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

Supporting document 1

Technological and Nutrition Risk Assessment – Application A1090

Voluntary Addition of Vitamin D to Breakfast Cereal

Executive summary

Vitamin D is a conditionally-essential nutrient that is synthesised in the skin with sun exposure or is consumed through dietary sources. Dietary sources are mainly animal-derived foods containing relatively small quantities of endogenous vitamin D and some fortified foods in Australia and New Zealand (ANZ). The purpose of this assessment was to examine the vitamin D status of ANZ populations and determine the health effects, or the potential risks to health, if permission were to be given for breakfast cereal to be fortified with vitamin D. Within these objectives, the bioavailability of the different forms of vitamin D, the technological efficacy of adding vitamin D to breakfast cereal, and the most appropriate method to ascertain potential effects on vitamin D status were also evaluated.

Two forms of vitamin D are permitted for addition to foods: D_2 (present in certain fungi and yeast) and D_3 (present in animal-derived foods and is the form synthesised in skin). Both forms have similar chemical and physical properties. They are considered to perform their technological function identically and have similar stability when included in a food matrix. Identity and purity specifications for both forms is defined in the ANZ Food Standards Code. Vitamin D activity is lost through some production processes and over the shelf life of breakfast cereal. Based on the requested maximum claim per normal serving of 25% regulatory RDI (10 µg) per reference quantity, and accounting for these losses, breakfast cereal containing a mean level of 5 µg/serve was used to estimate vitamin D intakes from breakfast cereal for the dietary intake assessment.

Vitamin D is metabolised through a complex pathway. The active metabolite, 1,25-dihydroxy vitamin D, is a hormone that maintains cellular calcium by regulating intestinal calcium absorption and mobilisation of calcium from bone. Vitamin D status is assessed by using the serum concentration of the metabolite 25-hydroxy vitamin D (25OHD) which circulates at more measurable concentrations (about 1000-fold greater than the 1,25-metabolite) and with a longer serum half-life compared to vitamin D or other metabolites. Measurement of total 25OHD in serum permits quantification of dietary vitamin D and that derived from sun exposure combined. Cut-off values for serum 25OHD concentrations that are associated with risk of adverse effects or disease, both high and low concentrations, are an area of current research.

Vitamin D requires no digestion and is absorbed in the intestine in association with fat and bile salts. As breakfast cereal contains between 1–5 g fat /100 g, consumption of vitamin D-fortified cereal with or without milk is likely to be absorbed with comparable efficacy.

Vitamin D_2 and D_3 are considered to be equally effective in raising serum 25OHD concentration up to intake levels of 25 µg/day when present in fortified foods and therefore the two forms were considered to have equivalent potency in this assessment.

Selection of criteria and assessment of national vitamin D status

For inadequate vitamin D status, serum 25OHD concentrations of 30 nM or less are clearly associated with increased risk of bone disease such as rickets and osteomalacia. There is debate about potential inadequacy associated with serum 25OHD concentrations above this value, in part due to problems with assays that over- or under-estimate serum 25OHD concentration. FSANZ adopted the recent US Institute of Medicine (IOM) view that 40 nM represents the average requirement. Therefore, by analogy with the usual FSANZ nutrient intake assessment procedures (the Estimated Average Requirement (EAR) Cut Point method), the proportion of the population with serum 25OHD < 40 nM provided an estimate of the prevalence of inadequate 25OHD concentrations in the ANZ populations.

UV-B exposure gives rise to maximal serum 25OHD concentration in the range of 120–160 nM. This range can be considered an upper physiological limit because, unlike orally consumed vitamin D, continued UV-B exposure degrades D_3 (and its precursor) in the skin to inert isomers. Very high oral doses (i.e. > 250 µg/day only as a vitamin D supplement) gives rise to serum 25OHD concentrations in the range of 220–700 nM and are associated with adverse health effects related to calcium regulation. The moderately high serum 25OHD concentration, that is, in the range 120–220 nM, has been proposed to be linked to other long term adverse health outcomes (e.g. cardiovascular disease) but results of studies are variable. Because of this uncertainty, the recent vitamin D review published by the IOM concluded that serum 25OHD concentrations in the range 125–150 nM would be of concern. Therefore, the prevalence of excess vitamin D was estimated in this assessment using the proportion of the population with serum 25OHD > 125 nM.

The most recent national biomedical surveys for Australians aged 12 years and over, for New Zealand children aged 5–14 years, and New Zealand adults aged 15 years were used to assess vitamin D status. These surveys reported that 80% or more of the adult population had adequate vitamin D status using a serum 25OHD concentration of 40 nM as a cut-off value. Prevalence of adequate status was lower (69%) in New Zealand children. The prevalence of low values was higher in winter than summer, varied with region, and was more common in indigenous and some migrant groups. The prevalence of Australians (aged 12 years and over) and New Zealand adults (aged 15 years and over) that had serum 25OHD > 125 nM was about 2%.

Effect of consumption of vitamin D-fortified breakfast cereal

Accurate food composition analyses for vitamin D are limited. In addition, because vitamin D metabolites are derived from both oral sources and sun exposure (which cannot be quantified), comparison of dietary intake with daily intake reference values (such as the Adequate Intake or Upper Level of Intake) are unsuitable to predict vitamin D status. Therefore, a unique approach was undertaken to determine potential health effects from consumption of vitamin D-fortified cereal. Using the mean level of vitamin D in breakfast cereal of 5 µg/ serve, vitamin D intakes from fortified breakfast cereal were predicted for a number of population groups and scenarios using ANZ food consumption survey data. These intakes were then used to predict the effect on serum 25OHD concentrations using a relationship reported in a recent study that found that serum 25OHD increased by 1.2 nM per 1 µg increment in vitamin D from fortified food (Black et al. 2012).

Estimated effects on serum 25OHD concentrations for ANZ populations were modelled under four scenarios: vitamin D intakes estimated from the mean or 90th percentile breakfast cereal consumption for brand loyal consumers (who always eats the same type of vitamin D fortified cereal) and for consumers who select from the range of products in the market over time, some of which have added vitamin D (market share model).

The mean consumption of vitamin D-fortified cereal gave a predicted annual mean serum 25OHD concentration in the range of 57.9–79.6 nM for a brand loyal consumer, and 52.8–72.7 nM for a consumer assuming a 35% market share of fortified breakfast cereal. High consumption (90th percentile) of vitamin D-fortified cereal gave a predicted serum 25OHD concentration in the range 62.2–88.5 nM for a brand loyal consumer and 54.3–75.8 nM for a consumer assuming a 35% market share of vitamin D-fortified cereal. These values were well within the physiological range of serum 25OHD concentration.

The predicted winter and summer mean serum 25OHD concentrations for adult ANZ populations were calculated independently to account for the known seasonal effects on serum 25OHD due to UV-B (sunlight) exposure. Using summer baseline values, the highest mean serum 25OHD concentrations that were predicted for the brand loyal, 90th percentile consumers ranged from 93.3–98.0 nM, starting from a baseline summer mean of 70.7–76.4 nM. This conservative estimate of serum 25OHD concentration from breakfast cereal consumption was still well under the 125 nM cut-off value. Using winter baseline values, none of the population groups had a baseline mean serum 25OHD < 40 nM (i.e. indicating inadequate status) and consumption of vitamin D fortified cereal was predicted to increase 25OHD status within the range 46–81.3 nM.

As a measure of the effect of the vitamin D fortification on the distribution of vitamin D status across the population over all seasons, the proportion of the population with serum 25OHD < 40 nM or > 125 nM was also estimated for the four breakfast cereal consumption scenarios.. For Australian adults, the proportion of the population with serum 25OHD < 40 nM decreased from a 13.4% baseline to 1.6% for the 90th percentile brand loyal consumers and to 12.4% for mean consumers of various brands (assuming 35% market share). Similarly, for New Zealand adults, the proportion with 25OHD < 40 nM decreased from a baseline of 20.9% to 1.5% for the 90th percentile brand loyal consumers and to 19.2% for mean consumers of various brands (assuming 35% market share). The proportion with serum 25OHD > 125 nM increased from a 1.3% baseline to 7.5% for the 90th percentile, brand loyal consumer in Australia and, for New Zealand adults in the same scenario, increased from 1.4% at baseline to 5.5%. Because this result represents the most conservative, worst-case scenario using a conservative estimate of high serum 25OHD concentration that would be of concern, it was concluded that increased risk of adverse health effects are unlikely.

On the basis of the above considerations, it was concluded that vitamin D (D_2 or D_3) fortification of breakfast cereal at the modelled level is unlikely to raise serum 25OHD levels above the physiological range and therefore the draft variation does not pose a risk to public health and safety. Additionally, vitamin D fortification of breakfast cereal has the potential to increase the vitamin D status of individuals whose vitamin D status is inadequate.

Terminology and abbreviations

General terms and abbreviations	
ANZ	Australia and New Zealand
ANZFSC	Australian New Zealand Food Standards Code
ABS	Australian Bureau of Statistics
IOM	US Institute of Medicine
NHMRC	National Health and Medical Research Council
NZ MoH	New Zealand Ministry of Health
EFSA	European Food Safety Authority
RTE	Ready-to-eat breakfast cereal
EAR	Estimated Average Requirement – the daily nutrient level estimated to meet the requirements of 50% of the healthy individuals in a particular life stage and gender group. The proportion of the population with intakes below the EAR is often used in population assessments to estimate the percentage with inadequate intake (providing the requirement distribution is thought to be normally distributed) because it approximates the result of the more complex Probability Approach.
RDI	Recommended Dietary Intake –- the average daily dietary intake level that is sufficient to meet the nutrient requirements of 97–98% of healthy individuals in a particular life stage and gender group; RDI = EAR +2$SD_{EAR.}$ Not used in population assessment of nutritional intakes (NHMRC & NZ MoH-2006).
Regulatory RDI	The regulatory recommended dietary intake is the quantity specified in Standard 1.1.1 that is used for calculations in the Nutrition Information Panel. It is not necessarily the same as the RDI for nutrients in the NHMRC and MoH report (NHMRC and NZ MoH 2006; NHMRC and NZ MOH 2006)). Nutrients such as vitamin D that have AI but no RDI might, nevertheless, have a labelling value currently termed 'RDI' in the Standard.
AI	Adequate Intake –- the average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate; used when there is insufficient data to define the RDI or EAR. In population assessments, the median intake of the population is described as being above or below the AI, but a quantifiable comparison is not done.
UL	Upper Level of Intake which is the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. In population assessments, the proportion with intake exceeding the UL is evaluated.
HPLC	high performance liquid chromatography
LC	liquid chromatography
MS or MS/MS	mass spectrometry or tandem mass spectrometry
RIA	radioimmunoassay
nM	nanomolar or nanomoles per litre
Abbreviations of surveys	
ANCNPAS	2007 Australian Children's Nutrition and Physical Activity Survey
ANNS	1995 Australian National Nutrition Survey
NZ NCNS	2002 New Zealand National Children's Nutrition Survey
AHS	2011–13 Australian Health Survey
NHMS	National Health Measures Survey, the component of the AHS that reported measurement of 25OHD serum concentration in the Australian population.
NNPAS	National Nutrition and Physical Activity Survey component of the AHS that food and nutrient intakes for the Australian population
NZ ANS	2008-09 New Zealand Adult Nutrition Survey

Vitamin D Terminology

Vitamin D terminology and definitions are summarised in the table below. Throughout this report, the term 'vitamin D' (i.e. without a subscript) will be used to mean either form of vitamin, i.e. D_2 or D_3. Vitamin D metabolites without a subscript means the metabolite could be derived from either D_2 or D_3 or the metabolite has not been measured specifically. The particular forms of vitamin D (i.e. D_2 or D_3), or metabolites of these forms, will be described and denoted with a subscript as required.

Vitamin D terminology and definitions			
Term used in this Report	**Other terms**	**Definition**	**Abbreviation**
vitamin D	calciferol	Includes both forms of vitamin D – D_2 or D_3	none
vitamin D forms	-	Refers specifically to the permitted forms of dietary vitamin D under the ANZFSC	
vitamin D metabolites	-	Includes both inactive and active products derived from vitamin D_2 or vitamin D_3	
7-dehydrocholesterol	-	Precursor to vitamin D_3 found in the skin of most terrestrial animals	none
previtamin D_3	precalciferol	Precursor of vitamin D_3, formed by action of UV-B (sunlight) on 7-dehydrocholesterol	none
vitamin D_3	cholecalciferol	Vitamin D that is photosynthesised in the skin of animals and humans	D_3
25-hydroxy vitamin D_3	25-hydroxy cholecalciferol	The main circulating metabolite derived from vitamin D_3	$25OHD_3$
1,25-dihydroxy vitamin D_3	1,25-dihydroxy cholecalciferol	Main physiologically active metabolite derived from vitamin D_3	$1,25(OH)_2D_3$
vitamin D_2	ergocalciferol	Vitamin D that is derived from certain fungi	D_2
25-hydroxy vitamin D_2	25-hydroxy ergocalciferol	The main circulating metabolite derived from vitamin D_2	$25OHD_2$
1,25-dihydroxy vitamin D_2	1,25-dihydroxy ergocalciferol	Main physiologically active metabolite derived from vitamin D_2	$1,25(OH)_2D_2$
25-hydroxy vitamin D	calcifediol or calcediol	Generic term to include both $25OHD_2$ and $25OHD_3$ metabolites	25OHD
1,25-dihydroxy vitamin D	Calcitriol	The active metabolite derived from either vitamin D_2 or vitamin D_3	$1,25(OH)_2D$
vitamin D binding protein	-	Vitamin D binding protein binds to vitamin D or its metabolites to produce a complex which circulates in the blood.	DBP

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1 Introduction

1.1 Background

Vitamin D is a pre-prohormone that exists in two forms – vitamin D_2 and vitamin D_3, referred to as D_2 and D_3 respectively. Both are metabolised to the hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which regulates calcium homeostasis and bone growth and maintenance. For most people living in Australia and New Zealand (ANZ), the main source of vitamin D is from photochemical synthesis in the skin by exposure to UV-B irradiation (or sunlight) (Nowson et al. 2012). Therefore vitamin D is conditionally essential in the diet. Vitamin D is present naturally in food especially animal-derived foods such as meat and eggs. Recent food composition work using sophisticated analytical methods indicates that the vitamin D content of the diet may be greater than previous estimates and so the relative contribution of dietary sources is unclear. Other sources of vitamin D in the diet are some fortified foods including table edible spreads and some dairy products.

The Application seeks permission for voluntary addition of vitamin D to breakfast cereal with vitamin D to a maximum claim per normal serving of 25% regulatory RDI (10 µg) per reference quantity specified in Schedule 1 of Standard 1.1.1. The purpose of this request stems from recent concerns that the ANZ populations may not be receiving adequate vitamin D, in part due to advice from public health and medical experts to reduce sun exposure to prevent skin cancers. This has led to some expert panels and advisory bodies to recommend that persons at risk of vitamin D deficiency should increase their vitamin D intakes. (Ministry of Health & Cancer Society of New Zealand 2012; Nowson et al. 2012; Ebeling et al. 2013). This can be achieved through greater sunlight exposure, use of vitamin D supplements, fortification of certain foods, or increased consumption of foods which naturally contain vitamin D.

1.2 Objectives

The risk assessment will examine the effects of voluntary fortification of breakfast cereal with vitamin D on the ANZ populations. The assessment aims to address the following risk assessment questions:

1) What is the likely effect of processing and storage on the lability of vitamin D in breakfast cereal?
2) Is vitamin D bioavailable from vitamin D-fortified breakfast cereal consumed with milk?
3) What is the evidence for vitamin D inadequacy in ANZ?
4) What is the risk to health from consumption of vitamin D-fortified breakfast cereal in Australia and New Zealand?
5) What is the benefit to health from consumption of vitamin D-fortified breakfast cereal in Australia and New Zealand?

1.3 Scope

Nutritional and physiological effects of vitamin D are influenced by several factors which are briefly reviewed in this assessment. These include:

- multiple sources (oral and photochemical synthesis in the skin)
- complex metabolism involving numerous metabolites
- problematic assay methods for measuring serum 25-hydroxy vitamin D (25OHD) and vitamin D content in food
- effects on calcium homeostasis that are linked to both inadequate and excess vitamin D status.

Inadequate exposure to UV-B (sunlight) irradiation may cause a reduced vitamin D status that could be improved by oral consumption of vitamin D-rich food or from vitamin D supplements. However, oral consumption of vitamin D may also exceed a safe level of intake particularly if a repeated daily dose is consumed. As vitamin D metabolites are derived from both oral sources and sun exposure (which cannot be quantified), comparison of dietary intake with daily intake reference values (such as the Adequate Intake (AI) or Upper Level of Intake (UL) are unsuitable to predict vitamin D status. Therefore, a unique approach was undertaken to determine potential health effects from consumption of vitamin D-fortified breakfast cereal by determining the intake of vitamin D from the fortified cereal and estimating the effect of consuming the cereal on serum 25OHD, the vitamin D metabolite that can be measured to reflect vitamin D status.

This assessment does not review the body of the evidence on which the current dietary reference values such as the Adequate Intake (AI) and the UL have been established.

