either of the intervention diets. Significant reductions in maternal blood cholesterol concentrations were reported for both intervention diets compared with the blood cholesterol concentration for the ad libitum diet. Infant blood cholesterol was not changed when the mothers were on either intervention diet compared with the infant blood cholesterol concentration when mothers consumed an ad libitum diet. There were significant correlations between plant sterol concentrations in diet and maternal blood, maternal blood and breast milk as well as between breast milk and infant blood. For cholesterol, there was only a significant correlation between concentration in the diet and maternal blood. No significant correlation was noted for cholesterol concentrations in maternal blood and breast milk or in breast milk and infants' blood. The fatty acid profile concentrations in maternal breast milk as reported by Mellies et al. (1978) was comparable with that reported by other studies in which the breast-feeding mothers followed similar *ad libitum*, polyunsaturated fat-rich or saturated fat-rich diets. The authors concluded that alterations in maternal dietary plant sterol dietary exposure lead to parallel changes in maternal blood and breast milk as well as in the breast-feeding infant's blood concentration of plant sterols. These changes, however, did not affect the cholesterol concentration in the infant's blood (Mellies et al. (1978).

For the parallel study (Laitinen et al. 2009) the intervention was daily consumption of spreads containing phytostanol esters (mean exposure of 1.1 ± 0.4 g total plant sterol equivalents) during pregnancy and for one month (mean exposure of 1.4 ± 0.9 g total plant sterol equivalents) post-partum. The mothers were less than seventeen weeks into their pregnancy at the start of the trial, were healthy, and, for those in the intervention arm of the trial but not the control arm, were counselled to follow a balanced diet that is appropriate for pregnant women. The subjects were followed through each trimester and then with their infants at 1, 6, and 12 months. The serum concentrations of total, LDL, and HDL cholesterol and triacylglycerides for the women did not differ between the two arms of the trial at any time point during the course of the study. At one month post-partum, the concentrations of total cholesterol in breast milk did not statistically differ between the two groups, although the concentration of desmosterol – a sterol precursor in the cholesterol biosynthetic pathway – was 23% lower ($p = 0.038$) in the breast milk of mothers in the intervention group. However, there was no difference between the concentrations of cholestenol – another sterol in the cholesterol biosynthetic pathway that is synthetically independent of desmosterol – in the breast milk of the mothers in the two groups. Furthermore, the breast milk concentrations of squalene and lathosterol, both of which are common precursors to cholestenol and desmosterol, did not statistically differ between the two groups. Therefore, the difference is likely to have arisen by chance. It is also worth noting that the concentrations of the plant sterols that were measured in the breast milk of the women did not statistically differ between the two groups.

All infants were born full-term without complications and were exclusively breastfed for an average of 11 ± 8 (SD) weeks in the intervention arm and for an average of 16 ± 2 weeks in the control arm of the trial. There were no differences in the gestational weight gain, height, or cognitive development between the infants born to mothers in the intervention group and those born to mothers in the control group. The mean serum β-carotene concentrations between the two infant groups were not statistically different at one month and six months of age; although when the concentrations were adjusted for total cholesterol, the mean serum β-carotene concentration was lower in the infants born to mothers in the intervention group at one month of age compared with the corresponding mean serum β-carotene concentration for the infants born to the mothers in the control group. The difference in the adjusted β-carotene concentrations at six months was not statistically significant. β-Carotene is transported in blood by LDL cholesterol. The apparent difference after adjusting for total cholesterol is likely to have arisen because of a difference in a lipoprotein cholesterol fraction such as HDL cholesterol between the two infant groups rather than an actual difference in serum β-carotene concentrations. Indeed, the mean serum HDL cholesterol concentration at one month post-partum for the infants born to the mothers in the intervention group was 17% higher than that of the infants born to the mothers in the control group. At six months post-partum both infant groups had the same serum HDL cholesterol concentration. It is also worth noting that any difference in serum β-carotene concentration between the infant groups at one month post-partum is unlikely to be nutritionally relevant because breast milk is the most important source of vitamin A for neonates (Stoltzfus and Underwood 1995).

The evidence indicates that consumption of 0.7 g/day of plant sterol equivalents does not pose a nutritional safety risk for pregnant women or infants. However, it should be noted that pregnant and lactating women and children under five years of age do not, generally, need to lower their cholesterol levels because growing children and developing embryos have an increased need for cholesterol for normal development (Berger et al. 2004; FSANZ 2012a).

4.2 Effect of plant sterols on fat-soluble vitamins and carotenoids

The reduction in plasma concentrations in some vitamins associated with consumption of foods that were fortified with plant sterols has been previously evaluated by FSANZ (FSANZ 2005; FSANZ 2006; FSANZ 2010b). The conclusion reached from those evaluations is that the reductions in vitamins D, K and E levels are not significant after adjusting for decreases in LDL cholesterol, which plays a role in transporting these and other fat soluble vitamins in blood (Schurgers and Vermeer 2002; Mardones and Rigotti 2004; Kono and Arai 2015). FSANZ also noted that a reported decrease of 20-25% (Fardet et al. 2016) in the serum concentration of carotenoids that is associated with the consumption of plant sterols "falls within a broad natural range considered to be typical of variable diets" (FSANZ 2010b). The importance of carotenoids to human health is related to the group referred to as provitamin A carotenoids because of their metabolic conversion to vitamin A in the intestinal mucosa of humans (Harrison 2012). Of the provitamin A carotenoids, β-carotene is the most important to human nutrition and to a lesser extent α-carotene because the former-mentioned carotenoid is more abundant in fruits and vegetables (Burns et al. 2003) and its bioconversion to vitamin A is higher than that of other provitamin A carotenoids (Institute of Medicine (U.S.) 2001; Weber and Grune 2012). A meta-analysis (Katan et al. 2003) of fourteen randomised placebo-controlled intervention studies using daily doses that ranged from 0.8 to 4.2 g of total plant sterol equivalents and lasted between 3 and 52 weeks suggests that the reduction in serum β-carotene concentration is not nutritionally relevant in that the pooled mean difference in serum vitamin A concentration (-0.1%) across the trials was not statistically significant (95% CI: -1.6 to 1.5%). Indeed, the mean serum vitamin A concentration of subjects (n = 25) receiving 8.9 g/day of total plant sterol equivalents, given as plant stanol esters, during a ten-week randomised, double-blind, placebo-controlled study (Gylling et al. 2010) was not significantly different (p = 0.32) compared with the control group (n = 24) that consumed a diet that did not differ in energy and macronutrients content but without added plant sterols. Similarly, the mean difference in serum vitamin A concentration between baseline and after intervention during a 15 week randomised double-blind, placebo-controlled trial (Tuomilehto et al. 2009) in which the subjects (n = 36) received 1.25 g/day for the first five weeks, then 2.5 g/day for the next five weeks, and 5 g/day total plant sterol equivalents for the last five weeks of the study was not statistically different (p = 0.55) to the mean change from baseline in serum vitamin A concentration for the control group (n = 35). This is consistent with the findings of an eight week randomised, double-blind, placebo controlled clinical trial (Davidson et al. 2001) with four parallel arms that showed daily doses of up to 9 g of total plant sterol equivalents, given in esterified form, did not significantly change serum vitamin A concentrations.

Four human clinical studies (Kaffe et al. 2012; Söderholm et al. 2012; Sialvera et al. 2013; Petrogianni et al. 2014) reporting the effects of plant sterols on the concentrations of fat-soluble vitamins have been published since the last assessment by FSANZ in 2012. In two (Kaffe et al. 2012; Petrogianni et al. 2014) of the four studies, the reported changes in circulating fat-soluble vitamin concentrations were confounded by the use of interventions that contained supplemental vitamins that were also measured in the blood of the subjects and in one case (Kaffe et al. 2012) the change was confounded by the use of plant sterols as a delivery vehicle for the fat-soluble vitamin being studied.