2 Food Technology Report

2.1 Chemistry of biologically inert vitamins: D_2 and D_3

Chemical structures for the two forms of vitamin D are provided in Figure 1. Structurally, the only difference between the two is that D_2 has a double bond between carbons 22 and 23 and a methyl group at carbon 24. The chemical and physical properties are shown in Table 1.

Vitamin D_2 is derived from plants, yeast and fungi through exogenous irradiation of ergosterol. D_2 is usually isolated for commercial use from yeast. The skin produces vitamin D_3 photochemically from 7-dehydrocholesterol, which is present in the skin of higher terrestrial animals, by the action of sunlight or artificial UV light. Vitamin D_3 for commercial use in food fortification or vitamin supplements is mainly derived from its precursor, 7-dehydrocholesterol which in turn is prepared from the lanolin in sheep wool. As D_2 and D_3 have chemically similar structures and properties (see Table 1), they are considered to technologically perform identically and have similar stability when included in a food matrix.

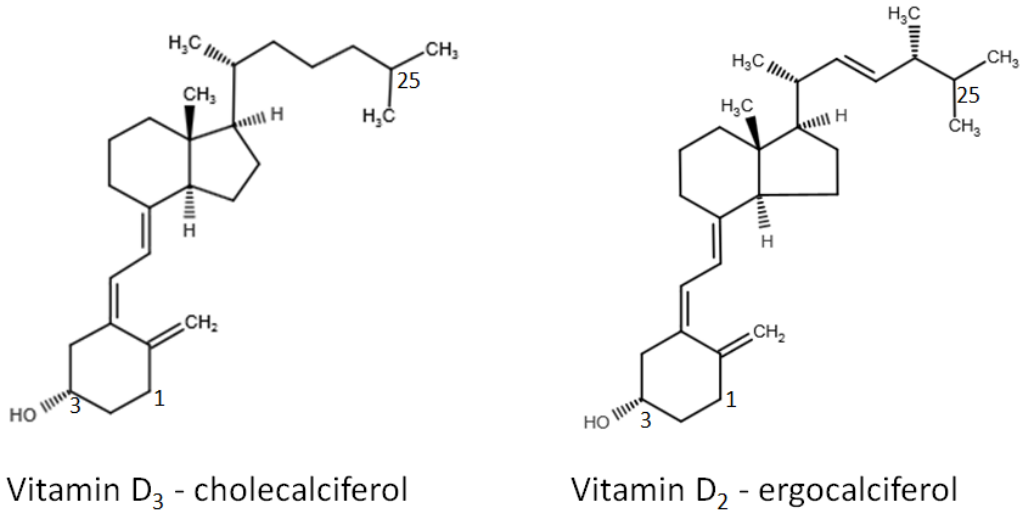

Figure 1: The structures of D_3 and D_2.

Position numbers 1 and 25 indicate sites where hydroxylation occurs to produce physiologically important metabolites of vitamin D (see Section 3.1)

Table 1: Chemical and physical properties of vitamin D_2 and D_3

Property	Vitamin D_2	Vitamin D_3
Molecular structure	$C_{28}H_{44}O$	$C_{27}H_{44}O$
Molecular weight (g/mol)	396.66	384.65
CAS number	50-14-6	67-97-0
Appearance	White crystals	White crystals
Melting point (°C)	115–119	84–89
Water solubility	Insoluble	Insoluble
Soluble in other solvents	Alcohol, chloroform and fatty oils	Alcohol, chloroform and fatty oils

Source: U.S. Pharmacopeial Convention, Food Chemicals Codex 9th Edition (2014)

2.2 Specifications

Both D_2 and D_3 are permitted forms of vitamin D for addition to foods in the Schedule to Standard 1.1.1 – Preliminary provisions – application, interpretation and general prohibitions.

Standard 1.3.4 – Identity and Purity requires that all vitamins permitted to be added to food meet appropriate specifications for identity and purity.

Clause 2 of Standard 1.3.4 contains a list of primary sources of specifications. One of these is the Food Chemicals Codex (U.S.Pharmacopeial Convention 2014) which contains specifications for both D_2 and D_3 as listed in Table 1 above.

The Application is for the voluntary fortification of vitamin D to breakfast cereal. The Australia New Zealand Food Standards Code (ANZFSC) already has permissions for the addition of vitamin D to a variety of foods as set out in the Table to clause 3 of Standard 1.3.2 – Vitamins and Minerals.

2.3 Analytical methods in foods

Vitamin D has been added to various foods for many years and there have been a variety of analytical methods to determine and quantify the levels of added vitamin to different food matrices. The Official Methods of Analysis for the Association of Official Analytical Chemists (AOAC) has variations on different analytical methods for determining the levels of addition of vitamin D added to food depending on the food matrix (Horwitz and Latimer 2012).

The current version (19th edition, 2005, revision 5, 2012) of the AOAC Official Methods of Analysis (Horwitz and Latimer 2012) contains a large number of analytical methods for the determination of vitamin D in various food, feed and vitamin preparations. Of relevance to this Application are those for:

- Vitamin preparations (method 979.24, liquid chromatography (LC) method)
- Multivitamin preparations (980.26, LC method)
- Milk and milk powder (981.17, LC method)
- Mixed feeds, premixes, and pet foods (982.29, LC method)
- Infant formulas and enteral products (995.05, LC method)
- Infant formulas and adult/paediatric nutritional formula (2011.11, ultra-high performance LC mass spectroscopy/mass spectroscopy (MS/MS or tandem MS) method
- Vitamin D_3 in selected foods (2002.05, LC method).

It is expected that suitably qualified and experienced analysts would be able to adapt these various analytical methods, essentially using high performance liquid chromatography (HPLC) to determine the amount of vitamin D added to breakfast cereal.

The use of HPLC coupled with tandem mass spectrometry to analyse for vitamin D in various food matrices, including cereal and cereal-based foods has also been described (Huang et al. 2009). D_3 and D_2 are separable and quantifiable by the HPLC method.

Therefore, there are a variety of analytical methods available which can either be used or adapted as required to analyse for vitamin D in breakfast cereal.

2.4 Application of vitamin D to breakfast cereal

Breakfast cereals have been fortified with vitamin D for many years in other countries so the technology is well known and commercialised. The methods are similar to those already employed to add a variety of fat soluble and water insoluble vitamins to breakfast cereal in Australia and New Zealand.

The Application provides information on two main methods to incorporate vitamin D into breakfast cereal. These methods are explained in more detail below.

2.4.1 Aqueous spray application

Since vitamin D is poorly soluble in water, emulsifiers are required to ensure a miscible homogeneous blend if the vitamin D was made up in an aqueous solution. This solution is then applied to the surface of the formed breakfast cereal product. The solution of heat labile vitamins such as vitamin D (and vitamin A) is applied after cooling of the breakfast cereal pieces. Spray application can be performed on a conveyor belt or in a tumbling drum with either process controlled to ensure homogeneity of application of the vitamin preparation.

2.4.2 Dry batching

This method of application requires that the dry form of the vitamin is mixed in with the initial flour ingredients used to produce the extruded breakfast cereal products or added into the cooked form of the cereal mass before final shape forming and drying of the breakfast cereal pieces.

Due to the processing involved (either as a coating process or incorporated into the flour mix used to produce the final product) unprocessed rolled oats sold for porridge will not be fortified with vitamin D, in the same way that current rolled oats are not fortified with other vitamins.

2.5 Stability of vitamin D during processing and losses with shelf life

The Application provides some details about both stability of D_3 during production of the breakfast cereal and over the shelf life of the product once produced and sold. Specifically information is provided on the percentage losses due to processing and shelf life storage. This information is important for breakfast cereal manufacturing so the appropriate amount can be added to ensure the final commercial product contains the claimed label amount of the vitamin even at the end of the designated commercial shelf life of the product. Food manufacturers add extra vitamins to compensate for these losses and the term used for the extra addition is ‘overage’. This is a well-known and understood practice when foods are fortified with nutrients including vitamins.

The Application notes that vitamin D is relatively unstable to heat and moisture and there are processing losses during extrusion in the range of 25–40% for extruded cereal products (Riaz et al. 2009). The Application used the figure of 30% for its example.

Vitamin D activity is also lost during the commercial shelf life of the product because it is unstable to heat, moisture, oxygen, contact with trace minerals and UV light (Ott 1988). Based on industry experience the average vitamin D loss is considered to be 10% per month at ambient temperature and humidity. Breakfast cereal products usually have a shelf life of between 9 and 12 months. The Application provides a theoretical worked example to indicate there is approximately a 70% loss of vitamin D content during storage of a fortified breakfast cereal at the end of its commercial shelf life at the worst case of 12 months. Therefore, the midpoint of the shelf life was estimated to be 5 µg /serve and was the figure used to estimate vitamin D intakes from breakfast cereal for the dietary intake assessment (see Section 5).

Breakfast cereal manufacturers would need to know the total vitamin D losses for their products during production and storage at various temperature and humidity conditions for the shelf life of their product so they can add sufficient vitamin D to compensate for the losses.

2.6 Summary – food technology

It was concluded that the manufacturing process of adding vitamin D during or after the formation of the extruded breakfast cereal product is well understood and practised as it has been commercialised in other countries around the world and uses processes already used to add other fat soluble, heat labile vitamins to breakfast cereal products.

Breakfast cereal manufacturers will need to determine what vitamin D losses occur during processing on their production facilities and are lost during storage of breakfast cereal throughout the shelf life of the product and compensate by adding extra vitamin D to ensure that the claimed amount is present.

There are analytical methods for the determination of vitamin D in various foods that can be modified as required so the levels in breakfast cereal can be determined. There are also specifications for both D_2 and D_3 forms in a primary source of specifications in the ANZFSC so no new specification is required.

3 Hazard Identification and Characterisation

3.1 Metabolism

Humans obtain vitamin D from several sources:

- Endogenous D3 produced by skin exposure to UV-B light which converts the precursor 7-dehydrocholesterol to D3 which then diffuses into the circulation over a period of days.
- Dietary D3 or D2 is absorbed in the small intestine by passive diffusion into intestinal cells where it is incorporated into chylomicrons for transfer into the circulation.

D_2 and D_3 are metabolised similarly although there is some debate about their relative bioequivalence which is discussed in Section 3.3. Therefore, the mechanism of vitamin D metabolism is described here as ‘vitamin D’ to represent both D_2 and D_3.

Figure 2 summarises the metabolism of vitamin D. Dietary and skin-derived vitamin D is transported via the circulation to the liver where the enzyme 25-hydroxylase adds a hydroxyl group at the 25-position to produce 25OHD. This metabolite circulates in association with vitamin D binding protein (DBP) in nanomolar quantities with a half-life in humans of about 15 to 20 days (Jones 2008).

The biologically active metabolite $1,25(OH)_2D$ – also called calcitriol – is produced by a second hydroxylation in the kidney. $1,25(OH)_2D$ is a steroid hormone which exerts its biological action through binding to its nuclear vitamin D receptor which directs the expression of genes involved in vitamin D metabolism and bone maintenance. The nuclear vitamin D receptor is present in the major target cells required for calcium homeostasis: intestinal cells where $1,25(OH)_2D$ stimulates calcium absorption; bone cells where $1,25(OH)_2D$ stimulates bone resorption to maintain plasma calcium concentrations; and kidney cells where $1,25(OH)_2D$ increases plasma calcium concentration by stimulating renal tubule reabsorption of calcium.

Production of $1,25(OH)_2D$ is tightly regulated through the endocrine system: conversion of 25OHD to $1,25(OH)_2D$ is increased by secretion of parathyroid hormone (for example in response to calcium deficiency) and is inhibited by elevated serum phosphorus and calcium concentrations. The $1,25(OH)_2D$ metabolite can be present in the circulation in picomolar quantities. Relative to vitamin D and 25OHD, it has a short half-life in serum of approximately seven hours which is due to feedback regulation though the kidney vitamin D receptor (VDR) causing relatively rapid degradation and clearance and/or due to lower affinity to DBP (Jones et al. 1998; Tsiaras and Weinstock 2011). For this reason, serum $1,25(OH)_2D$ is not a useful measure of vitamin D status or as a marker for health outcomes.

25OHD and $1,25(OH)_2D$ are further hydroxylated by two cytochrome P-450 enzymes CYP24 and CYP3A4 to metabolites which re-enter the blood though enterohepatic circulation. These metabolites are nutritionally inactive although have been studied for use in clinical applications. Vitamin D metabolites are progressively hydroxylated by cytochrome P-450 enzymes CYP24 and CYP3A4 leading to loss of hormonal activity and eventual excretion into bile (Prosser and Jones 2004; Schuster 2011).

The location of vitamin D or 25OHD stores in humans and terrestrial animals is unknown although there are liver stores in fish (Pierens and Fraser 2014). The fat solubility and long half-life of 25OHD has led to speculation that 25OHD may be sequestered into adipose tissue where it could be released back into the circulation with metabolism of fatty acids. Recent studies suggest that 25OHD is stored in skeletal muscle which may prevent it from degradation and play function role in metabolism (Abboud et al. 2013a; Abboud et al. 2013b). This is another area of vitamin D metabolism which is not fully characterised.

3.1.1 Serum 25OHD as a marker of exposure or vitamin D status

The vitamin D status in humans is usually assessed by measuring the concentration of the metabolite 25OHD in plasma or serum. Importantly serum 25OHD concentration is an indicator of both oral intake and UV photosynthesis combined.

UV-B exposure (sunlight) increases serum $25OHD_3$ to a maximal range of about 120–160 nM (EFSA Panel on dietetic products 2012a). Prolonged exposure to UV-B does not increase serum $25OHD_3$ beyond this range as both D_3 and pro-vitamin D are degraded in the skin to inert isomers with continued UV-B exposure. Therefore, concentrations up to about 120 nM can be considered to be in a physiological range.

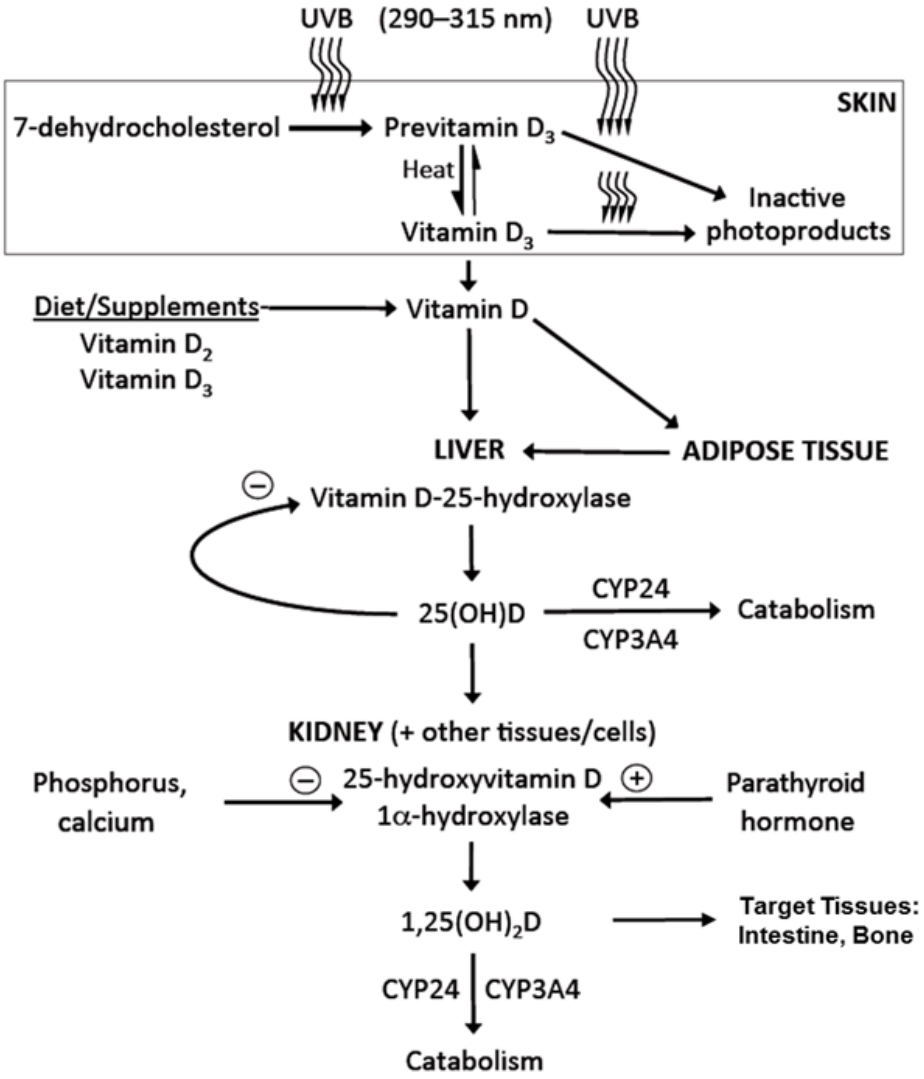

Figure 2: Metabolism of vitamin D

Adapted from an open access, online source (Tsiaras and Weinstock 2011).

Oral consumption of vitamin D – through vitamin D containing foods (fortified or naturally occurring) or dietary supplements (which usually contain greater amounts of vitamin D than that contained in fortified foods) – increases serum concentrations of 25OHD depending on the dose. For daily vitamin D intakes up to 250 µg/day, serum 25OHD concentration is usually in the range of 25–125 nM (Vieth, 1999) (See Section 3.5 for further discussion).

Serum 25OHD concentration is controlled by vitamin D supply (oral and photochemically synthesised) and the rate of metabolism by hydroxylases in the liver. The dietary factors that affect vitamin D metabolism are complex and not well understood. For example, vitamin D deficiency (as determined by low serum 25OHD concentration) can be induced from dietary calcium deficiency as a result of increased conversion of 25OHD to $1,25(OH)_2D$ needed to maintain intracellular and extracellular calcium concentrations (Clements et al. 1987). On the other hand, very high doses of vitamin D (for example, via supplement use) may also give rise to preferential catabolism of 25OHD to inactive metabolites or to sequestration into fat and muscle (IOM 2011). Despite complexities in the steady state conditions of serum 25OHD, it represents the only suitable marker of vitamin D status.

3.1.2 Assays for serum 25OHD

Serum 25OHD can be quantified by a number of methods including competitive protein binding assays, radio-immuno binding assays (RIA), chemiluminescent immuno-assay, and HPLC assays (Lai et al. 2010). Results are highly variable across different assay methods due to: 1) hydrophobic nature of vitamin D metabolites and binding to DBP which necessitates extraction and or protein precipitation steps leading to potential losses; 2) multiple metabolites ($25OHD_2$ and $25OHD_3$) contributing to total serum 25OHD concentrations but may be quantified differentially by some methods; and 3) cross-reactivity with 25OHD epimers (such as the C3-epimer of 25OHD which differs in the configuration of the hydroxyl group at the 3 position of vitamin D) and other hydroxylated metabolites. These variabilities have led to under- or overestimation of serum 25OHD concentrations, particularly with automated commercial assay methods (Roth et al. 2008; Wagner et al. 2009; Lai et al. 2010; Snellman et al. 2010; Connell et al. 2011).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the most accurate method but is not commonly used in commercial laboratories because of the technical expertise and expensive equipment required. The most common method used in clinical studies has been commercially available automated assays which utilise immuno-binding and chemiluminescence methodology to quantify 25OHD (either $25OHD_2$ or $25OHD_3$ or both). Results from these high-throughput methods are relatively rapid, inexpensive, and technically simple, but can produce inaccurate and unreliable measurements of 25OHD concentrations (Carter 2012). Because of its greater accuracy, recent studies have aimed to use the LC-MS/MS method or at least validate a commercial immuno-binding assay by measuring a subset of samples using LC-MS/MS (Daly et al. 2011).

Until 2009, no standard reference materials existed that allowed laboratories using different methods to calibrate their results to each other. As a result, combining 25OHD results from different studies (such as in systematic reviews) which are then used to set clinical cut-off values for serum 25OHD concentrations linked to adverse health effects has been controversial. The US National Institutes of Health's Vitamin D Standardisation Program (VDSP) is an international collaboration of scientists which has been set up to validate 25OHD assay methods (National Institutes of Health 2014). The program is on-going although some results have been published (Cashman et al. 2013). The National Health Measures Survey (NHMS), a component of the Australian Health Survey (AHS), includes measurement of 25OHD concentration in the Australian population. The AHS is a contributor to the VDSP and has therefore utilised methodology that is validated by appropriate standards and assay techniques.

3.2 Absorption

Dietary vitamin D is absorbed in association with lipids or bile salts by passive diffusion in the small intestine. Once taken up by intestinal cells, vitamin D is incorporated into chylomicrons which are vesicles that transport lipids and lipid-soluble nutrients in the blood. Vitamin D is also transferred from chylomicrons to DBP which enables transport of vitamin D metabolites in blood (Gropper and Smith 2013).

Vitamin D is soluble in lipids. Therefore the amount of fat in a meal may affect bioavailability with potentially higher vitamin D absorption from consumption in a higher fat food or meal. Few studies, however, have examined the question of whether absorption of dietary lipids with a vitamin D-fortified food may improve vitamin D absorption and the optimal amount of fat is not known. Most research has examined the effect of fat-containing meals or lipid vehicle on serum 25OHD concentration after administration of a vitamin D supplement (Tripkovic et al. 2012). These studies generally involve very high doses of vitamin D which are not comparable to amounts present in food.

It is also possible that fat has only minor influence on absorption of vitamin D contained in foods. Tangpricha et al. (2003) examined whether the fat ingested with vitamin D affected the absorption of vitamin D added to orange juice. Healthy adults given orange juice fortified with 25 μg vitamin D over 12 weeks showed increased serum 25OHD concentrations compared to adults given unfortified orange juice, thus suggesting that vitamin D administered in a fat-free food is absorbed. In a separate experiment (Tangpricha et al. 2003), adults given a single dose of 625 μg D_2 or D_3 in whole milk, skim milk, or in corn oil on toast showed comparable increases in 25OHD for each vehicle. Both experiments suggest that dietary fat has minimal effect on vitamin D absorbed from a vitamin D-fortified food.

The efficiency of vitamin D absorption specifically from fortified breakfast cereal has not been reported. Most breakfast cereal contain between 1–5 g fat per 100 g (and some higher if they are oat-based or include nuts) so that consumption of vitamin D-fortified cereal with or without milk is likely to result in absorption of vitamin D.

3.3 Biological effect of D_2 versus D_3

The relatively complex pathway involved in generating the active metabolite $1,25(OH)_2D$ means that differences in the biological action of D_2 and D_3 potentially arise from one or more of the following metabolic steps (see Figure 1) (Jones 2008; IOM 2011):

- absorption in the gut
- binding of vitamin D or vitamin D metabolites to DBP
- hydroxylation of vitamin D to 25OHD
- hydroxylation of 25OHD to the $1,25(OH)_2D$
- catabolic breakdown of 25OHD to inactive metabolites
- binding of $1,25(OH)_2D$ to vitamin D receptor on target cells
- biological responses in target cells (i.e. gene expression).

The IOM (2011) concluded that D_2 and D_3 behave identically for most of these steps and that at low doses, D_2 and D_3 have equivalent potency but at high doses, D_2 is less effective than D_3. This conclusion disagrees with the conclusions of earlier studies or reviews (Houghton and Vieth 2006) who suggested that D_2 and D_3 should be considered equivalent. Bioequivalence of D_2 and D_3 continues to be of current research interest particularly in relation to vitamin D supplements.

The bioequivalence of D_2 or D_3 is generally studied by measuring the ability of vitamin D doses to raise serum concentrations of 25OHD, usually measured as total 25OHD, or $25OHD_2$ plus $25OHD_3$. Tripkovic et al. (2012) reported a meta-analysis of randomized controlled trials (RCTs) that compared the effects of a range of doses of D_2 and D_3 on serum 25OHD measured by a variety of methods: LC-MS/MS, RIA, or HPLC). For high vitamin D doses (125–1250 μg/dose) administered as a single bolus, D3 lead to serum 25OHD concentrations measured at various time points (28 days – 24 weeks) that were significantly increased compared to the same amount of D_2. Although the reason for this difference is not known, possible explanations are preferential hepatic breakdown of D_2 to inactive metabolites or high levels of D_2 competitively binding to the 25-hydroxylase and thus decreasing serum $25OHD_3$.

For lower doses, Tripkovic et al. (2012) reported meta-analysis of five studies of the effect of D_2 or D_3 at doses of 25–100 μg/day administered over various periods (14 days to 12 months) on serum 25OHD concentration measured after the treatment period. In this case, the meta-analysis (which used a random effects analysis to account for heterogeneity between studies) showed that D_3 supplementation produced a weighted mean increase in serum 25OHD of 4.83 nM compared to D_2 supplementation but the result was not significant.

Given the complexity of factors that affect serum 25OHD concentrations, studies cited in Tripkovic (2012) plus four additional studies published subsequently (identified through keyword searching of Medline) were examined to determine comparability between the different studies. A number of variables potentially contributing to the effect of D_2 or D_3 on serum 25OHD concentration were identified, suggesting that direct comparisons between these studies are not straight-forward (Table 2). For this assessment, the most comparable studies listed in Table 2 are those that include:

(1) Healthy adults using a placebo-treated control group to account for non-treatment effects (e.g. vitamin D from sun exposure)
(2) A dosing period of at least 11–12 weeks to allow for steady state serum 25OHD to be established (i.e. four half-life periods with t½ for 25OHD equal to about 20 days)
(3) Daily D_2 or D_3 administered at 25 µg or less. This amount is considered to be most comparable to potential intakes from food sources (see Section 4)
(4) Use of LC-MS/MS or HPLC assays that will detect both $25OHD_2$ and $25OHD_3$
(5) Comparable baseline serum 25OHD concentrations to allow for different pharmacokinetic effects of vitamin D doses on low and high baseline 25OHD.

Table 2: Characteristics of studies comparing low D_2 and D_3 doses on serum 25OHD

Study [a]	Subjects	Control group	D_2 or D_3 (µg/day) Duration	Commencing	Assay	[25OHD] (nM)	
						Baseline [b]	Treatment Difference D_3 - D_2 [c]
Biancuzzo – 1 (2010)	Healthy adults	Placebo	25 µg 11 weeks	Late winter	LC-MS	39.5–49.5	5.5
Biancuzzo – 2 (2010)	Healthy adults	Placebo	25 µg 11 weeks	Late winter	LC-MS	41.5–49.0	-6.0
Binkley (2011)	Healthy adults Age 65+	None	40 µg 1 year	Not reported	HPLC	74.8–80.0	7.4*
Glendenning (2009)	Hip fracture inpatients	None	25 µg 3 months	Not reported	RIA, HPLC	37.2–42.2	16.4*
Holick (2008)	Healthy adults	Placebo	25 µg 11 weeks	Late winter	LC-MS	42.3–49.0	1.1
Trang (1998)	Healthy adults	Placebo	100 µg 2 weeks	Late winter/spring	RIA	39.8–43.7	9.6*
Logan (2013)	Healthy adults	Placebo	25 µg 24 weeks	Late summer	LC-MS	74–80	18*
Fisk – 1 (2012)	Healthy adults	Placebo	5 µg 4 weeks	Winter	LC-MS	31.3–48.0	7.0
Fisk – 2 (2012)	Healthy adults	Placebo	10 µg 4 weeks	Winter	LC-MS	30.9–41.9	6.1
Lehmann (2013)	Healthy adults	Placebo	50 µg 8 weeks	Winter	LC-MS	37.6–40.7	15.3*
Nimitphong (2013)	Healthy adults	None	10 µg 3 months	Winter	LC-MS	51.8–53.2	8.1

[a] Studies used in Tripkovic meta-analysis were Biancuzzo (2010), Binkley,(2011), Glendenning (2009), Holick (2008), and Trang (1998).
[b] Values are range across all treatment groups
[c] As reported or calculated from results using equation:
Difference $D_3 - D_2 = [(25OHD_3)_{post\text{-}treatment} - (25OHD_3)_{baseline}] - [(25OHD_2)_{post\text{-}treatment} - (25OHD_2)_{baseline}]$ where
* indicates that difference between D_2 and D_3 treatments was statistically significant (P value 0.05 or less).

Only studies published by Biancuzzo (2010) and Holick (2008) (shaded in grey in Table 2 below) meet all of the above study characteristics and both of these studies showed no significant difference between the D_2 and D_3 treatments in ability to raise serum 25OHD concentration.

Studies which showed that D_3 was significantly more effective at raising 25OHD compared to D2 were conducted in unhealthy subjects (Glendenning), or with no control group (Glendenning, Binkley, Nimitphong), or with short treatment periods (Trang, Lehmann), or using vitamin D doses greater than 25 µg/day (Binkley, Trang, Lehmann). Therefore, conclusions from these studies cannot be drawn for this assessment.

The study by Logan et al. (2013) took a different approach from all the other studies. Most of the studies in Table 2 were commenced in winter or late winter when baseline serum 25OHD concentrations are known to be at minimum levels due to low UV-B exposure. In contrast, Logan et al. studied the difference between D_2 and D_3 treatment commencing in late summer when baseline 25OHD concentrations are substantially higher. The study found that D_3 treatment was significantly different from D_2 treatment where D_3 maintained serum 25OHD at 80 nM but serum 25OHD was 18 nM lower with D_2 treatment.

The Logan study indicates possible differences between D_3 and D_2 on maintaining relatively high serum 25OHD concentrations at a dose level of 25 µg/day. However, in light of the other studies which show no difference between the effect of the two compounds, further studies particularly at lower dose levels which are comparable to the amount contained in food, would be needed to conclude that D_2 and D_3 are non-equivalent in raising serum 25OHD. Therefore, in this assessment D_2 and D_3 were considered to have equivalent potency.

3.4 Inadequate vitamin D

Severe vitamin D deficiency gives rise to rickets in infants and children and osteomalacia in adults and older children. Both conditions are characterised by undermineralised bone caused by prolonged elevation of parathyroid hormone (in response to low serum calcium or phosphorus) which promotes bone resorption to release calcium and phosphorus. Decreased bone mineralisation associated with low vitamin D status has also been linked to increased risk of osteoporosis although the aetiology of this disease in relation to vitamin D intakes is also complex and of current research interest (Reid et al. 2014; Reid 2014) .

Receptors for $1,25(OH)_2D$ are found on nearly every cell type suggesting additional functional roles for vitamin D and generating much research interest. Numerous observational studies and RCTs on the risk of cardiovascular diseases, diabetes and metabolic syndrome disorders, neurodegenerative diseases, and death that is associated with low vitamin D status have been published. Results are highly variable in part due to unreliable assay methodology for serum 25OHD, confounders that can affect serum 25OHD (such as season, ageing, latitude, adiposity, physical activity, smoking, and diet), or metabolic differences with different dosage level of vitamin D. Based on several recent systematic reviews (Fisk et al. 2012; Theodoratou et al. 2014; Autier et al. 2014; Bolland et al. 2014; Chowdhury et al. 2014) as well as the conclusions of EFSA (2012) and IOM (2011), the current body of evidence does not sufficiently support a link between vitamin D and any of these health relationships.

3.4.1 Determination of the dietary recommendations for inadequacy

The current ANZ recommendations for vitamin D intakes were set as Adequate Intake (AI) levels because evidence at the time was insufficient to derive an EAR or RDI (NHMRC & NZ MoH, 2006). The AI assumes minimal exposure to UV-B and thereby indicates a level of intake for individuals with greatest dietary requirement.

The AI for children and adults up to 50 years is 5 µg/day. For men and women 51–70 years, the AI is 10 µg/day, and for over 70 years is 15 µg/day. For population assessment, the median intake is compared to the AI. However, the AI applies only to the sub-section of the population who have minimal sunlight exposure and there are no other health based guidance values for dietary intake for other sections of the population.

The IOM updated their reference intakes for vitamin D and calcium in 2011. The IOM also reviewed the evidence showing an association between serum 25OHD concentration with disease status and concluded that only the relationship between bone disease and 25OHD was substantially supported by the body of evidence.

It was concluded that 25OHD > 50 nM represents the serum concentration at which practically all persons are sufficient in vitamin D intake, and 25OHD in the range 30–50 nM represents the population that is potentially at risk of deficiency. The risk of deficiency increases as serum 25OHD levels drop below 30 nM. The EAR and RDI were set by IOM by back-calculating the dietary intakes of vitamin D required to achieve the following concentrations assuming that sun exposure was minimal (IOM 2011):

- EAR: Serum 25OHD = 40 nM - meets needs of 50% of population;
- RDI: Serum 25OHD = 50 nM - meets needs of 97.5% of population

3.4.2 Recommended 25OHD cut-off values to define inadequate populations

Nutritional status can be assessed directly using biochemical or other measures rather than using indirect dietary assessment. Because vitamin D is unique in that it is obtained through dietary sources and also through sunlight exposure, dietary intake data do not provide a good basis for assessing vitamin D status. By contrast, serum 25OHD reflects vitamin D derived from both sunlight and diet.

Expert panels and advisory bodies have published various cut-off values for serum 25OHD concentration to indicate inadequate vitamin D status (summarised in Table 3). The Table clearly shows discrepancy in the application of terms such as ‘deficiency’ to different serum concentrations. Therefore this assessment will report results of surveys measuring 25OHD without using these terms.

The IOM 2011 decisions for the dietary recommendations described above were based on the assumption that a serum 25OHD distribution with a mean of 40 nM and a 97.5th centile of 50 nM represented the physiological requirement. Therefore, assuming normality, this distribution has a standard deviation of 5 nM. Consequently, 30 nM and 25 nM are two and three standard deviations below the mean respectively. Earlier clinical work has identified 30 nM as a point where rickets and osteomalacia are common. The current AI for Australia New Zealand for various population groups such as young adults was “*based on the amount of vitamin D required to maintain serum 25(OH)D at a level of at least 27.5 nmol/L with minimal exposure to sunlight”* (NHMRC & NZ MoH, 2006). In the United Kingdom, the Vitamin D Working Group notes that “*a serum 25(OH)D concentration of 25 nmol/L was a population protective level”* (i.e., the serum 25(OH)D concentration that individuals in the UK population should not fall below in terms of protecting bone health) (Scientific Advisory Committee on Nutrition 2014).