The other two studies were both placebo-controlled, randomised trials with parallel designs. Sialvera et al. (2013) tested the effects of 4 g/day of plant sterols in subjects (n = 53) with metabolic syndrome for two months on plasma antioxidant capacity and the others (Kaffe et al. 2012; Söderholm et al. 2012; Sialvera et al. 2013; Petrogianni et al. 2014) tested the effects of free plant sterols on serum lipids in normocholesterolemic subjects (n = 32) using an initial dose of 2 g/day for two-weeks and then 4 g/day for the final two-weeks of the study.

In both studies, the vitamin E mean concentrations in the intervention groups did not statistically differ from the vitamin E concentrations in the control groups (n = 55 and 31, respectively). Statistically significant changes in blood vitamin E concentrations have been reported for intervention trials with plant sterols. However, it should be noted that a meta-analysis (Katan et al. 2003) of 15 studies showed that the pooled estimate for the change in circulating vitamin E concentration associated with plant sterol interventions that ranged from 0.8 to 4.2 g total plant sterols equivalents per day and lasted for 3 to 52 weeks was not significant when the changes in plasma vitamin E concentrations were adjusted for the changes in blood cholesterol concentrations. A randomised, double-blind, placebo-controlled study (Gylling et al. 2010) with a parallel design in which mildly to moderately hypercholesteraemic subjects (n = 25) received 8.9 g of total plant sterol equivalents, given as plant stanol esters, showed that the γ-tocopherol serum concentration did not change both within the intervention group and between the groups over the ten-week trial. Interestingly, the more lipophilic α congener significantly ($p < 0.05$) decreased (16%) within the intervention group and also significantly ($p < 0.05$) differed (6 μM) from the control group (n = 24). The within group decrease and between group difference was not significant after adjusting the α-tocopherol concentrations for blood total cholesterol concentrations. The mean α-tocopherol concentration in the intervention group after ten-weeks was 34.86 ± 1.14 μM. The median, arithmetic and geometric mean (± SEM) concentrations derived from 4087 adults who participated in the 1999-2000 National Health and Nutrition Examination Survey were 25.94, 30.09 ± 0.45, and 27.39 ± 0.38 μM, respectively (Ford et al. 2006). It is also worth noting that the results of an eight week double-blind, placebo controlled randomised trial (Davidson et al. 2001) in which healthy subjects received 3 (n = 21), 6 (n = 21), and 9 (n = 23) g/day of total plant sterol equivalents showed that the unadjusted group mean plasma vitamin E concentrations of the subjects in the three intervention arms were not statically different to the unadjusted mean value of the control group (n = 21).

A fifty-two week randomised double-blind placebo-controlled parallel trial in which subjects received 1.6 g per day of esterified phytosterols revealed that the concentrations of the vitamin D_3 metabolite 25-hydroxy vitamin D_3 did not significantly differ at any time points within the intervention group (Hendriks et al. 2003). However, the differences from baseline at 26 weeks and at the end of the trial at 52 weeks were statistically significant when compared with the respective differences for the control group. The mean relative changes in 25-hydroxy vitamin D_3 from the group baseline concentration (82 ± 21 nM) for the subjects in the intervention arm of the trial were reductions of 17% at twenty-six weeks and 4% at the end of the trial whereas the mean relative changes from the baseline concentration in the control group (80 ± 26 nM) was a reduction of 8% at twenty-six weeks and an increase of 3% at the end of the trial (Hendriks et al. 2003). Nonetheless, a significant reduction in 25-hydroxy vitamin D_3 was not observed following a daily dietary exposure of 8.9 g of total plant sterol equivalents for 10 weeks in a randomised, double-blind, placebo-controlled study (Gylling et al. 2010). Similarly, a randomised, double-blind, controlled study that delivered 0, 3, 6 and 9 g of total plant sterol equivalents per day in parallel arms to healthy subjects for 8 weeks did not show significant reductions in 25-hydroxy vitamin D_3 at week 4 or at the end of the trial in the intervention groups compared with their baseline (Davidson et al. 2001). Therefore, there is no dose-response relationship between the dietary exposure to plant sterols and 25-hydroxy vitamin D_3 and the observations made by Hendriks et al. (2003) are most likely attributed to chance.

Although shorter in the intervention period compared with that reported by Gylling et al. (2010) or by Davidson et al. (2001), the findings of a randomised, double-blinded placebo-controlled study by Mensink et al. (2010), in which subjects received up to 9 g/d of total plant sterols equivalents for 4 weeks, were consistent with the longer studies.

No significant changes have been reported in vitamin K concentration in the blood serum following plant sterols consumption by hypercholesterolaemic (Sanchez-Muniz et al. 2009), mildly hypercholesterolaemic (Korpela et al. 2006) and healthy subjects (Plat and Mensink 2000; Davidson et al. 2001).

The current evidence shows that the concentrations of fat-soluble vitamins and carotenoids in the blood remain within the broad natural range of variation. Therefore, the current assessment agrees with the previous conclusion reached by FSANZ and confirms that the reduction in serum carotenoid concentration in the blood caused by consuming plant sterols does not pose a health risk to the adult population.

5 Dietary exposure assessment

5.1 Background

Dietary exposure to a novel food/ingredient such as plant sterols[2] is calculated from (1) the concentration of the ingredient in the foods requested and (2) consumption data for the foods that have been collected through a national nutrition survey. The methodology for conducting dietary exposure assessments (DEAs) has been established at FSANZ. Details are provided in Appendix 1.

Generally, predicting dietary exposure to a novel ingredient using food consumption amounts reported in national nutrition surveys (NNSs) could be done by assuming:

1. all foods that are permitted and requested to be permitted to contain the novel ingredient do indeed contain it; or

2. only a proportion of these foods contain the novel ingredient, with the proportion determined based on predicted market share for the foods likely to contain the novel ingredient ('a market share model').

The first assumption will provide a conservative exposure estimate for the whole population because, in reality, only a subset of foods permitted to contain plant sterols will actually contain them and only some people will choose these products. The second assumption gives an estimate of long-term exposure to plant sterols across the population as a whole, taking into account consumers and non-consumers of plant sterols. However, market share estimates are unlikely to reflect consumption patterns among those individuals who are regular and/or brand loyal consumers of foods with added plant sterols. Neither approach estimates dietary exposure in those consumers who deliberately alter their eating habits to include the manufacturers recommended number of serves of foods with added plant sterols.

For these reasons, recent comprehensive DEAs for plant sterols added to foods reported in previous applications, particularly Applications A1019 (FSANZ 2010b) and A1024 (FSANZ 2010a) were mainly based on:

- assessing the dietary exposure to plant sterols from the recommended number of serves of different foods containing plant sterols

[2] The term plant sterols has been used in the DEA to include the total amount of phytosterols, phytostanols and phytosterols, and phytostanols following hydrolysis of any phytosterol esters and phytostanols esters, as defined as 'total plant sterol equivalents' in section 1.1.2—2 in the Code.

- analysing consumption data from NNSs and calculating the mean and 95th-percentile dietary exposure to plant sterols that could be experienced if conventional products (e.g. edible oil spreads, breakfast cereal, low fat milk, low fat yoghurt and cheese (FSANZ 2010b) were substituted with plant sterol-containing products.

Both 2010 assessments concluded that there was no public health risks associated with consumption of foods with added plant sterols in target or non-target populations. However, because only limited foods with added plant sterols were available at the time of the NNSs used for previous exposure assessments, the DEA for these Applications could not estimate plant sterol exposures for persons reported as consuming food with added plant sterols.

Consumption data for foods with added plant sterols have now become available through more recent NNSs (2011–12 Australian National Nutrition and Physical Activity Survey (NNPAS), 2008-09 New Zealand Adult Nutrition Survey), which were used in this assessment.