Therefore there is agreement that serum concentrations in the range 25–30 nM are too low for most or all of the population and this is consistent with the serum 25OHD distribution articulated by the IOM. Practitioners and others who give advice to individuals commonly use a concentration that covers most of the population.

For example, the RDI is typically used when giving dietary advice to individuals even though this is not used in population assessments. The identification of 50 nM as a concentration at which advice to individuals is given (Table 3) is therefore also consistent with the distribution articulated by the IOM.

FSANZ concludes that there is currently considerable international consensus for viewing the physiological requirement distribution for serum 25OHD as having a mean of 40 nM and a standard deviation of 5 nM. Therefore, the criterion for adequacy for this assessment was taken to be the percentage of the population groups with serum 25OHD < 40 nM. This value was chosen because the most recent EAR from the IOM was calculated assuming that 40 nM was a suitable level for 50% of the population. This is analogous to using the EAR Cut-point method used in other FSANZ nutrient intake assessments to estimate the prevalence of inadequate intakes.

Table 3: Expert panel recommendations for serum 25OHD concentrations to indicate vitamin D status

Panel (reference)	Recommendation
IOM (Ross et al. 2011)	At risk of deficiency 25OHD < 30 nM Potentially at risk of deficiency 25OHD = 30-50 nM Practically all persons sufficient 25OHD > 50 nM No additional benefit 25OHD > 75 nM
NZ MOH (Ministry of Health & Cancer Society of New Zealand 2012)	25OHD < 30 nM are 'deficient'. 25OHD ≥ 50 nM seems prudent. 25OHD > 125 nM is not recommended.
Working Group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis Australia (Nowson et al. 2012)	Adequacy 25OHD ≥ 50 nM[1] Mild deficiency 25OHD = 30–49 nM Moderate deficiency 25OHD = 12.5–29 nM Severe deficiency 25OHD < 12.5 nM
The Endocrine Society (US) (Holick et al. 2011)	Deficiency 25OHD < 50 nM Insufficiency 25OHD = 52.5–72.5 nM
UK SACN (SACN 2007)	Increased risk of deficiency 25OHD < 25 nM

[1] At the end of winter; 10–20 nM higher at the end of summer, to allow for seasonal decrease.

3.5 Excess vitamin D

Hypercalcaemia (elevated calcium in blood) and subsequent hypercalciuria (excessive calcium excretion) are indicators of toxicity linked to high vitamin D intakes. Hypercalcaemia is caused by increased bone resorption (causing more circulating calcium and phosphorus) and impaired bone mineralisation through a mechanism that is not fully understood. Although production of active metabolite $1,25(OH)_2D$ does not increase with elevated 25OHD concentration because of tight endocrine regulation, it is thought that very high serum 25OHD concentration and breakdown metabolites in blood may displace DBP-bound $1,25(OH)_2D$ releasing more free $1,25(OH)_2D$ for uptake by target cells. This results in increased mobilisation of calcium from bone cells, and increased calcium absorption in intestinal cells (Jones 2008).

Long term health outcomes associated with prolonged hypercalcaemia or hypercalciuria are soft tissue calcification and eventually renal and cardiovascular damage. These outcomes are achieved only through high oral intakes (as in supplements) and not through sun exposure or, because of the relatively low vitamin D content of food, through dietary intakes.

Recently it has been suggested that moderately high 25OHD (approximately 25OHD > 75 nM but lower than that giving rise to hypercalcaemia or hypercalciuria) might be associated with other long term adverse health outcomes such as increased risk of tuberculosis, schizophrenia (in relation to neonatal serum 25OHD concentrations), cardiovascular disease, total cancer mortality, and all-cause mortality (Hathcock et al. 2007; Durup et al. 2012; EFSA Panel on dietetic products 2012a; Spedding et al. 2013). However, because of nonstandard assay methods for serum 25OHD, potential confounding effects (such as calcium and phosphorus intakes or other underlying disease effects), and the problem that moderately high serum 25OHD overlaps with the concentration thought to be beneficial for bone health and calcium homeostasis, it has not been possible to determine whether or not a causal relationship exists between 25OHD concentration in this range and disease risk.

3.5.1 Determination of excess vitamin D

3.5.1.1 Using the UL

Excess vitamin intakes are usually assessed against the UL, where established, which is defined as the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population.

Current recommendations for the vitamin D UL for adults and children set by the NHMRC/NZ MoH, EFSA, and the IOM, are in the range of 80–100 µg/day for adults and 25–80 µg/day for children and adolescents (NHMRC and NZ MoH 2006; IOM 2011; EFSA Panel on dietetic products 2012a). The recommendations are based on studies showing the highest vitamin D intake (i.e. in a supplementary dose) that does not cause hypercalcaemia (i.e. the no observable adverse effect level or NOAEL) with application of different uncertainty factors to account for small sample numbers, variability in measurement of blood or urinary calcium, or potential but as yet uncharacterised links to other adverse health outcomes. For this reason, expert bodies using the same data derive slightly different recommended ULs.

The main limitation of using the UL to assess vitamin D excess is that it does not account for the contribution of vitamin D from sun exposure. For example, an individual who consumes dietary and/or supplementary vitamin D may be well below the UL but could reach unsafe serum 25OHD concentrations if sun exposure is also factored in. Measurement of serum 25OHD provides a method to quantify vitamin D from all sources – food, sun exposure and vitamin supplements.

3.5.1.2 Using serum 25OHD

The IOM and EFSA reviewed the use of serum 25OHD concentration as a measure of safe intakes. The IOM review (2011) determined that toxicity linked to hypercalcaemia is found with serum 25OHD concentration greater than about 500 nM. The IOM concluded that there may be ‘reason for concern’ for persons with concentrations of serum 25OHD > 125 nM and that serum 25OHD levels above approximately 125 to 150 nM ‘should be avoided’. The IOM also noted that emerging data suggests that adverse outcomes may occur with serum 25OHD above approximately 75 nM but due to lack of data and considerable uncertainty, conclusions for serum 25OHD in the range of 75–125 nM could not be drawn.

EFSA (2013) determined that serum 25OHD concentrations linked to hypercalcaemia varied over a wide range (220–700 nM) and unlike the IOM, concluded that there was no clear association between serum 25OHD concentrations and other adverse health outcomes. Therefore, EFSA decided that serum 25OHD concentrations could not be used as a marker or indicator for adverse effects.

The Working Group of the Australian and New Zealand Bone and Mineral Society and Osteoporosis Australia (Nowson et al. 2012) also concluded that vitamin D toxicity is characterised by hypercalcaemia which occurs with 25OHD concentration in the range of 220–500 nM. The Working Group also acknowledged adverse health effects may not always be associated with hypercalcaemia and that emerging evidence suggest that adverse effects may be linked to higher serum 25OHD under some circumstances. No range of serum 25OHD concentration was defined for these effects and it was concluded that further research was needed.

The conclusions of the IOM, EFSA, and the Osteoporosis Australia Working Group suggest that serum 25OHD concentration up to 250 nM can be considered to have minimum risk of adverse health effects. However, adverse health effects with prolonged, moderately high serum 25OHD are not well understood. Therefore, based on the conclusion of the IOM that serum 25OHD above approximately 125–150 nM should be avoided, serum 25OHD levels above 125 nM can be used as a conservative concentration to assess the potential risk of harm due to vitamin D intake.

3.6 Summary – hazard identification and characterisation

Both forms of vitamin D (D_2 and D_3) are absorbed in the intestine and require no digestion. The metabolism of vitamin D is complex and involves many metabolites with the active metabolite $1,25(OH)_2D$ functioning in the maintenance of calcium homeostasis. Vitamin D status is measured by the serum concentration of the metabolite 25OHD. Studies comparing the ability of D_3 and D_2 to raise serum 25OHD indicate that, at vitamin D levels present in food, both forms are considered to have equivalent ability to elevate 25OHD serum levels.

The criterion for adequacy was taken to be the proportion of the population groups with serum 25OHD < 40 nM. This value was chosen because the most recent EAR from the IOM was calculated assuming that 40 nM was a suitable level for 50% of the population. Therefore, calculating the proportion of the population with serum values less than 40 nM is analogous to using the EAR Cut-point method in other FSANZ nutrient intake assessments to estimate the prevalence of inadequate intakes. As noted above, the dietary recommendations derived from this serum value assumed minimal sunlight exposure, but the serum values themselves include 25OHD generated from sunlight exposure.

The criterion for excess was taken to be the proportion of population groups with serum 25OHD concentrations above 125 nM. As noted above, this should not be interpreted as a true UL but exceedance of this concentration is considered by some expert bodies to be avoided.

4 Vitamin D in food

4.1 Content

Similar to measurement of serum 25OHD, assays for vitamin D in food are particularly difficult due to a methodology that (1) is laborious and expensive so that only a few samples can be analysed, (2) involves extraction steps that have been validated only for a few food matrices, (3) does not reliably quantify all vitamin D (D_2 and D_3) or metabolites (25OHD), and (4) does not utilise standard reference materials.

As a result, the total amount of vitamin D in food is not fully known and international food composition databases are considered to be inaccurate.

Recent research using accurate assay methods provides more reliable estimates of total amounts of vitamin D and 25OHD in food. Endogenous D_3 is present in relatively small quantities in animal-derived foods such as fish (various species, 0.6–135 µg/100 g), meat (0.120 µg/100 g) or in eggs (2.11–2.93 µg/100 g) (Schmid and Walther 2013; Liu et al. 2013; Liu et al. 2014). Small amounts of the 25OHD metabolite are also present in animal-derived foods such as beef (0.269 µg/100 g) and chicken (0.14 µg/100 g) (Liu et al. 2013; Taylor et al. 2014). Oral 25OHD is considered to be five-fold more potent in raising serum 25OHD than vitamin D itself (Taylor et al. 2014). Therefore, the relatively small amounts of 25OHD in food may make a significant contribution to total vitamin D status in individuals who consume animal-derived foods (Ovesen et al. 2003; Cashman et al. 2012; Taylor et al. 2014).

Both vitamin D and 25OHD are present in much higher levels in eggs of chickens fed vitamin D-fortified feeds (Liu et al. 2013; Schmid and Walther 2013; Liu et al. 2014). Up until recently, D_2 has usually been considered to be an insignificant and rare form of vitamin D in foods, present mostly in mushrooms (Urbain et al. 2011), and not a major contributor to vitamin D intakes. A recent survey suggests that D_2 may also be consumed from animal-derived food (Cashman, 2014). The origin of D_2 in animal-derived foods includes that from animals fed grasses or hay (where D_2 is produced by UV exposure to fungi present) and that from intensively-reared animals whose feed is supplemented with D_2. Although, the presence of D_2 from these sources in Australian and New Zealand animals has not been demonstrated, overall these data suggest that total intakes of vitamin D from non-fortified foods is probably underestimated.

For these reasons, accurate data for vitamin D intakes from food for the ANZ populations was not available. Others have previously estimated average daily dietary intake of vitamin D in Australian and New Zealand adults to be 2–3 µg/day compared to North America where more vitamin D fortification is permitted or mandated and intakes are in the range 3–6 µg/day (Nowson et al. 2012).

4.2 Effect of consuming vitamin D fortified foods on serum 25OHD

Numerous studies have shown that consumption of vitamin D-fortified foods increases serum 25OHD concentrations and several systematic reviews have been published (O'Donnell et al. 2008; O'Mahony et al. 2011; Black et al. 2012). Although fortification of breakfast cereal is permitted in several countries, no quality studies on the effect of consumption of vitamin D-fortified breakfast cereal on serum 25OHD have been reported. However, based on studies of other fortified foods, it is reasonable to conclude that vitamin D consumed through vitamin D-fortified cereal will also increase serum 25OHD concentrations.

A recent systematic review of 16 studies measured the effect of consumption of vitamin fortified foods on serum 25OHD concentrations as measured by RIA and HPLC methods (Black et al. 2012). The study included a meta-analysis with random-effects analysis to determine the overall weighted mean difference. Interventions of fortified foods included yoghurt drinks, orange juice, milk and milk powder, dairy products, and wheat bread with daily doses of D_2 or D_3 ranging from 3 to 25 µg (per 100 g or serving, or daily dose). Overall, foods fortified with vitamin D were found to increase serum 25OHD concentration in a dose dependent manner where a 1.2 nM increase in 25OHD resulted from every 1 µg vitamin D per day consumed (Figure 3).

Although a high degree of statistical heterogeneity was shown in the analysis (which might be due to factors such as seasonal effects), it was noted that the use of different assay types may impact on the validity of individual study outcomes but was not a contributor to statistical heterogeneity of the meta-analysis. Therefore, it was concluded that the dose-response provides a reasonable estimate of potential increases in serum 25OHD with dietary vitamin D.

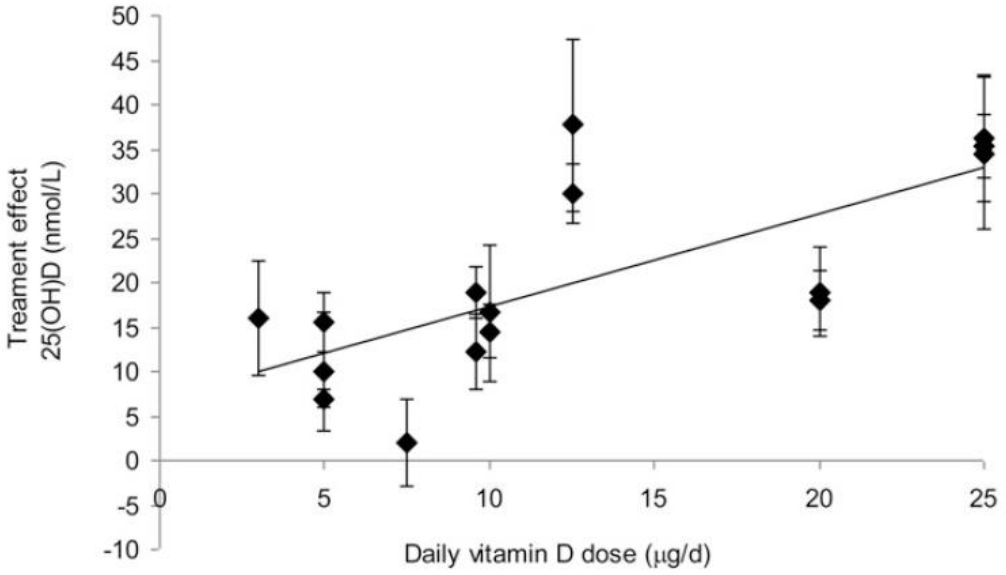

Figure 3: Effect of consumption of vitamin D fortified food on serum 25OHD concentrations

Each diamond represents results of one study in which vitamin D dose (in fortified food) was between 3 and 25 µg. (Reproduced with permission from Black et al. 2012)

5 Estimated vitamin D intakes from fortified breakfast cereal

5.1 Introduction

A conventional nutrient intake assessment was not undertaken for vitamin D for the ANZ populations as there were limited ANZ derived analytical data on the vitamin D (D_2, D_3, $25OHD_2$ and $25OHD_3$) content of food (see Section 4.1). Rather, the potential impact of fortification of breakfast cereal with vitamin D was estimated by first determining each population group's consumption of breakfast cereal, and second, estimating the potential increase in vitamin D intake that could occur should breakfast cereal be fortified at the midpoint level estimated by the Applicant. Third, this increase was then used to predict the incremental increase in serum 25OHD due to fortified breakfast cereal consumption (see Section 6.2) and fourth, to compare resulting estimated serum 25OHD levels for nominated population groups to cut-off values linked to inadequate or excess intakes (as summarised in Section 3.4 and 3.5).

5.2 Assessment of breakfast cereal consumption

5.2.1 Breakfast cereal consumption data

FSANZ uses food consumption data from the most recent National Nutrition Surveys (NNS) in our custom built dietary modelling analytical program to estimate food consumption and dietary intake of nutrients for the Australian and New Zealand populations. The design of each of these surveys varies somewhat and key attributes of each are summarised in Appendix 1 (includes links to reports and/or reference).

Only ready-to-eat (RTE) breakfast cereal was included in the calculations for the amount of cereal consumed. RTE breakfast cereal consumption included the following types of breakfast cereal:

- extruded, puffed or flaked cereal
- single or mixed grain cereal (corn, oat, wheat, rice)
- fortified or unfortified cereal, with our without added sugar
- toasted or natural mueslis
- cereal with added fruit and/or nuts
- bran based cereal.

Porridge or similar cooked grain cereal, breakfast bars and breakfast drinks were excluded from the breakfast cereal consumption estimates as these products are unlikely to be fortified due to the processes needed to apply the fortificant (either as a coating process or incorporated into the flour mix used to produce the final product, see section 2.4).

Mean and 90th percentile RTE breakfast cereal consumption amounts were derived for each age group assessed for consumers of RTE breakfast cereal only, as defined above.

5.2.2 Age groups assessed

A range of age groups was selected for this assessment of potential vitamin D intakes from breakfast cereal, based on the NHMRC/NZ MoH nutrient reference value age groups that would normally be used for nutrient intake assessments, within the limitations of each Australian and New Zealand NNS.

For the Australian population the following age groups were used:

- 2007 Australian National Children's Nutrition and Physical Activity Survey (ANCNPAS): 2–3 years, 4–8 years, 9–13 years, 14–16 years
- 1995 NNS: 17 years and above.

For the New Zealand population the following age groups were used:

- 2002 New Zealand National Children's Nutrition Survey (NZ NCNS): 5–8 years, 9–13 years, 14 years
- 2008-09 New Zealand National Nutrition Survey (NZ NNS): 15 years and above.

5.2.3 Proposed concentration of vitamin D in breakfast cereal

RTE breakfast cereals currently contain no appreciable vitamin D or 25OHD content from their ingredients. The Applicant has requested fortification of breakfast cereals with vitamin D (as D_3) to a maximum claim per reference quantity of 25% regulatory RDI (10 µg cholecalciferol) as set out in the ANZFSC. However, the permitted forms of vitamin D for fortification in the ANZFSC are D_2 and D_3. Therefore for the purpose of estimating vitamin D intakes from breakfast cereal, the vitamin D content of breakfast cereal was taken to equal the sum of the amount of D_2 and D_3 added as fortificants.

For the purposes of Standard 1.3.2 – Vitamins and Minerals, the reference quantity of breakfast cereal is a 'normal serving'. Given the variability in weight of RTE breakfast cereal, it is noted that a serving of a puffed, flaked or extruded type breakfast cereal can weigh approximately 30–45 g, whereas a serve of muesli style breakfast cereal can weigh approximately 60–75 g (FSANZ 2014).

A concentration of 5 µg per normal serving was assumed for the purposes of this nutrient intake assessment, estimated as the approximate concentration of vitamin D expected to remain at the mid-point of the shelf life of the product (see Section 2.5). This assumption takes into account brand loyal consumers who always choose the same brand of potentially fortified RTE breakfast cereal, but may select the cereal at different points in its shelf life over time.

Using the lower end of the serve size range of 30 g for RTE breakfast cereal, and an average concentration of 5 µg of vitamin D per serving resulted in a concentration of approximately 17 µg /100 g of breakfast cereal. This is considered the most conservative approach for modelling purposes (high concentration of vitamin D per serve for a brand loyal consumer).

5.3 Results

5.3.1 Breakfast cereal consumption and potential vitamin D intake from breakfast cereal

Four scenarios to predict vitamin D intakes from breakfast cereal were modelled based on:

(1) estimated mean RTE breakfast cereal consumption for consumers who always selects the same type of vitamin D fortified cereal (brand loyal model)
(2) estimated 90th percentile RTE breakfast cereal consumption for consumers who always selects the same type of vitamin D fortified cereal (brand loyal model)
(3) estimated mean RTE breakfast cereal consumption for consumers who select various brands from the range of products in the market over time, some of which have added vitamin D (market share model)
(4) estimated 90th percentile RTE breakfast cereal consumption for consumers who select various brands (market share model).

The Applicant reported the permissions for voluntary vitamin D fortification in other countries. The USA and Canada currently permit voluntary fortification of a range of foods with vitamin D, including breakfast cereal, margarines, milk, milk products and milk analogues, grain products and pasta, fruit juices and drinks. Voluntary vitamin D fortification is permitted for all foods in the UK and EU (except unprocessed foods and alcoholic beverages) and a number of Asian countries. Uptake of this voluntary permission, or market share of cereal fortified with vitamin D, was estimated to range from approximately 7% for the EU, to 35% for the USA.

The maximum market share value of 35% was assumed for the market share model for the purposes of this assessment. This represents a scenario where 35% of RTE breakfast cereal are fortified with vitamin D and the concentration of vitamin D used in the model is reduced proportionally, representing the fact that consumers would select from a range of different fortified and unfortified cereal over time.

5.3.1.1 Breakfast cereal consumption

Estimated mean and 90th percentile RTE breakfast cereal consumption (Table 4) was determined for each Australian and New Zealand population group assessed. The proportion of the population consuming RTE breakfast cereal appeared to decrease as age increased. Each New Zealand population group assessed had a lower proportion of RTE breakfast cereal consumers than similar Australian population groups. However, mean and 90th percentile consumption of breakfast cereal increased as age increased for Australian and New Zealand children's population groups because the quantity consumed per occasion increased. Australian children's population groups appeared to have a slightly higher estimated mean consumption and similar 90th percentile consumption of RTE breakfast cereal compared to similar New Zealand children's population groups.

New Zealanders aged 15 years and above had a similar estimated consumption of RTE breakfast cereal compared to Australian's aged 17 years and above at both the mean and 90th percentile.

Table 4: Mean and 90th percentile breakfast cereal consumption for ANZ population groups and predicted vitamin D intakes from RTE breakfast cereal, brand loyal consumer

National Nutrition Survey	Population group	% consumers to respondents	RTE Breakfast cereal consumption (g/day)		Predicted intake of vitamin D from RTE breakfast cereal* (µg/day)	
			Mean	*P90*	*Mean*	*P90*
2007 ANCNPAS^	2–3 years	76	26	45	4.4	7.7
	4–8 years	71	31	56	5.3	9.4
	9–13 years	63	42	75	7.2	12.8
	14–16 years	55	52	96	8.8	16.3
1995 ANNS	17 years & above	41	58	111	9.9	18.8
2002 NZ NCNS	5–8 years	56	39	60	6.6	10.2
	9–13 years	47	47	80	7.9	13.6
	14 years	40	59	90	10.0	15.3
2008-09 NZ NNS	15 years & above	34	57	108	9.7	18.4

* Based on a concentration of vitamin D in breakfast cereal of 17 µg/100 g.
^ Average of Day 1 and Day 2 consumption.

5.3.1.2 Comparison to 2011–12 NNPAS results

In the 2011–12 National Nutrition and Physical Activity Survey (NNPAS) component of the AHS, summary results published by the ABS indicated RTE breakfast cereal was consumed by 36% of the population aged 2 years and above, with a further 7% eating porridge on day 1 of the survey. Children aged 2–3 years were most prevalent consumers of RTE breakfast cereal (54%), followed by 4–8 year olds (52%). Overall, 42% of Australians aged 2–18 years and 35% of Australians aged 19 years and above consumed RTE breakfast cereal.

ABS published median consumption amounts rather than mean amounts from Day 1 records so these cannot be compared to the results in Table 4 for the 1995 NNS and the 2007 ANCNPAS. Median RTE cereal consumption increased as age increased for Australians aged 2–18 years, from 26 g/day for children aged 2–3 years to 53 g/day for Australians aged 14–18 years. Australians aged 19 years and above consumed a median of 48 g/day of RTE breakfast cereal (median intake was not reported for the 2–18 year age group as a whole).

For adults, noting the small difference in age groups reported, the proportion of the population consuming RTE breakfast cereal was slightly higher in 1995 (41%) compared with 2011–12 (35%). Median intakes of RTE breakfast cereal were similar in both 1995 (45 g/day) and 2011–12 (48 g/day) which suggests that consumption patterns have not changed appreciably.

5.3.2 Predicted intake of vitamin D from breakfast cereal

5.3.2.1 Brand loyal consumer model

The intake of vitamin D from voluntary fortification at the Applicant's mid-point (17 µg/100 g) was predicted. This deterministic method of estimating vitamin D intake from RTE breakfast cereal assumed that all RTE breakfast cereal was fortified at the specified level and that brand loyal always chose the same fortified breakfast cereal. Potential intakes of vitamin D were proportional to RTE breakfast cereal consumption and were predicted to range from 4.4 µg/day (Australians aged 2–3 years) to 10.0 µg/day (New Zealanders aged 14 years) at mean levels of RTE breakfast cereal consumption, and from 7.7 µg/day (Australians aged 2–3 years) to 18.8 µg/day (Australians aged 17 years and above) at the 90th percentile of RTE breakfast cereal consumption (see Table 4).

5.3.2.2 Market share model

Potential intake of vitamin D from RTE breakfast cereal was predicted (Table 5) for the Australian and New Zealand populations assuming the maximum reported market share of 35%. Predicted mean intakes of vitamin D from RTE breakfast cereal ranged from 1.5 µg/day for Australian children aged 2–3 years up to 3.5 µg/day for Australians aged 17 years and above and New Zealand children aged 14 years. Predicted 90th percentile intakes of vitamin D from RTE breakfast cereal ranged from 2.7 µg/day for Australian children aged 2–3 years, up to 6.6 µg/day for Australians aged 17 years and above.

Table 5: Predicted mean and 90th percentile vitamin D intakes from fortified RTE breakfast cereal consumption for Australian and New Zealand population groups, assuming a 35% market uptake of voluntary fortification permissions

National Nutrition Survey	Population group	Predicted intake of vitamin D from RTE breakfast cereal* 35% market share scenario (µg/day)	
		Mean	*P90*
2007 ANCNPAS^	2–3 years	1.5	2.7
	4–8 years	1.9	3.3
	9–13 years	2.5	4.5
	14–16 years	3.1	5.7
1995 ANNS	17 years & above	3.5	6.6
2002 NZ NCNS	5–8 years	2.3	3.6
	9–13 years	2.8	4.8
	14 years	3.5	5.4
2008–09 NZ NNS	15 years & above	3.4	6.4

* Based on a concentration of vitamin D in breakfast cereal of 17 µg/100 g, multiplied by 0.35 to represent 35% market share.
^ Average of Day 1 and Day 2 consumption.

5.3.3 Use of dietary supplements by Australian and New Zealand populations

The amount if vitamin D from supplements was not taken into account in the assessment of vitamin D intakes from the diet, however any effect of consuming supplements on vitamin D status would have been captured in individuals' serum 25OHD measurements, where national nutrition surveys included a biomedical component. It is noted that the proportion of the Australian and New Zealand populations that reported consuming vitamin D supplements was low in all surveys collecting information on supplement use.

Both the 2002 NCNS and 2007 ANCNPAS collected detailed information on children's use of dietary supplements. Approximately 19% of Australian children aged 2–16 reported consuming at least one dietary supplement on either or both days for the 2007 ANCNPAS. No vitamin D only supplements were reported as consumed in the 2007 ANCNPAS. About 5% of New Zealand children aged 5–14 years reported consuming a dietary supplement in the 2002 NCNS, the most frequently consumed being multivitamins & minerals (2.1%) and vitamin C (2%). The consumption of vitamin D only supplements by New Zealand children was not reported (MoH, 2003).

In the 2011–12 NNPAS component of the AHS, 29% of Australians aged 2 years and above reported taking at least one dietary supplement on the day prior to interview. Multi-vitamin and/or -mineral supplements were the most commonly taken dietary supplements, being consumed by around 16% of the population and fish oil supplements taken by around 12% of the population. Thirteen percent of Australians aged 2–18 years reported consuming multi-vitamin and/or -mineral supplements. Less than 1% of Australians aged 2–18 years reported consuming vitamin D supplements as identified in that survey. No respondents aged 2–8 years reported consuming vitamin D supplements. The ABS reported that persons with high serum 25OHD levels reported in the 2011–12 NNMS were not taking vitamin D supplements (ABS 2014a).

For the 2008–09 NZ NNS, survey respondents were asked whether they took any supplements at any time during the last 12 months. For each identified type of supplement, the frequency with which it was consumed was also recorded. *Any* supplement use in the last year was reported by 47.6% of New Zealanders aged 15 years and above. Of the total population, 30.7% were *regular* supplement users (consumed at least one supplement daily, more than once per week or once per week) and a further 16.9% were *occasional* supplement users (consumed supplements less than once per week). The most frequently consumed supplements were oils at 16.4% and multi-vitamins/multi-minerals at 14.8%. Single vitamins were reported to be consumed by 8.6% of the population. Use of vitamin D supplements specifically was not reported.

5.3.4 Assumptions in the assessment

FSANZ aims to make as realistic an estimate food consumption or nutrient intake as possible. However, where significant uncertainties in the data exist, conservative (or 'worst-case') assumptions are generally used to ensure that any assessment does not underestimate food consumption or nutrient intake, where the risk assessment is focused on the potential for high levels of nutrient intake.

Assumptions made in undertaking this assessment included:

- RTE breakfast cereal was assumed to be fortified with vitamin D (as D_2 and or D_3), at a concentration of 17 µg/100 g (Applicant's midpoint amount per minimum serve), a value considered to be at the upper end of the range of possible concentrations.
- porridge and other similar cooked grain cereal, breakfast bars and breakfast drinks were assumed not to be fortified with vitamin D (not included in the Application).

For the purposes of risk assessment, it is preferable to estimate nutrient intakes from all foods over a long period of time (usually estimated using specific statistical adjustments). In the context of the above vitamin D intakes predicted from consuming fortified breakfast cereal, it is noted that these are likely to be overestimates of intakes based on long term food consumption patterns as they were based on one or two days of records only.

For more information on FSANZ dietary intake assessment, assumptions, limitations and uncertainties of the concentration and food consumption data, see the FSANZ document, *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

5.4 Summary – vitamin D intake assessment

Potential dietary intake of vitamin D from RTE breakfast cereal was predicted by deriving mean and 90th percentile RTE breakfast cereal consumption for each population group from Australian and New Zealand NNS and assuming all RTE breakfast cereal was fortified with vitamin D at the midpoint estimated by the Applicant or that an uptake of voluntary permissions of 35% of all RTE breakfast cereal was achieved (35% market share). Assuming a brand loyal consumer always selected RTE breakfast cereal that was fortified, predicted mean intake of vitamin D from RTE breakfast cereal ranged from 4.4 µg/day (Australians aged 2–3 years) to 10.0 µg/day (New Zealanders aged 14 years), and from 7.7 µg/day (Australians aged 2–3 years) to 18.8 µg/day (Australians aged 17 years and above) at the 90th percentile of RTE breakfast cereal consumption. Using a 35% market share scenario, predicted mean intakes of vitamin D ranged from 1.5 µg/day (Australians aged 2–3 years) to 3.5 µg/day (New Zealanders aged 14 years and Australians aged 17 years and above) and ranged from 2.7 µg (Australians aged 2–3 years) to 6.6 µg/day (Australians aged 17 years and above) at the 90th percentile RTE breakfast cereal consumption.

6 Scenario modelling: effect on serum 25OHD

This section estimates the potential effect of consumption of vitamin D-fortified cereal on serum 25OHD measured for the Australian and New Zealand populations.

6.1 Serum 25OHD results from Australia and New Zealand national surveys

Only data from the national surveys conducted in the two countries were used in this assessment and these are described in the subsequent sections. Methodology details, graphical results and distributions stratified according age, region, and country of birth are reported in Appendix 2.

As the laboratory methods used in the available Australia and New Zealand national surveys had not been calibrated to each other, prevalence data between the two countries were not directly comparable. FSANZ also notes that different definitions of terms such as ‘deficient’ are found in the scientific literature. Therefore, to avoid confusion about the proportion of persons categorised as ‘deficient’, the results below report data in terms of 25OHD concentration and not descriptive terms to indicate low vitamin D status.

6.1.1 Australia Health Survey (2011–12)

The distribution of serum 25OHD results from the 2011–12 NHMS component of the AHS is shown in Table 6.

The AHS results indicate that, 13.4% of adults were estimated to have serum 25OHD serum concentrations less than 40 nM, with results weighted to account for various response rates across the population groups. However there were important variations in the prevalence of serum 25OHD concentration <40 nM according to age, region, season and country of origin (Appendix 2).

Other key results from the AHS results indicate that:

- 18–34 year olds have the greatest prevalence of 25OHD < 30 nM and therefore may be considered at risk of deficiency (Appendix 2, Figure A1)
- a greater proportion of the population from Australian southern states (ACT, TAS, NSW, and VIC) have 25OHD < 50 nM (Appendix 2, Figure A2)
- several population groups born outside Australia (all Asian regions and North Africa & Middle East) show a much greater proportion (49.9–67.4%) with 25OHD < 50 nM compared with individuals born in Australia or born in other regions (17.0–30.1%) (Appendix 2, Figure A3)
- as expected, the proportion of the population with 25OHD < 50 nM increased during the winter and spring months, particularly in Australian southern states (NSW, VIC, TAS, SA, ACT) (Appendix 2, Figure A4).
- Less than 2% of the Australian population (unweighted proportion) had serum 25OHD of 125 nM or greater.

Table 6: Distribution of serum 25OHD concentration determined in the 2011-13 AHS for persons 12 years and above, including pregnant women[1]

Serum 25OHD (nM)	Males (%)	Females (%)	Persons (%)
<20	2.3	1.9	2.1
20 to <30	4.2	4.6	4.4
30 to <40	7.3	6.3	6.8
40 to <50	10.5	10.0	10.2
50 to <60	15.0	14.8	14.9
60 to <70	17.5	18.1	17.8
70 to <80	17.7	15.4	16.5
80 to <90	10.6	12.3	11.5
90 to < 100	6.3	7.4	6.8
100 to <110	3.5	4.2	3.8
110 to <120	1.6	1.6	1.6
120 to <130	*0.8	0.6	0.7
> 125 **	1.2	*1.0	1.3 **
Total	100.0	100.0	100.0
Proportion <40 nM**	13.2	12.8	13.4**

[1] Table adapted from Table 5.3 in *AHS: Biomedical Results for Nutrients 2011-13* (ABS 2014a): http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0062011-12?OpenDocument (Accessed 8 July 2014)

* Indicates that the ABS regards this value as unreliable.