5.2 Objective and approach

This DEA estimates plant sterol exposure from foods which contain added plant sterols. Baseline dietary exposure was estimated from the consumption of plant sterol-containing foods as reported in recent NNSs for Australian and New Zealand populations assuming they contained added plant sterols at the proposed maximum permitted amount. The Applicant requested permission to add plant sterols to breakfast cereals at a content of no less than 0.8 g and no more than 2 g per serve. The maximum of 2.2 g total plant sterol per serving provides for an *average quantity* of 2 g per serving to be declared without needing to exceed the maximum permitted amount of 2 g per serving on some occasions (see Call for Submissions paper). Therefore, dietary exposures were calculated using 2.2 g/serving as a conservative estimate.

Two approaches were used:

1. estimation of the total predicted dietary exposure based on the baseline exposure and an additional serving of portion-controlled breakfast cereals which contained 2.2 g plant sterols per serve (the per portion approach for Australian and New Zealand populations);

2. estimation of the total predicted dietary exposure by scenario modelling based on the baseline exposure and additional plant sterol exposure from breakfast cereal consumption, where the amount of plant sterols added to breakfast cereal was based on 2.2 g/serve but calculated on a per kg of cereal basis (the scenario modelling approach for the Australian population).

The latter approach was a more refined estimation because it was based on breakfast cereal consumption reported in the 2011-12 NNPAS and factors in persons who would consume greater than one serving of breakfast cereal per day.

5.3 Methods

5.3.1 Food consumption data

Dietary exposure to plant sterols was estimated using food consumption data from the most recent NNSs for the Australian and New Zealand populations:

- **2002 New Zealand National Children's Nutrition Survey** (2002 NZ NCNS): a 24-hour recall of 3,275 New Zealand children aged 5–14 years, with a second 24-hour recall undertaken for 15% of respondents (Ministry of Health 2003; Ministry of Health 2005)
- **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 2011; University of Otago and NZ Ministry of Health 2011)
- **2011–12 Australian National Nutrition and Physical Activity Survey** (2011-12 NNPAS), a component of the 2011–13 Australian Health Survey (AHS): a 24-hour recall of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (ABS 2014).

Specific consumption data for plant sterol-containing products (e.g. breakfast cereal, yoghurt, milk, edible oil spreads including margarine, and cheese) were collected in each of these surveys. In the NNPAS survey plant sterol-containing spreads, unflavoured milks and processed cheese were reported to be consumed. In the NZ surveys only plant sterol-containing spreads were reported to be consumed.

Additional attributes of each nutrition survey are summarised in Appendix 1.

5.3.2 Concentrations of plant sterols in foods

Previous FSANZ estimates of dietary plant sterol exposure did not include the contribution of the intrinsic amounts of plant sterols naturally occurring in foods since, at the time, there was limited analytical data for intrinsic amounts of plant sterols in foods and it was assumed that intrinsic plant sterols would make a minimal impact on exposure estimates. Dietary plant sterol exposure for populations on a typical Western-type diet (i.e. derived from non-enriched foods) has been reported to be between 150 to 360 mg per day (Chan et al. 2006). In the absence of more up-to-date concentration data for intrinsic plant sterols in foods, this approach has also been used in this assessment.

The estimations of baseline dietary exposures took into account only foods in which there are existing Code permissions to add plant sterols and the maximum permitted levels (MPLs) defined in the Code were used as the plant sterol concentration (Table 1). There was no reported consumption of breakfast cereals with added plant sterols in any of the surveys, possibly because these products were not available at the time of the survey. Therefore, plant sterols from breakfast cereals did not contribute to the baseline exposure estimate.

Amounts of plant sterols permitted to be added to foods are defined in terms of total plant sterol equivalents and not the plant sterol preparations actually added to foods. Therefore, no correction factor for phytosterol concentration in the foods needed to be applied to the MPLs for the DEA, except for cheese and processed cheese which on a plant sterol equivalents basis would be 54 g/kg (using a 0.6 conversion factor based on molecular weights).

One of the objectives of the DEA was to estimate plant sterol exposures if the permission for portion-controlled breakfast cereals of 2.2 g/serve was extended to all breakfast cereals at 2.2 g/serve. The plant sterol amounts used for this calculation needed to be converted from 2.2 g/serve to a per kilogram basis, which resulted in a higher concentration than currently permitted in the Code (19 g/kg). Serve sizes of most breakfast cereals range between 30 and 50 grams, which converted to a concentration of 40–73 g plant sterols/kg. The upper end of this range of 73 g/kg was used in the dietary exposure calculation for all breakfast cereals as a conservative estimate.

Table 1: Foods permitted to add plant sterols

Food[1]	Standard	Prescribed form[2]	Maximum Permitted Level	Conditions for PS addition
Breakfast cereals	Schedule 25	Total PS equiv	19 g/kg	The total fibre content is no less than 3 g/50 g serve and contains no more than 30 g/100 g of total sugars
Yoghurt	section 2.5.3—5	Total PS equiv	1.0 g/package (capacity no more than 200 g)	With no more than 1.5 g total fat/100g
Milk	section 2.5.1—6	Total PS equiv	4 g/L	With no more than 1.5 g total fat/100g
Edible oil spread (including margarine)	section 2.4.2—2	Total PS equiv	82 g/kg	The total saturated and trans fatty acids are no more than 28% of the total fatty acid content of the food
Cheese and processed cheese	section 2.5.4—4	Tall oil phytosterol esters[3] (Total PS equiv)	90 g/kg (54 g/kg)	With no more than 12 g total fat/100g

Abbreviations: PS = plant sterols; equiv = equivalents

[1] See Appendix 2 for corresponding Harvest food classification names and codes.

[2] Section 1.1.2—2 in the Code defines 'total plant sterol equivalents' as the total amount of phytosterols, phytostanols and phytosterols, and phytostanols following hydrolysis of any phytosterol esters and phytostanols esters.

[3] A previous FSANZ assessment concluded that vegetable-oil derived and tall-oil derived phytosterols were equivalent in terms of cholesterol lowering effects and food safety (FSANZ 2010a)

5.3.3 Age groups assessed

Mean and 90th percentile (P90) plant sterol exposures were derived for the age groups listed in Table 2. The adult age group (18 years and above) was split so that results could be reported for the intended target group of individuals aged 45 years and above.

Table 2: Population sub-groups used in this assessment

Country	Survey	Population surveyed	Population age-groups analysed
Australia	2011–12 NNPAS	2 years and above	2–4 years 5–12 years 13–17 years 18–44 years 45+ years 2+ years (all ages)
New Zealand	2002 NZ NCNS	5–14 years	5–12 years 13–14 years 5+ years (all ages)
	2008 NZ ANS	15 years and above	15–17 years 18–44 years 45+ years 15+ years (all ages)

5.3.4 Calculation of dietary exposure estimates

5.3.4.1 Baseline dietary exposure

Plant sterol exposure estimates were derived using Harvest, FSANZ's custom-built dietary modelling computer program. Results were derived using the first 24-hour recall consumption data only from all three surveys. All results were weighted to make them representative of the respective populations. The two day average exposure was also derived based on consumption data from the 2011–12 NNPAS for the 64% of respondents with two days of data (applying a different set of sample weights to make this survey sub-sample representative of the population). The two day average exposures better reflect longer term estimates of dietary exposure and therefore are a better estimate of chronic dietary exposure. The two day average for the New Zealand surveys was not derived due to low numbers of respondents for Day 2 of the surveys. The proportions of consumers of plant sterols, the mean and P90 plant sterol exposures were calculated for consumers of the foods listed in Table 1.

In all populations groups assessed, breakfast cereals or yoghurts with added plant sterols were not consumed in any of the surveys presumably because these products were not available on the market (Figure 2 and Tables A5 and A6 in Appendix 3).

5.3.4.2 Estimation of dietary exposure (per portion approach)

In this first approach, dietary exposure estimates were based on a proposed maximum permitted amount of 2.2 g of plant sterol equivalents per serving of breakfast cereal. Plant sterol dietary exposures estimated from the nutrition surveys (i.e. baseline dietary exposure) were added to this amount to derive an estimated maximum total dietary exposure per day. This exposure estimate assumed that persons would consume one serving of breakfast cereal per day in addition to other foods containing plant sterols.

5.3.4.3 Estimation of total dietary exposure for all breakfast cereals (scenario modelling approach)

In the second approach, scenario modelling was used to calculate exposure from reported consumption of foods that contained added plant sterols (i.e. baseline exposure) plus reported consumption of breakfast cereals that met the sugar and fibre criteria (see Table 1) assuming that those breakfast cereals contained added plant sterols. The scenario model assumed that persons who reported consuming breakfast cereal in the survey would consume the same amount of cereal containing added plant sterols at the maximum concentration. To calculate exposure in Harvest by this approach, new codes were assigned to these foods and a semi-probabilistic[3] calculation undertaken. (see Appendix 2).

Two day average exposures were calculated in the scenario as an estimate of chronic dietary exposure. As with the baseline exposure estimate, two day average for the New Zealand surveys was not derived due to low numbers of Day 2 respondents in the surveys.

[3] Semi-probabilistic refers to a dietary exposure method where individual food consumption data is matched with a single point chemical concentration per food or food group, to generate a range of individual dietary exposures. When individual records of food consumption are used, information can be generated on the distribution of food chemical dietary exposures in the population in addition to data on mean and percentile exposures for all respondents or consumers only. This method is particularly useful if a chemical is present in a wide variety of foods (FSANZ, 2009).

The proposed maximum amount of 2.2 g/serve was used as the plant sterol concentration for breakfast cereals in the scenario, with the serving size assumed to be 30 g of cereal giving a concentration of 73 g/kg cereal (see section 5.3.1). Because consumption amounts of oats and porridge were reported in the NNPAS as either uncooked or cooked amounts, the proposed amount of plant sterols to be added per kg of cereal was adjusted for exposure calculations taking hydration factors into account (Table 3).

Table 3: Plant sterol concentrations used in the scenario model to estimate dietary plant sterol exposure from breakfast cereals with added plant sterols

Cereal Types (as reported in NNPAS)	Proposed maximum amount	Assumed weight	Concentration used in scenario (g/kg)
All breakfast cereal meeting sugar and fibre criteria[1] including muesli and flaked biscuits		30 g serve	73
Oats and porridge "uncooked"	2.2 g/serve	40 g serve[2]	55
Oats and porridge "as prepared" or "cooked"		165 g serve[2]	13.3

[1] As specified in Schedule 25 (see Table 1)
[2] Based on a label review of 15 oats and porridge-type cereals (including single sachets) currently on the market in Australia and New Zealand where average dry weight servings were 40 g with 125 mL of liquid added.

5.4 Dietary exposure assessment results

5.4.1 Baseline plant sterol exposure from foods with added plant sterols

The national nutrition survey data showed that the number of plant sterol consumers as a proportion of respondents was about 5% for both Australian and New Zealand adult populations (Appendix 3). Based on Day 1 results, the 45+ age groups had the highest proportion of consumers (6.6% and 7.6% of respondents for Australian and New Zealand respondents, respectively). Children had the lowest proportion of consumers of foods with added plant sterols (31 consumers or 2% of respondents for Australians aged 2–17 years, which is consistent with previous FSANZ assessments (FSANZ 2010b). New Zealand children (5–14 years) had a very low proportion of consumers of foods with added plant sterols (7 consumers or 0.2% of respondents).

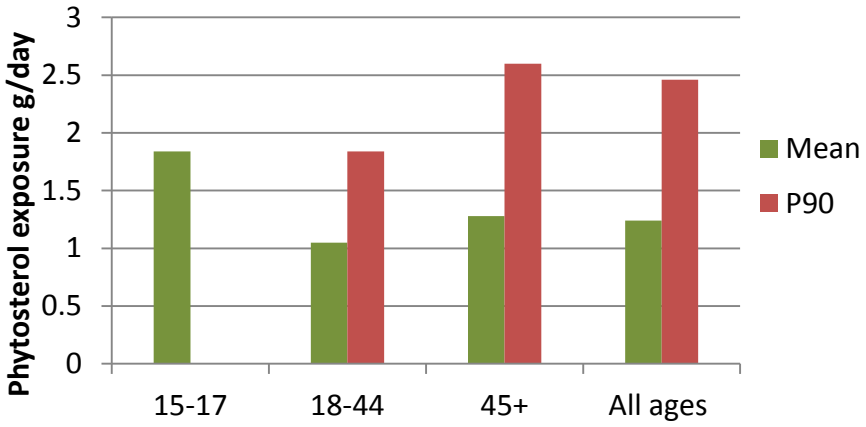
Estimated plant sterol dietary exposure from foods currently permitted to contain plant sterols, consumed in the NNSs for age groups assessed (baseline exposures) are shown in Table 4 and Figure 1, with detailed numerical data presented in Appendix 3. These estimates do not capture any breakfast cereal consumption despite the current permission as none were reported as consumed in the nutrition surveys, and for children only include consumption of edible oil spreads. Figure 1 does not show exposure estimates for the 2002 NZ NCNS, and they are not discussed here in detail, because there were too few persons (n = 7) consuming foods with added plant sterols, although these data are provided in Appendix 3.

Based on one day of consumption data, the mean baseline exposures were 0.7–0.90 g/day and 1.1–1.3 g/day for Australian and New Zealand adults (aged 18+ years) respectively, expressed as plant sterol equivalents. The P90 baseline exposures were 1.56–1.95 g/day and 1.84 and 2.60 g/day for Australian and New Zealand adults (aged 18+ years) respectively, expressed as plant sterol equivalents. Results for specific age groups are shown in Appendix 3.

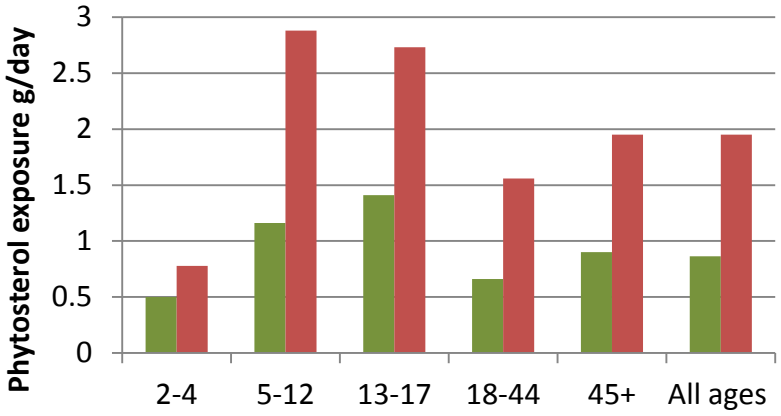
Based on one day of consumption data for Australian children[4] (aged 2–17 years), the mean and P90 baseline exposures for consumers of plant sterols were 0.5–1.4 g/day and 0.8–2.7 g/day respectively, expressed as plant sterol equivalents.

Nearly all plant sterol exposure was derived from consumption of edible oil spreads (Figure 2). However, types of edible oil spreads differed slightly with Australians mainly consuming reduced salt versions (with either regular fat or reduced fat content) and New Zealand population groups mainly consuming reduced or standard fat versions with standard salt content. Only the adult age group (18+ years) of the Australian population consumed unflavoured milks or processed cheese containing added plant sterols. However, the percent contribution of these foods to total plant sterol exposure was very low (< 3%). This could be due to the Australian survey being conducted more recently (2011–12) and the availability of more products containing plant sterols like milk and cheese on the market compared to when the New Zealand surveys were conducted.

A: New Zealand Adults (2008 NZ ANS), Day 1



B: Australia (2011-12 NNPAS), Day 1



[4] Results for New Zealand children excluded from this range due to the low numbers of consumers of foods with added plant sterols in the 2002 NZ NCNS. See Table A3 in Appendix 3.