**These values were derived from ABS Table Builder using AHS Core Content – Risk Factors and Selected Health Conditions, 2011–12. Table Builder is an online tool available from the ABS used (under subscription) to construct data tables from survey results. It allows the user to conduct more specific analyses than published in ABS reports. Because cells in the table are randomly adjusted to avoid the release of confidential data meaning discrepancies may occur between sums of the component items and totals.

6.1.2 New Zealand Adult Nutrition Survey (2008–09)

The NZ ANS indicated that 20.9% of New Zealand adults (15 years and over) had serum 25OHD < 40 nM. The prevalence of 25OHD > 125 nM was 1.4% (data provided to FSANZ from the NZ MoH).

Additional data from the NZ ANS were reported using cut-off values of 25OHD < 25 nM, 25OHD = 25-49 nM, and 25OHD ≥ 50 nM. Selected results reproduced from the NZ MoH report are shown in Appendix 2 and are summarised as follows:

- most New Zealanders have serum 25OHD ≥ 50 nM (Appendix 2, Figure A5) with about 5% of adults having 25OHD < 25 nM and another 27% were in the 25–49 nM range
- there was no significant difference in prevalence of 25OHD < 25 nM by age (from 15 years to 75 years and above) (Appendix 2, Figure A6). Unlike Australian results, the young adult group did not have the highest prevalence among the adult groups.
- there were no significant differences in the prevalence of population that had 25OHD < 25 nM according to region, although the prevalence tended to be higher in the central and southern regions. (Appendix 2, Figure A7)
- the prevalence of 25OHD < 25 nM was highest during the winter months reaching a peak of about 16% in September. (Appendix 2, Figure A8).

6.1.3 The 2002 New Zealand Children's Survey

Results of serum 25OHD measured in children aged 5–14 years as part of the 2002 New Zealand Children's Survey were reported by Rockell et al. (2005). These authors used 17.5 nM and 37.5 nM to classify serum 25OHD concentration (Table 7) which were selected on the basis of cut-off values used in a US survey (3rd U.S. National Health and Nutrition Examination Survey or NHANES III). However, 37.5 nM is below the value 40 nM selected by FSANZ. Therefore it can be stated that the prevalence of inadequate vitamin D status in New Zealand children was slightly greater than 31%.

The key results from the New Zealand Children's Survey (see also Appendix 2, Table A1) show that:

- Maori and Pacific adolescents (11–14 years), particularly girls, have the highest prevalence of low vitamin D status compared to all children in this age bracket
- as expected, the proportion of children and adolescents with low 25OHD concentration was greater in the winter months.

Table 7: Mean serum 25OHD and proportion below two cut-off values, 2002 New Zealand Children's Survey [a]

Population group (age in years)	Mean serum 25OHD (nM)	Proportion (%)	
		25OHD < 17.5 nM	25OHD < 37.5 nM
All children (5–14)	50	4	31
Boys			
(5–14)	52	3	27
(5–6)	57	1	19
(7–10)	53	3	24
(11–14)	50	4	33
Girls			
(5–14)	47	4	36
(5–6)	48	1	29
(7–10)	51	2	31
(11–14)	42	7	43

[a] Source: Adapted from Table 2 and Table 4 in Rockell et al. (2005). Data were adjusted for survey weighting.

6.2 Predicted effects of voluntary fortification of breakfast cereal with vitamin D

Estimated serum concentrations of 25OHD following consumption of vitamin D fortified RTE breakfast cereal was derived from serum 25OHD data published as part of the national surveys described above and using daily vitamin D intakes calculated in Section 5.3 in the following scenarios:

- Scenario (1): Mean consumer, brand loyal
- Scenario (2): High consumer (90th percentile), brand loyal
- Scenario (3): Mean consumer, various brands (i.e. 35% market share)
- Scenario (4): High consumer (90th percentile), various brands (i.e. 35% market share)

The incremental daily vitamin D intakes (μg/day) from RTE cereal were then converted to serum 25OHD concentration using the conversion: increment of 1 μg/day increases serum 25OHD by 1.2 nM (Black et al. 2012) as described in Section 4.2.

6.2.1 Effect on mean population serum 25OHD concentrations

The predicted effects of each scenario on the mean population serum 25OHD concentrations for specified age groups are reported in Tables 8–11. For both Australian and New Zealand children (Tables 8 and 10, respectively), the predicted increase in serum 25OHD is presented as a range because the age groups used to determine cereal intake do not correspond with the age groups for which 25OHD was reported (different surveys).

For all specified age groups, the predicted change in mean serum 25OHD concentration ranged from minor for mean cereal consumers assuming a 35% market share of cereal with added vitamin D (Scenario 3) to a substantial increase for brand loyal, high consumers of breakfast cereal (Scenario 2). The predicted change for all scenarios and all Australian and New Zealand age groups did not approach the cut-off value of 125 nM, the concentration that was selected to assess safety of vitamin D intakes (see Section 3.5.1). Results for New Zealand children were considerably lower than that reported for New Zealand adults, or the Australian adults and children probably because the measurements for the 2002 NZ CNS were conducted using RIA methodology whereas the other two surveys used LC-MS/MS (see Section 3.2.1). High variability between different methodology measuring serum 25OHD concentration has been reported (see Section 3.1.1).

Table 8: Predicted mean serum 25OHD concentrations in Australian children (12 – 17 years) consuming vitamin D fortified breakfast cereal

Dietary intake		**Serum 25OHD concentration (nM)**		
Modelling scenario	*Vitamin D intake from cereal (μg/day)* [a]	*Calculated increase in serum 25OHD*	*Mean baseline* [b]	*Predicted mean with fortified cereal*
(1) Mean consumer, brand loyal	7.2– 8.8	8.6–10.6	69.0	77.6–79.6
(2) High consumer, brand loyal	12.8–16.3	15.4–19.5	69.0	84.4–88.5
(3) Mean consumer, market share (35%)	2.5–3.1	3.0–3.7	69.0	72.0–72.7
(4) High consumer, market share (35%)	4.5–5.7	5.4–6.8	69.0	74.4–75.8

[a] *See Tables 4 and 5 (Section 5). Values are the intake range for age groups 9–13 years and 14–16 years whereas mean serum 25OHD concentration was reported for the age group 12–17 years.*

[b] *Source 2011–13 Australian Health Survey(ABS 2014a) . Mean serum 25OHD concentration as reported for 12–17 years. The standard deviation was calculated from the reported interquartile range (56.0, 80.4) to be 9.0 (with rounding).*

Table 9: Predicted mean serum 25OHD concentrations in Australian adults (18 years and above) consuming vitamin D fortified breakfast cereal

Dietary intake		Serum 25OHD concentration (nM)		
Modelling scenario	*Vitamin D intake from cereal (µg/day)* [a]	*Calculated increase in serum 25OHD*	*Mean baseline* [b]	*Predicted mean with fortified cereal*
(1) Mean consumer, brand loyal	9.9	11.9	65.7	77.6
(2) High consumer, brand loyal	18.8	22.6	65.7	88.3
(3) Mean consumer, market share (35%)	3.5	4.2	65.7	69.9
(4) High consumer, market share (35%)	6.6	7.9	65.7	73.6

[a] *See Tables 4 and 5 (Section 5). Values taken from the 17 years and over population group since this age range is most comparable to AHS age group (18 years and over) for which serum 25OHD was reported.*

[b] *Source 2011–13 Australian Health Survey (ABS 2014a). Mean serum 25OHD concentration as reported for 18 years and over. The standard deviation was calculated from the reported interquartile range (50.0, 80.0) to be 11.1 (with rounding).*

Table 10: Predicted mean serum 25OHD concentrations in New Zealand children (5 – 14 years) consuming vitamin D fortified breakfast cereal

Dietary intake		Serum 25OHD concentration (nM)		
Modelling scenario	*Vitamin D intake from cereal (µg/day)* [a]	*Calculated increase in serum 25OHD*	*Mean baseline* [b]	*Predicted mean with fortified cereal*
(1) Mean consumer, brand loyal	6.6–10.0	7.9–12.0	50.0	57.9–62.0
(2) High consumer, brand loyal	10.2–15.3	12.2–18.4	50.0	62.2–68.4
(3) Mean consumer, market share (35%)	2.3–3.5	2.8–4.2	50.0	52.8–54.2
(4) High consumer, market share (35%)	3.6–5.4	4.3–6.5	50.0	54.3–56.5

[a] *See Tables 4 and 5 (Section 5). Values are the intake range combined for age groups 5–8 years, 9–13 years, and 14 years to compare to averaged serum 25OHD concentration for three age ranges (5–6 years, 7–10 years, 11– 4 years) reported from the New Zealand Children's Nutrition Survey (Rockell et al. 2005).*

[b] *Source: New Zealand Children's Nutrition Survey (Rockell et al. 2005). Serum 25OHD concentration averaged from reported values for 5–14 years (values similar across all three age ranges). The standard deviation was calculated from reported 99% confidence interval (54,45) to be 1.7 (with rounding).*

Table 11: Predicted mean serum 25OHD concentrations in New Zealand adults (15 years and above) consuming vitamin D fortified breakfast cereal

Dietary intake		Serum 25OHD concentration (nM)		
Modelling scenario	*Vitamin D intake from cereal (µg/day)* [a]	*Calculated increase in serum 25OHD*	*Mean baseline* [b]	*Predicted mean with fortified cereal*
(1) Mean consumer, brand loyal	9.7	11.6	63.0	74.6
(2) High consumer, brand loyal	18.4	22.1	63.0	85.1
(3) Mean consumer, market share (35%)	3.4	4.1	63.0	67.1
(4) High consumer, market share (35%)	6.4	7.7	63.0	70.7

[a] *See Tables 4 and 5 (Section 5).*

[b] *Source: 2008–09 New Zealand Adult Nutrition Survey (Ministry of Health 2012a). Mean serum 25OHD concentration reported for 15 years and over. The standard deviation was calculated from reported 95% confidence interval (61.4, 64.5) to be 0.8 (with rounding).*

6.2.2 Effect on summer and winter 25OHD concentrations

Winter and summer mean serum 25OHD concentrations from the Australian and New Zealand nutrition surveys were used to estimate the effect of vitamin D addition to breakfast cereal when the population is known to have minimal and maximal serum 25OHD concentrations due to variable UV-B exposure. The analysis shown in Section 6.2.1 was repeated and the results are summarised in Table 12.

ANZ population groups analysed showed baseline mean serum 25OHD concentrations which ranged from 52.4–61.8 nM for the winter season. This range was well above the cut-off value of 40 nM which was determined to be the appropriate concentration to indicate inadequate serum 25OHD concentrations for ANZ populations (see Section 3.4). As expected, the four modelling scenarios for vitamin D fortified cereal consumers increased the predicted winter mean 25OHD serum concentration range to 56.5–81.3 nM.

For the summer analysis, the baseline mean serum 25OHD ranged from 70.7–76.4 nM. As expected the four modelling scenarios for vitamin D fortified cereal consumers increased the predicted summer mean 25OHD serum concentration range to 74.9–98.0 nM with the highest range for brand loyal, high consumers of vitamin D fortified cereal. None of the four modelling scenarios increased the predicted mean serum 25OHD above 125 nM, the serum 25OHD concentration considered to be indicative of excessive intakes for the population.

Table 12: Predicted mean winter and summer serum 25OHD concentrations for ANZ populations consuming vitamin D fortified breakfast cereal

Modelling scenario	Predicted mean serum 25OHD concentration (nM)					
	Australia [a]		New Zealand [b]			Combined range across populations [c]
	12-17 years	*18+ years*	*5-14 years*	*15-17 years*	*18+ years*	
Winter Baseline mean	61.8	58.2	43	52.4	54.0	52.4–61.8
(1) Mean consumer, brand loyal	70.4–72.4	70.1	51–55	64.0	65.6	64.0–72.4
(2) High consumer, brand loyal	77.2–81.3	80.8	55–61	74.5	76.1	74.5–81.3
(3) Mean consumer, market share (35%)	64.8–65.5	62.4	46–47	56.5	58.1	56.5–65.5
(4) High consumer, market share (35%)	67.2–68.6	66.1	47–50	60.1	61.7	60.1–68.6
Summer Baseline mean	76.4	70.7	58	75.9	72.2	70.7–76.4
(1) Mean consumer, brand loyal	85.0–87.0	82.6	66–70	91.2	83.8	82.6–91.2
(2) High consumer, brand loyal	91.8–95.9	93.3	70 –76	98.0	94.3	93.3–98.0
(3) Mean consumer, market share (35%)	79.4–80.1	74.9	61–62	80.0	76.3	74.9–80.1
(4) High consumer, market share (35%)	81.8–83.2	78.6	62–65	83.6	79.9	78.6–83.6

[a] Baseline winter and summer serum 25OHD from Australian Bureau of Statistics, 2011–13, *Australian Health Survey, Core Content - Risk Factors and Selected Health Conditions, 2011–12.* Findings based on ABS Table Builder data.

[b] Baseline winter and summer serum 25OHD for ages 15 years and older was sourced from the 2008–09 *New Zealand Adult Nutrition Survey* and provided to FSANZ from the NZ MoH. Baseline values for 5–14 years was from Rockell et al (2005).

[C] Excluding values from New Zealand children aged 5–14 years since this survey used an assay for serum 25OHD that was not comparable to the other surveys.

6.2.3 Effect on the population distribution of annual mean serum 25OHD concentrations

The effect of consumption of vitamin D-fortified cereal on distribution of annual mean serum 25OHD concentrations reported for Australian (ABS 2014a) and New Zealand (Ministry of Health 2012a) adults was determined. Distribution fitting software (Palisade @RISK Add-In for Microsoft Excel) was used to estimate the decrease in the proportion with 25OHD < 40 nM and the increase in the proportion with 25OHD > 125 nM in each scenario modelled for Australian and New Zealand adults (aged 18 years and over, and 15 years and over, respectively). For the brand loyal scenarios (1) and (2), the estimated proportion with 25OHD < 40 nM or > 125 nM was calculated from the baseline proportion plus the estimated increase with consumption of vitamin D-fortified cereal. For the 35% market share scenarios (3) and (4), the estimated proportions were weighted to 65% of the population. Results are shown in Figure 4 and Table 13.

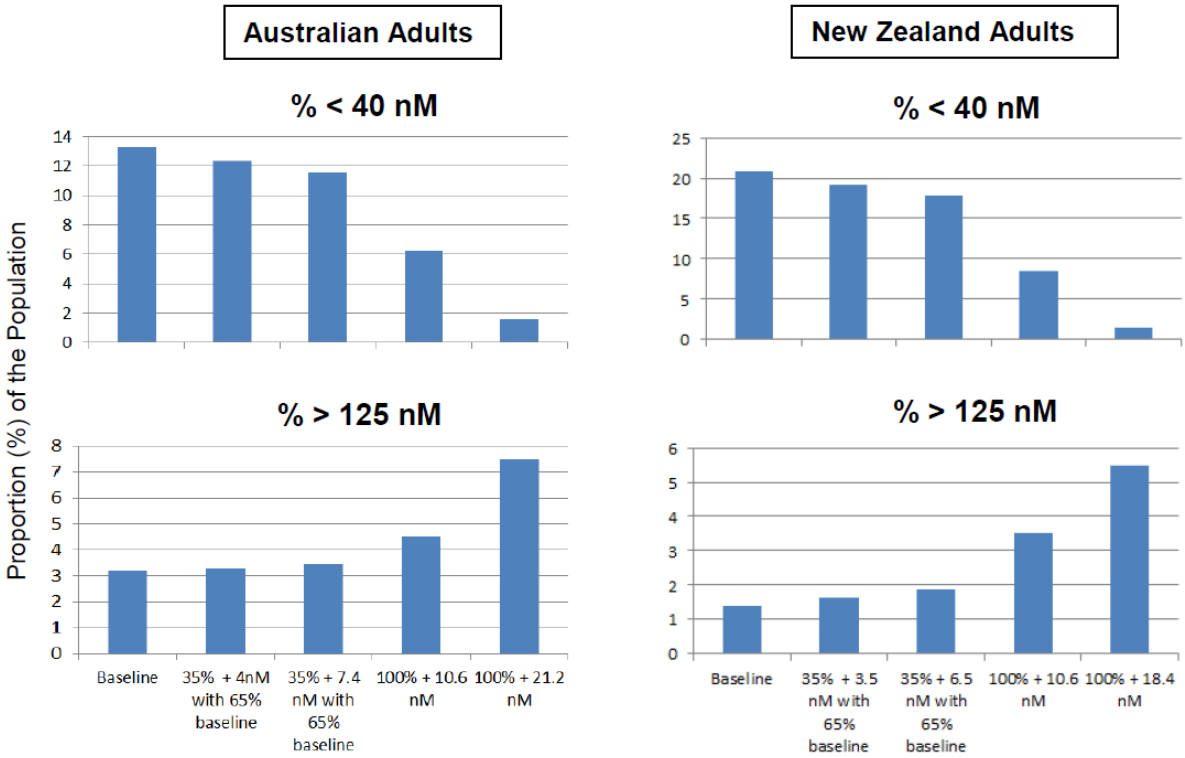

Figure 4: Predicted effect of vitamin D added to breakfast cereal on the population distribution of annual mean serum 25OHD concentrations for Australian aged 18 years and over and New Zealanders aged 15 years and over

Source for baseline distribution: Australian Bureau of Statistics, Australian Health Survey: Biomedical Results for Nutrients 2011-13. Table 5.1 (ABS 2014a) and Ministry of Health 2008–09 New Zealand Adult Health Survey (Ministry of Health 2012a).

Table 13: Tabulated results for distribution fitting - pproportions of population with serum 25OHD concentrations < 40 nM or >125 nM under the four modelling scenarios

Modelling Scenario:	Baseline	35% market share		Brand loyal	
		(3) Mean consumer	(4) P90 consumer	(1) Mean consumer	(2) P90 consumer
Australian adults (18 years and over)					
Predicted increase in serum 25OHD (nM)	0	4.0	7.4	10.6	21.2
Proportion (%) serum 25OHD < 40 nM	13.4 *	12.4	11.6	6.2	1.6
Proportion (%) serum 25OHD > 125 nM	3.2 **	3.3	3.5	4.5	7.5
New Zealand adults (15 years and over)					
Predicted increase in serum 25OHD (nM)	0	3.5	6.5	10.6	18.4
Proportion (%) serum 25OHD < 40 nM	20.9	19.2	17.8	8.4	1.5
Proportion (%) serum 25OHD > 125 nM	1.4	1.6	1.9	3.5	5.5

* This value is derived from ABS Table Builder using AHS Core Content – Risk Factors and Selected Health Conditions, 2011–12.

**This value differs from the Table Builder-derived value (1.3%) for the proportion with serum 25OHD > 125 nM due to a rounding error which occurs in the distribution fitting.

6.2.3.2 Impact on proportion with serum 25OHD concentration < 40 nM

For Australians aged 18 years and above, the prevalence of serum 25OHD < 40 nM was found to decrease from a baseline level of 13.4% down to 1.6% for the highest vitamin D intakes predicted from the modelling scenarios (Table 13). Similarly, data from the New Zealand adult survey shows the prevalence of serum 25OHD < 40 nM was found to decrease from a baseline level of 20.9% down to 1.5% for the highest vitamin D intakes predicted from the modelling scenarios. Therefore, it can be concluded voluntary fortification of breakfast cereal with vitamin D will potentially improve the vitamin D status of persons who have low vitamin D status and are possibly deficient.

6.2.3.2 Impact on proportion with serum 25OHD concentrations > 125 nM

For Australians aged 18 years and above, the prevalence of serum 25OHD > 125 nM was found to increase from a baseline of 3.2% (or 1.3% as determined from Table Builder analysis which does not include rounding error) to 7.5% for the highest vitamin D intakes from the modelling scenarios (Table 13). Similarly, data from the New Zealand adult survey shows the prevalence the prevalence of serum 25OHD > 125 nM was found to increase from a baseline level of 1.4% up to 5.5% for the highest vitamin D intakes predicted from the modelling scenarios.

6.3 Data gaps and limitations

The conclusions of the risk assessment were based on three components that factor into the potential effects on vitamin D status: (1) RTE breakfast cereal consumption (Section 5.2.1), (2) assumed vitamin D concentration in fortified breakfast cereal (Section 5.2.3), and (3) estimated effects of consuming vitamin D fortified breakfast cereal on serum 25OHD, particularly in relation to elevated concentrations (Section 6).

Limitations in relation to (1) – RTE breakfast cereal consumption – are:

- Age of the adult food consumption data – The 1995 NNS is the most recent comprehensive set of quantitative data on food consumption patterns for Australian adults and older teenagers currently available to FSANZ for modelling purposes. There is greater uncertainty when assessing consumption of foods that have been introduced to the market since the 1995 and 1997 NNSs were conducted, or for which there may have been changes in food consumption patterns over time. However summary reports for the 2011–12 NNPAS and 2008–09 ANS referred in this report indicate overall breakfast cereal consumption patterns remain similar.

- Estimating usual consumption – Estimating food consumption from one or two days of dietary data tends to overestimate the average amounts eaten of less frequently consumed foods. This is likely to have a less impact in relation to breakfast cereal consumption as this is a relatively commonly consumed food group.

- Lack of market share information – There is no identification of foods by factors such as brand, production method or treatment process available to FSANZ so only broad market share assumptions, relating to the overall proportion of the breakfast cereal category or sub categories that may be voluntarily fortified in Australia and New Zealand, could be made. In this case the assumed market share of 35% coverage for voluntary fortification of RTE breakfast cereal was based on the maximum figure reported in other countries.

Limitations in relation to (2) – assumed vitamin D concentration in fortified breakfast cereal – are:

- Food manufacturers add extra vitamins to compensate for these losses and the term used for the extra addition is 'overage'. This is a well-known and understood practice when foods are fortified with nutrients including vitamins. Breakfast cereal manufacturers would need to know the total vitamin D losses for their products during production and storage at various temperature and humidity conditions for the shelf life of their product so they can add sufficient vitamin D to compensate for the losses. Based on a theoretical worked example provided by the Applicant, a level of 5 µg vitamin D/serving was assumed for fortified breakfast cereal as consumed for modelling purposes, which may over- or underestimate actual values.

Limitations in relation to (3) – estimated effects of consuming vitamin D fortified breakfast cereal on serum 25OHD – are:

- Baseline concentrations for serum 25OHD concentrations were taken from the most recent surveys in Australia and New Zealand measuring serum 25OHD but these did not completely align with age ranges in the food consumption surveys. The surveys cover 12 years and above or 18 years and above (some data was not available for 12-17 years)for the Australian population, and 5 years and above for the New Zealand population. For New Zealand children, a different assay was used to measure 25OHD so these values were not comparable to other surveys.

- Limitations related to dose response equation – The efficacy of a given amount of dietary vitamin D to raise serum 25OHD may be impacted by factors on serum 25OHD such as sun exposure and dietary calcium or phosphorus intake. It was not possible to account for these effects in the meta-analysis used to define the dose response equation used in this assessment (Black et al. 2012).

- Additional dietary vitamin D intake will increase serum 25OHD concentration to a greater degree in those with low vitamin D status compared to those with high vitamin D status (25OHD > 50 nM) (NHMRC and NZ MoH 2006). As a result, the predicted proportions of the populations with 25OHD > 125 nM following fortification may be overestimated.

- The Black et al. (2012) meta-analysis was also based on a relatively limited number of fortification studies so it is possible that the conversion used in this assessment may change as more data emerges using improved assays methods.

7 Conclusions

7.1 Risk Characterisation

Vitamin D is obtained from both dietary sources and from synthesis in the skin with UV-B (sunlight) exposure. The best measure of vitamin D status is serum 25OHD concentration which accounts for all sources of vitamin D if measured using a reliable and validated assay. Recent results from ANZ surveys measuring serum 25OHD concentration indicated that the majority of the ANZ population have adequate vitamin D status. However, the prevalence of low values was higher in winter than summer, varied with region, and was more common in indigenous and some migrant groups. Therefore, certain population groups may benefit from additional vitamin D from dietary sources.

Because vitamin D can be obtained from dietary and other sources, the exposure assessment used a two-step process. First breakfast cereal consumption data were used to estimate vitamin D intakes under several scenarios for consumption of vitamin D fortified cereal. Vitamin D intakes were then used to predict potential increases in serum 25OHD concentration.

Serum 25OHD < 40 nM was determined to be an appropriate cut-off value to indicate low vitamin D status and risk of inadequate intakes. The predicted mean serum 25OHD across all ANZ populations and under all modelling scenarios ranged from 52–88 nM which is considered to be within physiological levels of serum 25OHD and indicates adequate vitamin D status. For Australian adults, the prevalence of serum 25OHD < 40 nM was found to decrease from a baseline level of 13.4% down to 1.6% for the highest vitamin D intakes predicted from the modelling scenarios. Similarly prevalence of serum 25OHD < 40 nM was found to decrease from 20.9% down to 1.5% for New Zealand adults.

Serum 25OHD > 125 nM was determined to be an appropriate cut-off value to indicate high vitamin D status and risk of excess intakes. For Australian adults the prevalence of serum 25OHD > 125 nM was found to increase from a baseline of 1.3% to 7.5%, and for New Zealand adults from 1.4% to 5.5% , for the highest vitamin D intakes from the modelling scenarios. Highest intakes were predicted with brand loyal, high cereal consumers which represented a conservative, worst-case scenario for vitamin D intake. The indicative proportion of persons exceeding 125 nM remained under 10% across all population groups and modelling scenarios.

Modelling under the four scenarios indicated that no persons were likely to reach serum 25OHD of 220–500 nM – the concentration for which the Working Group of the Australian and New Zealand Bone and Mineral Society and Osteoporosis Australia (Nowson et al. 2012) concluded that adverse effects associated with hypercalcaemia may occur.

Therefore, it is concluded that fortification of breakfast cereal with vitamin D (D_2 or D_3) at the modelled level does not pose a risk to public health and safety. Additionally, vitamin D fortification of breakfast cereal has the potential to increase the vitamin D status of individuals who may have less than adequate vitamin D levels.

7.2 Specific responses to assessment questions

Specific responses to assessment questions listed in Section 1.2 were summarised from the relevant sections of this assessment report.

Question 1) What is the likely effect of processing and storage on the lability of vitamin D3 in breakfast cereal?

Section	*Summary response/conclusion*
2.5	Vitamin D is relatively unstable to heat and moisture and is also degraded during the commercial shelf life of the product due to due to oxidation and storage at ambient temperature and humidity. Current best estimate is a 30% loss on manufacture and 10%/month loss on storage thereafter. A theoretically worked example indicates an approximate post-manufacture loss of 70% at the end of shelf life. Based these manufacturing and storage losses, and on the requested maximum claim per normal serving of 25% regulatory RDI (10 µg) per reference quantity, the midpoint of the shelf life was estimated to be 5 µg/serving and this was the figure used to estimate vitamin D intakes for the dietary intake assessment.

Question 2) Is vitamin D bioavailable from vitamin D-fortified breakfast cereal consumed with milk?

Section	*Summary response/conclusion*
3.2	Vitamin D requires no digestion and is absorbed in the intestine in association with fat and bile salts. Because it is soluble in lipids, it is possible that fat-containing meals facilitate absorption although this has mainly been examined in relation to vitamin D consumed as a supplement. Studies on absorption of vitamin D in food suggest that the fat content in food has minimal effect on absorption. There are no specific data examining whether milk facilitates the absorption of vitamin D from breakfast cereal. As breakfast cereal contains between 1–5 g fat /100 g, consumption of vitamin D-fortified cereal with or without milk is likely to be absorbed with comparable efficacy.
3.3	There is current debate on the relative bioavailability of D_2 compared to D_3. This question has been addressed mainly by determining the ability of the two forms to increase serum 25OHD but some research is obfuscated by assays that do not quantify $25OHD_2$ and $25OHD_3$ equally. Based on a recent RCT and meta-analysis, vitamins D_2 and D_3 present in fortified foods up to levels of 25 µg/day have been shown to be equally effective in raising serum 25OHD concentration. Only high levels of D_2 consumed as a supplement in doses of 125–1250 µg are less effective at raising serum 25OHD than equivalent amounts of D_3. These amounts are not comparable to levels in food (endogenous or fortified).

Question 3) What is the evidence for vitamin D inadequacy in Australian and New Zealand?

Section	*Summary response/conclusion*
6.1	Evidence for vitamin D inadequacy has been reported for the ANZ population through several nationally-based surveys measuring serum 25OHD concentrations. Because different assay methodologies and different cut-off values to describe vitamin D status were used in these surveys, prevalence data are not directly comparable (i.e. using a common cut-off value to indicate inadequacy). Nationally-based surveys reported that: • 13.4% of Australians (12 years and above) and 20.9% of New Zealand adults (15 years and above) have 25OHD < 40 nM • 31% of New Zealand children (5–14 years) have serum 25OHD < than 37.5 nM The survey data indicate that the majority of persons in ANZ have adequate vitamin D status but that prevalence of low vitamin D was higher in winter than summer, varied with region, and was more common in indigenous and some migrant groups.

Question 4) What is the risk to health from consumption of vitamin D-fortified breakfast cereal in Australia and New Zealand?

Section	*Summary response/conclusion*
3.5	Excessive nutrient intake is usually assessed against the UL but for vitamin D this does not account for the contribution of vitamin D from sun exposure and the recommended UL varies between different expert bodies. Therefore, risk of excessive vitamin D intake was determined from expert body conclusions on serum 25OHD concentrations that may be linked to adverse effects. Very high vitamin D intakes (i.e. > 250 µg/day only as a vitamin D supplement) give rise to serum 25OHD concentrations in the range of 220–700 nM , which are associated with adverse health effects related to calcium regulation. Moderately high serum 25OHD, that is, in the range 125–220 nM, has been proposed to be linked to other long term adverse health outcomes (e.g. cardiovascular disease) but results of studies are variable. Because of this uncertainty, the recent vitamin D review published by the IOM concluded that serum 25OHD concentrations in the range 125–150 nM would be of concern. Therefore, the criterion for excess was taken to be the proportion of population groups with serum 25OHD concentrations above 125 nM for this risk assessment.
6.2	The reported population distribution of serum 25OHD concentration in each survey was used to estimate the proportion of persons that would exceed serum 25OHD > 125 nM. Four modelling scenarios were used to estimate the predicted increase in serum 25OHD for high (90^{th} percentile) and mean cereal consumption. The proportion of the Australian adult population with serum 25OHD > 125 nM was increased from a baseline level of 1.3% to 7.5% for the scenario with the highest vitamin D intakes (90^{th} percentile, brand loyal cereal adult consumers). For New Zealand adults, the proportion increased from a baseline of 1.4% to 5.5% for the highest vitamin D intakes. This modelling scenario is likely to represent a conservative, worst-case scenario of the effect on serum 25OHD status. Therefore, FSANZ concludes that there is minimal risk to health from mean and high consumption of voluntarily vitamin D-fortified cereal in Australia and New Zealand at the maximum claim requested by the Applicant.

Question 5) What is the benefit to health from consumption of vitamin D-fortified breakfast cereal in Australia and New Zealand?

Section	*Summary response/conclusion*
3.4	The proportion with serum 25OHD less than 40 nM was used to estimate the prevalence of inadequate 25OHD serum concentrations in the ANZ populations. This value was chosen because the most recent EAR from the IOM was calculated assuming that 40 nM was a suitable level for 50% of the population and is analogous to using the EAR Cut Point method used in other FSANZ nutrient intake assessments to estimate the prevalence of inadequate intakes. It should be noted however, the results of ANZ national surveys report vitamin D status using different cut-off values for serum 25OHD concentration.
6.2	Mean consumption of vitamin D-fortified cereal gave a predicted serum 25OHD concentration in the range of 57.9–79.6 nM for a brand loyal consumer, and 52.8–72.7 nM for a consumer assuming a 35% market share of fortified breakfast cereal. High consumption (90^{th} percentile) of vitamin D-fortified cereal gave a predicted serum 25OHD concentration in the range 62.2–88.5 nM for a brand loyal consumer and 54.3–75.8 nM for a consumer assuming a 35% market share of fortified cereal. These ranges were well within the physiological range of serum 25OHD concentration. The modelled scenarios showed that the proportion with serum 25OHD < 40 nM in Australia would decrease from a baseline of 13.4% down to 1.6% for highest vitamin D intakes estimated for brand loyal, 90^{th} percentile cereal consumers. Similar reduction was estimated for New Zealand adults where the baseline proportion with serum < 40 nM was reduced from 20.9% to 1.5 % for the highest consumption of vitamin D fortified cereal.

Appendix 1 – Key attributes of nutrition surveys used in this assessment

2007 Australian Children's Nutrition and Physical Activity Survey (2007 ANCNPAS)

The 2007 ANCNPAS collected data on nutrition and physical activity for 4,487 children aged 2–16 years across Australia. The survey was conducted over a seven month time period, from February to August 2007. The results of the 2007 ANCNPAS were weighted to represent the overall population of Australian children because stratified sampling with non-proportional samples was used.