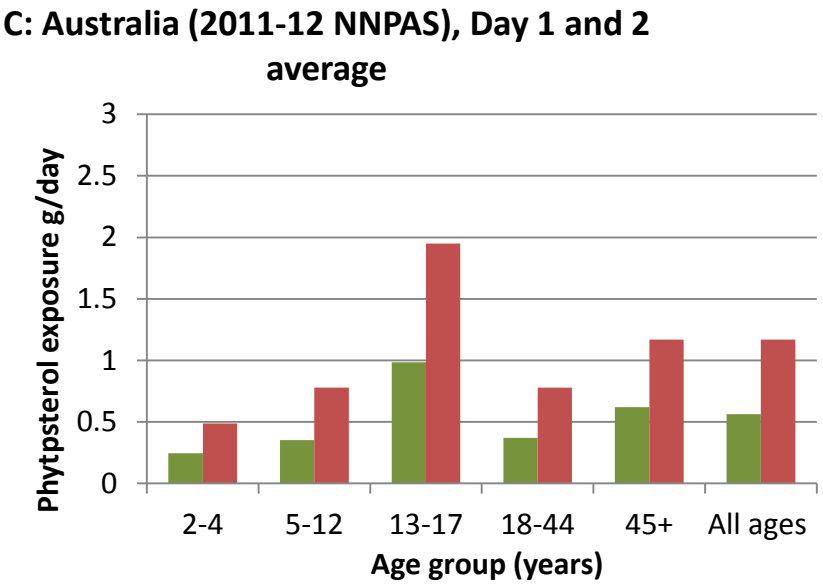
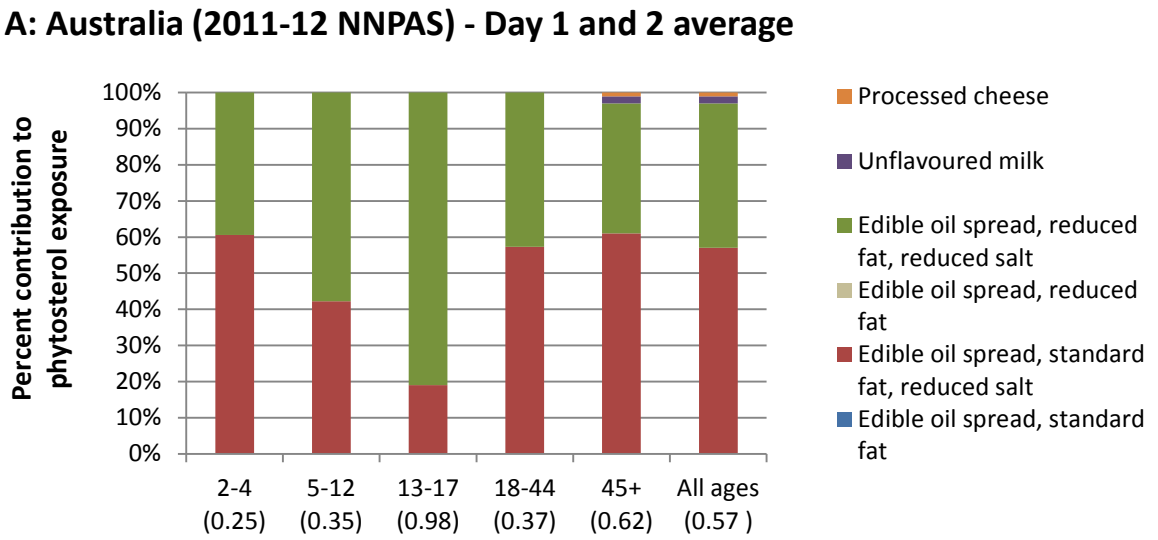
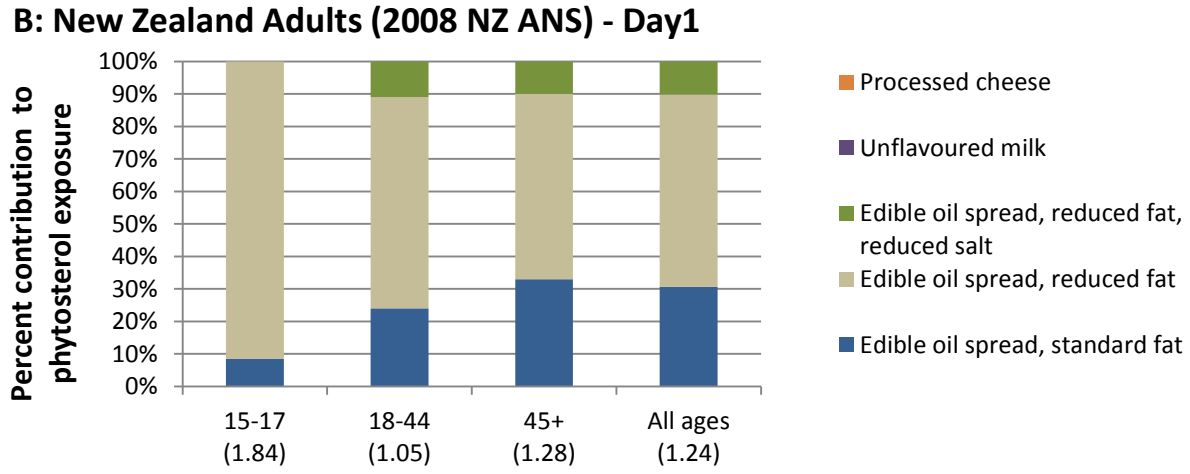


Figure 1: Baseline estimated dietary exposure to plant sterols for persons consuming foods with added plant sterols, by age groups. Results were derived from NNSs, as indicated. Results for New Zealand Children (2002 NCNS) and the P90 for New Zealand Adults aged 15-17 years (2008 ANS) are not shown due to limited numbers of consumers (see Appendix 3).





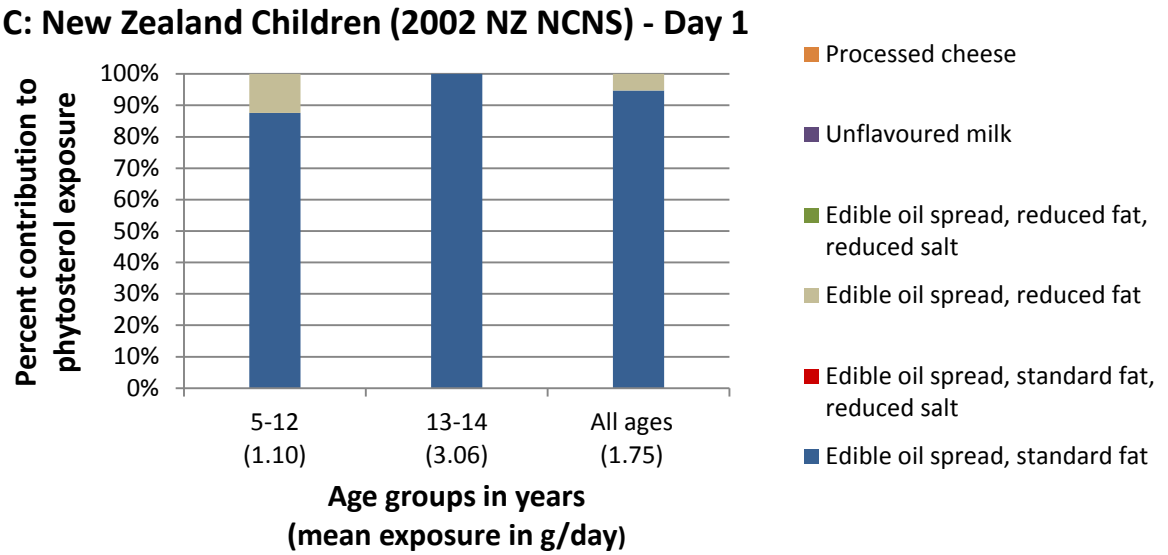


Figure 2: Per cent contribution of foods with added plant sterols to the population baseline estimated dietary exposure (mean exposure grams/day). Results were derived from NNSs, as indicated.

5.4.2 Estimated total dietary plant sterol exposure – per portion approach

If a serving of breakfast cereals containing 2.2 g plant sterols was consumed in addition to other foods containing added plant sterols (i.e. baseline exposure), the estimated mean total dietary exposure to plant sterols would not exceed 4.0 g/day across all population groups and the maximum P90 exposure would be 4.8 g/day and 5.1 g/day for New Zealand and Australian consumers[2] respectively, expressed as plant sterol equivalents (Table 4).

Table 4: Estimated total dietary exposures based on consumption of baseline foods containing added plant sterols and consumption of a portion-controlled breakfast cereal with added plant sterols at the MPL – Day 1 only, by age[1,2]

Survey	Age groups	Estimated Exposure Baseline foods only[3] (g/day)		Estimated Exposure Baseline foods + 2.2 g/day from portion-controlled breakfast cereal[3] (g/day)	
		Mean	P90	Mean	P90
2011-12 NNPAS	2–4 years	0.5	0.8	2.7	3.0
	5–12 years	1.2	2.9	3.4	5.1
	13–17 years	1.4	2.7	3.6	4.9
	18–44 years	0.7	1.6	2.9	3.8
	45+ years	0.9	2.0	3.1	4.2
	2+ years (all ages)	0.9	2.0	3.1	4.2

Survey	Age groups	Estimated Exposure Baseline foods only[3] (g/day)		Estimated Exposure Baseline foods + 2.2 g/day from portion-controlled breakfast cereal[3] (g/day)	
		Mean	P90	Mean	P90
2008 NZ ANS	15–17 years	1.8	na	4.0	na
	18–44 years	1.1	1.8	3.3	4.0
	45+ years	1.3	2.6	3.5	4.8
	15+ years (all ages)	1.2	2.5	3.4	4.7

[1] Abbreviations: na = not available (too few consumers)
[2] Results for New Zealand children (2002 NZ NCNS) excluded due to the low numbers of consumers of foods with added plant sterols. See Table A3 in Appendix 3
[3] Expressed as plant sterol equivalents

Because of the methodological differences, the exposure estimates shown in Table 4 (above) cannot be directly compared to the preceding assessments (e.g. (FSANZ 2010a; FSANZ 2010b). The overall range of plant sterol dietary exposures previously estimated by FSANZ was 1.9–4.8 g/day, which was of the same order of magnitude as the current estimate. It was concluded in previously assessments that there was no safety risk to the Australian and New Zealand populations.

5.4.3 Estimated total dietary plant sterol exposure - scenario modelling approach

Estimated total dietary plant sterol exposures were determined for Day 1 and Day 2 consumers of foods with added plant sterols as reported in the Australian 2011–12 NNPAS. The exposure estimates using the scenario model approach were not derived for the New Zealand population due to low numbers of respondents for Day 2 of the New Zealand surveys.

Estimated total dietary exposures calculated from the “Baseline” scenario (where foods included edible oil spreads, cheese, low fat milk, and yoghurt) ranged from 0.25–0.98 g/day and 0.78–1.95 g/day for mean and P90 estimates, respectively (Table 5). As these exposure estimates are based on an average of Day 1 and Day 2 consumption amounts, and therefore represent an estimate of chronic exposure, these exposure estimates are lower than the baseline exposures calculated from Day 1 consumption only (Table 4).

Estimated dietary exposures calculated from the “Breakfast cereal + Baseline” scenario (where foods included the baseline foods plus breakfast cereals with the proposed addition of plant sterols, as specified in Table 3) ranged from 2.05–3.67 g/day and 4.34–7.41 g/day for mean and P90 estimates respectively, expressed as plant sterol equivalents (Table 5).

FSANZ concluded in the Hazard Assessment (Section 3) that there was no justification for establishing an ADI for plant sterols. The Nutrition Assessment (Section 4) reported that human trials indicated that dietary exposures of up to 9 g/day of plant sterols did not affect blood concentrations of fat soluble vitamins. The proportion of survey respondents for which the estimated daily exposure was greater than 9 g/day was calculated (Table 5).

From the “Baseline” scenario, there were no age groups where respondents had estimated dietary plant sterol exposures greater than9 g/day. From the “Breakfast cereal + Baseline” scenario, the proportion of respondents with estimated dietary plant sterol exposures greater than 9 g/day ranged from 0 to 2.6% survey respondents. The proportion of children respondents aged 2–17 years with exposures greater than 9 g/day was 0 to 1.1 %.

The dietary exposure estimates for plant sterols for the high consumers (i.e. the P90 exposure) in the “Breakfast cereal + Baseline” scenario model were higher than the dietary exposure estimates for which no safety risk to the Australian and New Zealand populations was previously concluded by FSANZ (FSANZ 2010a, FSANZ 2010b). However, the proportion of the population (i.e. based on survey respondents) that would have exposures greater than 9 g /day, an amount that was shown to have no adverse health effects in human populations (see Section 4.2), was in the range of 0 to 2.6% across all age groups.

5.5 Dietary exposure assessment conclusion

Plant sterol dietary exposure was estimated using two approaches. The first per portion approach estimated exposure based on baseline consumption of foods with added plant sterols, as reported in the most recent NNSs and an added consumption of 2.2 g/day of plant sterols from portion-controlled breakfast cereal. This approach assumed that only persons choosing to consume breakfast cereals at the recommended serving size per day with a plant sterol concentration of 2.2 g/serve would be exposed to additional dietary plant sterols. Estimated total dietary exposures across all population groups (for which data were available) were in the range that would be unlikely to pose a risk to Australian and New Zealand populations.

For the second scenario modelling approach, it was assumed that all breakfast cereals meeting the sugar and fibre criteria would contain plant sterols at the proposed maximum permitted level. The approach was based on Day 1 and Day 2 consumption amounts of breakfast cereal and it accounted for persons who may consume more than the recommended serving amount. Estimated dietary exposures were higher than that derived in the first approach and higher than that derived in previous FSANZ assessments. However, the proportion of respondents in the Australian population that may have estimated dietary exposures greater than 9 g total plant sterol equivalents per day, an amount that has been shown in humans to have no adverse effects, is considered low (0-2.6% across different age groups). Given that the scenario employed to estimate the P90 exposure represented a conservative estimate in that it was assumed all breakfast cereals eligible to add plant sterols contained the maximum amount proposed to be permitted, it is unlikely that the P90 exposure estimates indicate a risk to the Australian population.

Given that no health based guidance value has been established for plant sterols to allow comparisons for risk characterisation purposes, the DEA supports the conclusion that addition of plant sterols at 2.2 g/serve to breakfast cereals would not pose a safety risk to the Australian and New Zealand populations.

Table 5: Estimated total dietary exposure to plant sterols from foods with added plant sterols – results from scenario modelling assuming plant sterols added at the MPL

Scenario	Age groups (years)	No. Consumers (No. Respondents)	% Consumers (as % of survey respondents)	Estimated total dietary exposure, consumers only* (g/day)		Proportion of survey respondents with exposure > 9 g/day (%)
				Mean	P90	
Baseline	2–4	7 (301)	2	0.25	na	0.0
	5–12	22 (812)	3	0.35	0.78	0.0
	13–17	15 (494)	3	0.98	1.95	0.0
	18–44	81 (3066)	3	0.37	0.78	0.0
	45–85	298 (3062)	10	0.62	1.17	0.0
	2+	422 (7735)	5	0.57	1.17	0.0
Breakfast cereal + Baseline	2–4	166 (301)	55	2.05	4.95	0.0
	5–12	348 (812)	43	2.27	4.34	0.7
	13–17	165 (494)	33	3.18	6.57	1.1
	18–44	1166 (3066)	38	3.67	7.41	2.4
	45–85	1626 (3062)	53	3.25	6.57	2.6
	2+	3472 (7735)	45	3.23	6.42	2.1

* Estimated total dietary exposure for consumers only, expressed as plant sterol equivalents, based on consumption of baseline foods with added plant sterols and consumption of breakfast cereals with the addition of plant sterols at 2.2 g/serve with adjustments specified in Table 3.

6 Uncertainties in the risk assessment

The available data on plant sterols in food technology, toxicology and nutrition studies are generally sufficient to provide a high level of confidence in the conclusions of this report in regards to the safety and suitability of fortifying breakfast cereals with increased amounts of plant sterols. These conclusions are extended mainly to the adult population, while there is no significant benefit or adverse effect for children or pregnant and lactating women. Based on the available knowledge on the mode of action of plant sterols, the current extensive experience on using foods fortified with plant sterol in the general population and an absence of adverse effects in children or pregnant women and animals and their offspring, there is no basis for postulating a risk to these population subgroups.

7 Conclusions

Breakfast cereals are not subjected to very high processing temperatures and oxidising conditions and because plant sterols are very stable compounds very few losses of plant sterols added to breakfast cereals are expected. Adding plant sterols at higher levels than currently permitted in breakfast cereals is concluded to be technologically feasible as methods are available to incorporate them. There are analytical methods available and specifications in the Code for plant sterols.

A review of the recent literature has not identified evidence to alter the conclusion reached previously by FSANZ, that a specified ADI is not justified for plant sterols for the general population. FSANZ has no toxicological concerns regarding the addition of plant sterols to breakfast cereals up to the concentrations proposed in the Application, for consumption by the general population. However, appropriate risk management measures are required for individuals with phytosterolaemia (sitosterolaemia).

The current evidence shows that for consumption of plant sterol-fortified food by humans at the dose proposed in the Application and higher, the concentrations of fat-soluble vitamins remain unchanged in the blood when adjusted to the changes in the total and LDL cholesterol; and carotenoids remain within the broad natural range of variation. Therefore, consuming plant sterol-fortified food at the proposed dose is not considered to pose a nutritional risk for humans.

Two approaches were used to estimate plant sterol exposure from breakfast cereals containing added plant sterols. The first 'per portion' approach estimated total dietary exposure based on consumption of foods with existing permissions to add plant sterols (i.e. the baseline exposure, estimated from the consumption of plant sterol-containing foods as reported in recent National Nutrition Surveys for Australian and New Zealand populations) and consumption of a serving of breakfast cereal containing 2.2 g of plant sterols. Across all surveys and age groups assessed for Australian (aged 2 years and over) and New Zealand (aged 15 years and over) populations, the total estimated dietary exposures to plant sterols by this approach were 2.7–4.0 g/day and 3.0–5.1 g/day for the mean and P90 exposures respectively, expressed as plant sterol equivalents.

The second scenario modelling approach estimated chronic plant sterol exposure if assuming all breakfast cereals contained plant sterols at the proposed maximum permitted amount of 2.2 g/serve. Using this second approach the total estimated dietary exposures to plant sterols for Australian consumers aged 2 years and above was 3.2 g/day and 6.5 g/day for the mean and P90 exposures respectively, expressed as plant sterol equivalents. It was also predicted that 0-2.6% of the Australian population aged 2 years and up would be exposed to more than 9 g/day of added dietary plant sterols equivalents, an amount that has been shown in humans to cause no adverse health effects. This estimate represents a conservative estimate since it assumed that persons who reported consuming breakfast cereal in the survey would consume the same amount of cereal containing added plant sterols at the proposed maximum permitted amount. This scenario accounts for the brand loyal consumer. Occasional ingestion of plant sterols at these levels is unlikely to pose any safety concerns.

Overall, the available data for plant sterols are considered to provide a high level of confidence in the safety and suitability of plant sterol fortified breakfast cereal products at the proposed maximum concentration, for all population groups.

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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'.

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 (FSANZ 2009). Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments FSANZ uses the food consumption data from each person in the NNSs 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[5].

FSANZ has developed a custom-built computer program 'Harvest' to calculate dietary exposures. Harvest is a newly built program and replaces the program 'DIAMOND' that had been used by FSANZ for many years. Harvest has been designed to replicate the calculations that occurred within DIAMOND using a different software package. Harvest was used for this assessment to extract the exposure data for plant sterols in foods with added plant sterols for Australian and New Zealand consumers. Dietary exposure assessments for all previous Applications for the addition of plant sterols to foods (e.g.A1019 *Phytosterol esters in low fat cheese*) were conducted using the DIAMOND program.

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)[6].

A1.1 Food consumption data used

The most recent food consumption data available were used to estimate plant sterol exposures for the Australian and New Zealand populations. The NNS data used for these assessments were:

The 2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)
The 2002 New Zealand National Children's Nutrition Survey (2002 NZ NCNS)
The 2008–09 New Zealand Adult Nutrition Survey (2008 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[7].

[5] http://www.foodstandards.gov.au/science/riskanalysis/Pages/default.aspx
[6] http://www.foodstandards.gov.au/science/exposure/Pages/dietaryexposureandin4438.aspx
[7] http://www.foodstandards.gov.au/science/exposure/Pages/dietaryexposureandin4438.aspx

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

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

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

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

A1.2 Limitations of dietary exposure assessments

Dietary exposure assessments based on 2011-12 NNPAS, 2002 NZ NCNS 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).

Appendix 2: Food classifications used in Harvest modelling

Foods that are permitted to contain added plant sterols are specified in the Code. However, these foods are coded in Harvest according to classification names and codes that can vary slightly from the Code and may also be split into sub-groups. To estimate plant sterol exposure from foods with added plant sterols, foods were assigned to the relevant Harvest food classification codes as listed in Table A1. New classification codes were created for the scenario model where plant sterols added at the proposed maximum amount of 2.2g/serve would be added to all breakfast cereals meeting the sugar and fibre criteria (see Schedule 25 of the Code) and included oats and porridge.

It is important to note that one of limitations of collecting food consumption data from nutrition surveys is that (1) consumers often do not know the exact product that is consumed, and/or (2) Harvest classifications do not match the criteria for the permission to add plant sterols specified in the Code. This is likely to be a factor for cheese, milk and yoghurt. Nevertheless consumption data from nutrition surveys for these foods were included in exposure estimates because people in the survey reported consuming the plant sterol versions of these foods.

Table A1: Existing and new classification names and codes used for the dietary exposure assessment[1]

Existing Classifications - Food Standards Code				
Standards in the Code			**Harvest**	
Category	**Standard**	**MPL**	**Classification Name**	**Code**
Breakfast cereals	Schedule 25	19 g/kg	Breakfast biscuits & flakes, choc/cocoa, PSE	6.3.2.1.1
			Breakfast biscuits & flakes, no choc/cocoa, PSE	6.3.2.2.1
			Puffed &/or extruded cereal, choc/cocoa, PSE	6.3.1.1.1
			Puffed &/or extruded cereal, no choc/cocoa, PSE	6.3.1.2.1
Yoghurt	section 2.5.3—5	1.0 g/200 g package (5 g/kg)	Ferm & renn milk prod, flav, froz, low/skim, PSE	1.2.2.5.4.2
			Ferm & renn milk prod, low/skim, choc, PSE	1.2.2.4.1.2
			Ferm & renn milk prod, low/skim, coffee, PSE	1.2.2.4.2.2
			Ferm & renn milk prod, low/skim, fruit, PSE	1.2.2.4.3.2
			Ferm & renn milk prod, low/skim, other flav, PSE	1.2.2.4.4.2
			Ferm & renn milk, unflav, low/skim, PSE	1.2.1.4.1
Milk	section 2.5.1—6	4 g/L	Liquid milk, phytosterol esters (PSE)	1.1.2.5
Edible oil spread (including margarine)	section 2.4.2—2	82 g/kg	Edible oil spread, red fat, PSE	2.2.2.1.2.1
			Edible oil spread, standard fat, PSE	2.2.2.1.1.1
			Margarine & similar products, PSE	2.2.1.3.1
Cheese	section 2.5.4—4	90 g/kg	Processed cheese, whole fat (=>15%), PSE added	1.6.3.1.2
New Classifications – for scenario model[2]				
Breakfast cereals	na	73 g/kg	Breakfast cereal that meets sugar and fibre criteria	6.3.4
		73 g/kg	Flaked biscuits	6.3.5
		13.3 g/kg	oats, rolled, cooked	6.1.1.1
		55 g/kg	oats, rolled, uncooked	6.1.1.2

[1] Abbreviations: PSE = phytosterols esters, choc = chocolate, ferm = fermented, renn = renneted, prod= products, flav = flavoured, unflav = unflavoured, froz = frozen, red = reduced, MPL= maximum permitted level (concentration used in dietary exposure estimation); na = not applicable.

[2] For the scenario model, only the identified breakfast cereals and oats consumed as breakfast cereal or porridge were assumed to contain plant sterols at the specified concentration. Breakfast cereals or oats used in mixed food recipes (e.g. oats in Anzac biscuits) were assumed to not contain plant sterols.

Appendix 3: Results of dietary exposure assessment

See the main report (Section 5) for the estimated dietary exposure to plant sterols that takes into account the higher requested amount proposed to be added to breakfast cereal.

The baseline dietary exposure estimates derived from NNSs (as indicated) is shown in the tables below.

Table A1 Estimated baseline dietary exposure to plant sterols for Australian consumers of foods with added plant sterols - 2011-12 NNPAS, Day 1 and Day 2 average, by age

Age Group (number of respondents)	Number of consumers	Consumers as a proportion of respondents	Mean exposure[#]		P90 exposure[#]	
			g/day	*g/kg bw/day**	*g/day*	*g/kg bw/day**
2–4 years (301)	7	2.2%	0.25	0.016	0.49	0.026
5–12 years (812)	22	2.7%	0.35	0.014	0.78	0.039
13–17 years (494)	15	3.0%	0.98	0.015	1.95	0.030
18–44 years (3066)	81	2.6%	0.37	0.004	0.78	0.008
44–85 years (3062)	298	9.7%	0.62	0.008	1.17	0.017
2+years (all ages) (7735)	422	5.4%	0.57	0.008	1.17	0.017

[#] Expressed as plant sterol equivalents

* Individual respondents' exposures are divided by their own body weight before deriving mean and P90 dietary exposures and are expressed as plant sterol equivalents.

Table A2: Estimated baseline dietary exposure to plant sterols for Australian consumers of foods with added plant sterols - 2011-12 NNPAS, Day 1 only, by age*

Age Group (number of respondents)	Number of consumers	Consumers as a percentage of respondents	Mean exposure[#]		P90 exposure[#]	
			g/day	*g/kg BW/day***	*g/day*	*g/kg BW/day^*
2–4 years (495)	10	1.9%	0.45	0.029	0.78	0.045
5–12 years (1291)	20	1.5%	1.16	0.033	2.88	0.074
13–17 years (746)	11	1.4%	1.41	0.021	2.73	0.038
18–44 years (4818)	92	1.9%	0.66	0.009	1.56	0.017
45+ years (4804)	318	6.6%	0.90	0.012	1.95	0.026
2+years (all ages) (12153)	451	3.7%	0.86	0.012	1.95	0.028

[#] Expressed as plant sterol equivalents

* Day 1 results are shown in Table A2.2 to permit comparison to New Zealand data (which is Day 1 only).

^ Individual respondents' exposures are divided by their own body weight before deriving mean and P90 dietary exposures and are expressed as plant sterol equivalents.

Table A3: Estimated baseline dietary exposure to plant sterols for New Zealand children consuming foods with added plant sterols - 2002 NZ NCNS, by age groups

Age Group (number of respondents)	Number of consumers	Consumers as percent of respondents	Mean exposure[#]		P90 exposure[#]^	
			g/day	*g/kg BW/day**	*g/day*	*g/kg BW/day**
5–12 years (2640)	5	0.18	1.10	0.036	na	na
13–14 years (635)	2	0.37	3.06	0.052	na	na
5–14 years (all ages) (3275)	7	0.22	1.75	0.041	na	na

[#] Expressed as plant sterol equivalents
* Individual respondents' exposures are divided by their own body weight before deriving mean and P90 dietary exposures and are expressed as plant sterol equivalents.
^na = not available (not reported as <10 consumers)

Table A4: Estimated baseline dietary exposure to plant sterols for New Zealand adults consuming foods with added plant sterols - 2008 NZ ANS, by age groups

Age Group (number of respondents)	Number of consumers	Consumers as percent of respondents	Mean exposure[#]		P90 exposure[#]^	
			g/day	*g/kg BW/day**	*g/day*	*g/kg BW/day**
15–17 years (266)	4	1.7%	1.84	0.028	na	na
18–44 years (2256)	51	2.3%	1.05	0.013	1.84	0.025
44+ years (2199)	167	7.6%	1.28	0.016	2.60	0.034
15+ years (all ages) (4721)	223	4.7%	1.24	0.016	2.46	0.033

[#] Expressed as plant sterol equivalents
* Individual respondents' exposures are divided by their own body weight before deriving mean and P90 dietary exposures and are expressed as plant sterol equivalents.
^ na = not available (not reported as <10 consumers)

Table A5: Amounts of foods with added plant sterols consumed – baseline foods only*

Survey	Age groups	Mean consumption for consumers of foods with added plant sterols (g/day)			
		Edible oil spread standard fat**	Edible oil spread reduced fat**	Unflavoured milk	Processed cheese
NNPAS 2011-12 (Day 1&2)	2–4 years (n=7)	3.5	2.4	Not consumed	Not consumed
	5–12 years (n=22)	3.4	4.9	Not consumed	Not consumed
	13–17 years (n=15)	4.8	11.8	Not consumed	Not consumed
	18–44 years (n=81)	4.6	4.0	83.3	Not consumed
	45+ years (n=298)	7.3	6.2	93.7	29.0
	All ages (n=422)	6.6	6.0	90.8	29.0
NZ ANS 2008 (Day 1)	15–17 years (n=4)	7.3	27.9	Not consumed	Not consumed
	18–44 years (n=51)	10.7	13.6	Not consumed	Not consumed
	45+ years (n=167)	17.0	15.9	Not consumed	Not consumed
	All ages (n=223)	13.8	15.6	Not consumed	Not consumed
NZ NCNS 2002 (Day 1)	5–12 years (n=5)	13.4	13.8	Not consumed	Not consumed
	13–14 years (n=2)	37.3	Not consumed	Not consumed	Not consumed
	All ages (n=7)	22.0	13.8	Not consumed	Not consumed

* Other food groups to which plant sterols were permitted to be added, where there was no consumption for any population groups for either country have not been included in this table. These are yoghurts and breakfast cereals.
** Includes salt reduced

Table A6: Food contributors to the baseline dietary exposure estimate for foods containing added plant sterols

Survey	Age groups	% Contribution to plant sterol dietary exposure					
		Edible oil spread, standard fat		Edible oil spread, reduced fat		Unflavoured milk	Processed cheese
		Standard salt	Reduced salt	Standard salt	Reduced salt		
NNPAS 2011-12 (Day 1&2)	2–4 years (n=7)	0	61	0	39	0	0
	5–12 years (n=22)	0	42	0	58	0	0
	13–17 years (n=15)	0	19	0	81	0	0
	18–44 years (n=81)	0	55	0	41	0	0
	45+ years (n=298)	0	61	0	36	2	1
	All ages (n=422)	0	57	0	40	2	1
NZ ANS 2008 (Day 1)	15–17 years (n=4)	8.5	0	91	0	0	0
	18–44 years (n=51)	24	0	65	11	0	0
	45+ years (n=167)	33	0	57	10	0	0
	All ages (n=223)	31	0	59	10	0	0
NZ NCNS 2002 (Day 1)	5–12 years (n=5)	88	0	12	0	0	0
	13–14 years (n=2)	100	0	0	0	0	0
	All ages (n=7)	95	0	5	0	0	0