In contrast to other national nutrition surveys used to date by FSANZ (e.g. the 1995 NNS), respondents in the 2007 ANCNPAS completed two 24-hour recalls on non-consecutive days. The availability of two days of food consumption data to calculate an average consumption amount over two days provides a more realistic estimate of long term consumption of infrequently consumed foods, because it takes account of those who may eat a food on one day of the survey but not on the other. Using only one 24-hour recall may capture an unusual eating occasion for an individual that does not describe how they normally eat.
Reference: Department of Health and Ageing, 2008, 2007 *Australian National Children's Nutrition and Physical Activity Survey- Main Findings*. Commonwealth of Australia, Canberra. http://www.health.gov.au/internet/main/publishing.nsf/Content/phd-nutrition-childrens-survey

1995 Australian National Nutrition Survey (1995 ANNS)

The 1995 NNS provides comprehensive information on dietary patterns of a sample of 13,858 Australians aged 2 years and above. The survey used a 24-hour recall method for all respondents, with 10% of respondents also completing a second 24-hour recall on another, non-consecutive day. The data were collected over a 13 month period. These data are used unweighted in calculations. It is the most recent NNS for Australians aged 17 years and above currently in FSANZ's modelling program (data from the 2011–13 Australian Health Survey not yet available for use, see below). Only the data from respondents aged 17 years and above from this survey were used in this assessment.
Reference: McLennan W & Podger A, 1999, *National Nutrition Survey. Foods Eaten. Australia. 1995*. (ABS Catalogue number 4804.0), Commonwealth of Australia, Canberra. http://www.abs.gov.au/ausstats/abs@.nsf/PrimaryMainFeatures/4804.0?OpenDocument

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

The 2002 NCNS provides comprehensive information on the dietary patterns of a nationally representative sample of 3,275 New Zealand children aged 5–14 years, including sufficient numbers of children in the Māori and Pacific groups to enable ethnic-specific analyses. The survey was conducted using a 24-hour recall methodology and collected data on dietary supplements as well as foods and beverages. A repeat 24-hour diet recall was obtained from a subsample of 15% of respondents. The results of the 2002 NCNS were weighted to represent the overall population of New Zealand children because stratified sampling with non-proportional samples was used in the survey.
Reference: Ministry of Health, 2003, *NZ Food NZ Children: Key results of the 2002 National Children's Nutrition Survey.* Wellington: Ministry of Health.
http://www.moh.govt.nz/notebook/nbbooks.nsf/0/658d849a2bac7421cc256dd9006cc7ec/$FILE/nzfoodnzchildren.pdf

2008–09 New Zealand Adult Nutrition Survey (2008–09 NZ ANS)

The New Zealand 2008–09 Adult Nutrition Survey was carried out from October 2008 to October 2009. The Survey involved face-to-face 24-hour recall interviews with 4721 New

Zealanders aged 15 years and above living in private dwellings, with 25% of respondents completing a second 24-hour recall.
Reference: University of Otago and Ministry of Health, 2011, A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey. Wellington: Ministry of Health. http://www.health.govt.nz/publication/focus-nutrition-key-findings-2008-09-nz-adult-nutrition-survey

More recent Australian nutrition survey data

More recent food consumption data for Australia have been collected. However, these data are not yet available in FSANZ's dietary modelling program for use in risk assessments. Where relevant, published summary data from this more recent survey have been provided.

The 2011–12 National Nutrition and Physical Activity Survey (NNPAS) was conducted as part of the 2011–13 Australian Health Survey (AHS). The 2011–12 NNPAS was conducted from 29 May 2011 to 9 June 2012 (with a pause between 6 August 2011 and 2 October 2011 while the 2011 Australian Census was conducted). Twelve thousand, one hundred and fifty three Australians aged 2 years and above living in private dwellings undertook a 24-hour recall interview, with 63.6% of respondents completing a second 24-hour recall.
Reference: Australian Bureau of Statistics, 2014, 2011-12 *National Nutrition and Physical Activity Survey: Main Findings*. ABS, Canberra
http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4364.0.55.007~2011-12~Main%20Features~Key%20Findings~1

Neither the 2008–09 NZ ANS nor the 2011–12 NNPAS reported vitamin D intakes from food. Estimating vitamin D status using the biomedical results collected from both surveys was considered a more accurate estimate of vitamin D status in the Australian and New Zealand populations as this measure takes into account the overall effect of diet, supplement use and sunlight exposure.

Appendix 2 – Serum 25OHD results as reported in ANZ Surveys

2011–13 Australia Health Survey (AHS)

The National Health Measures Survey (NHMS) component of the Australian Health Survey (AHS) conducted by the Australian Bureau of Statistics (ABS) analysed serum 25OHD for the population aged 12 years and above. Blood was collected from adults 12 years and above, including pregnant women and serum 25OHD was measured using tandem Mass Spectrometry (LC-MS/MS) methodology. Assays were conducted in collaboration with the international Vitamin D Standardisation Program, VDSP (Cashman et al. 2013) such that additional controls (quantification of D_2 and D_3, and use of standard reference materials) were used to allow comparison to other international analyses.

Selected 25OHD results from AHS (ABS 2014a) are presented in Figures 1–4 of this Appendix. Serum 25OHD was measured for ages 12 and above (Figure 1) or 18 years and above (Figure 2–4). Sampling and assay methods as reported (ABS 2014a; ABS 2014b; ABS 2014c). The proportion of the population with serum levels within different ranges was reported in AHS tables using the cut-off values indicated in Figure 1, and as published on the ABS website at
http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0062011-12?OpenDocument

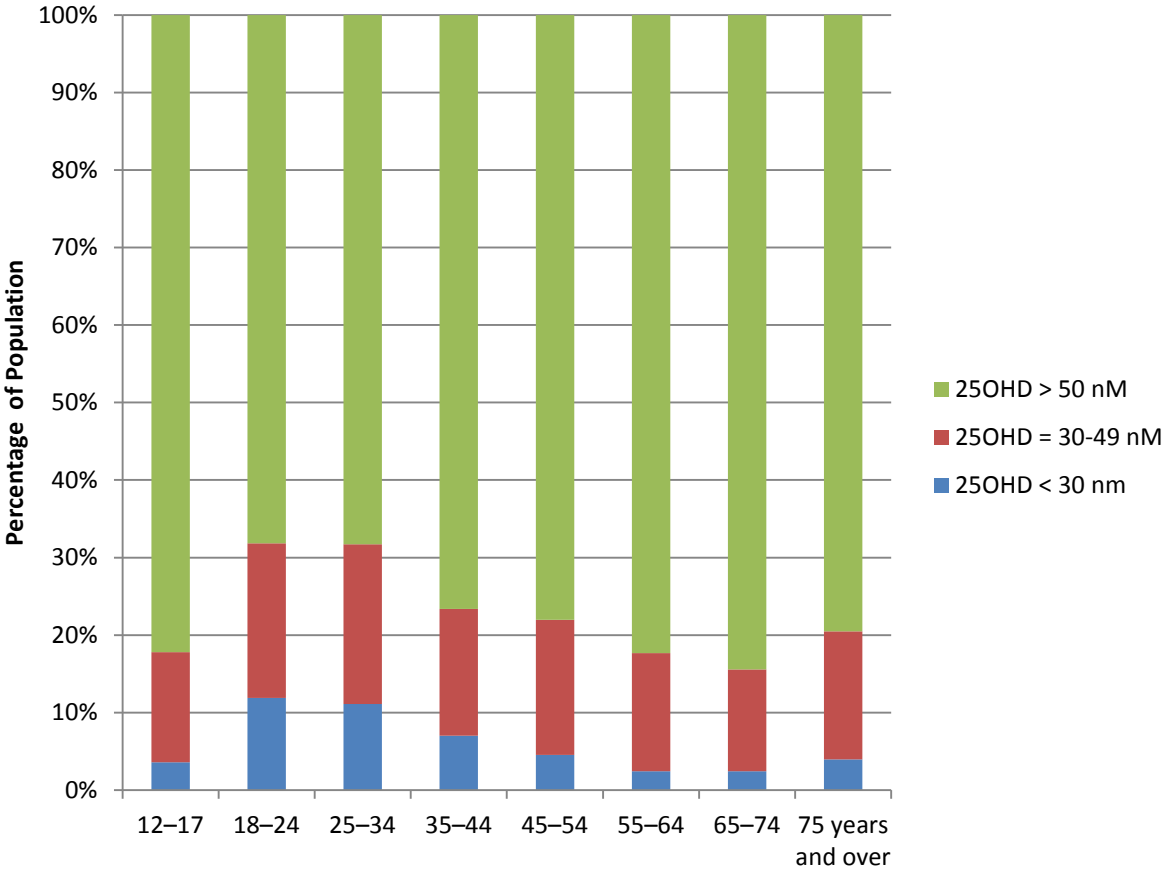

Figure A1: Vitamin D status of Australian population 12 years and above, by age

Source: Australian Bureau of Statistics, Australian Health Survey: Biomedical results for Nutrients 2011–12, Table 1.1 Nutrient biomarkers(a) by age then sex, means and medians.

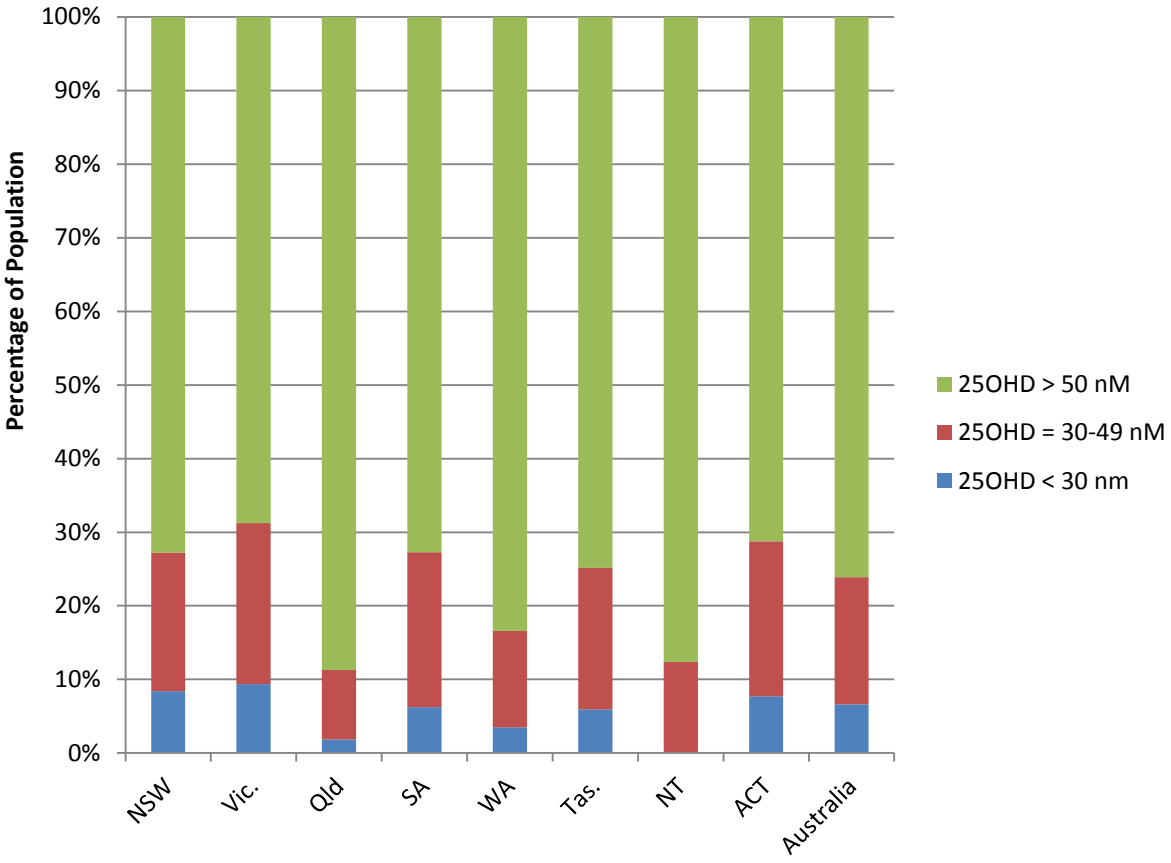

Figure A2: Vitamin D status of Australian population aged 12 years and above by state or territory

Source: Australian Bureau of Statistics, Australian Health Survey: Biomedical results for Nutrients 2011–12, Table 2.1 Nutrient biomarkers(a) by State and Territory, means and medians.

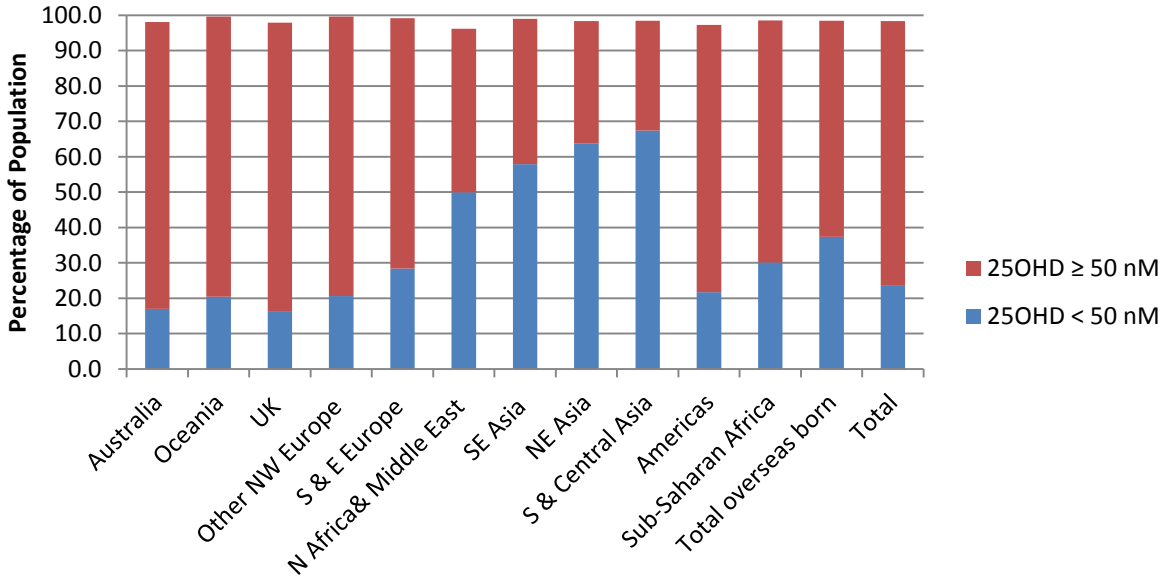

Figure A3: Vitamin D status of Australian population aged 12 years and above by country of birth.

Source: Australian Bureau of Statistics, Australian Health Survey: Biomedical results for Nutrients 2011–12, Table 10.1 Vitamin D by selected population characteristics.

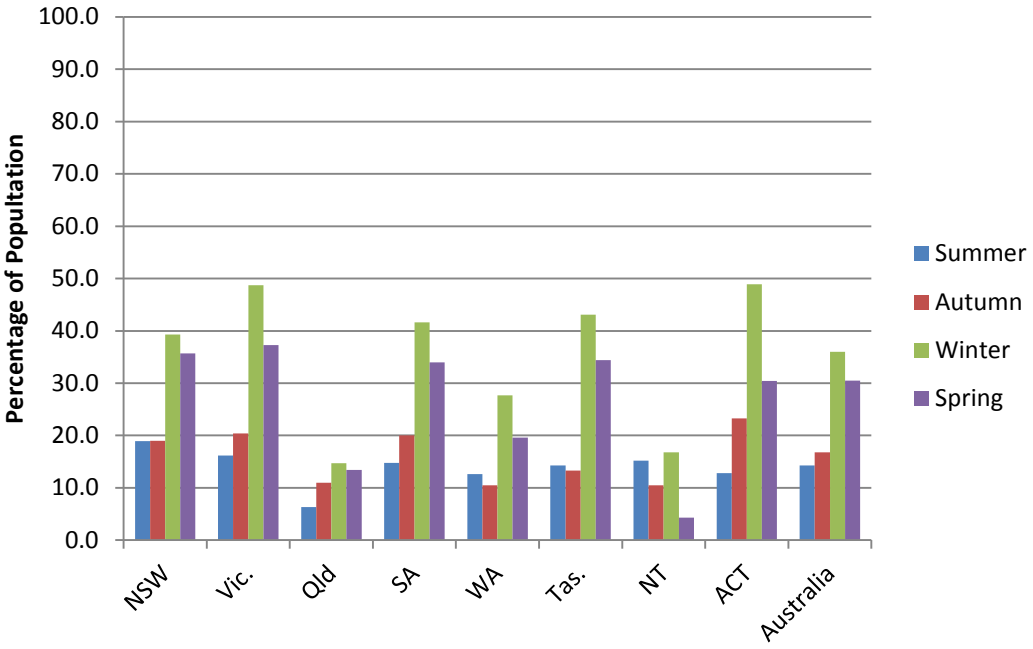

Figure A4: Seasonality of vitamin D status: percentage of popultation (aged 18 years and above) with serum 25OHD < 50 nM, by state or territory. *Source: Australian Bureau of Statistics, Australian Health Survey: Biomedical results for Nutrients 2011–12, Table 8.1 Vitamin D by selected population characteristics*.

2008–09 New Zealand Adult Health Survey

The vitamin D status of the New Zealand population aged 15 years and above was measured as part of the 2008–09 New Zealand Adult Nutrition Survey (Ministry of Health 2012a). Blood samples were analysed for 25OHD using the HPLC-tandem MS method. Data were reported using cut-off values of 25OHD < 25 nM, 25OHD = 25–49 nM, and 25OHD > 50 nM.

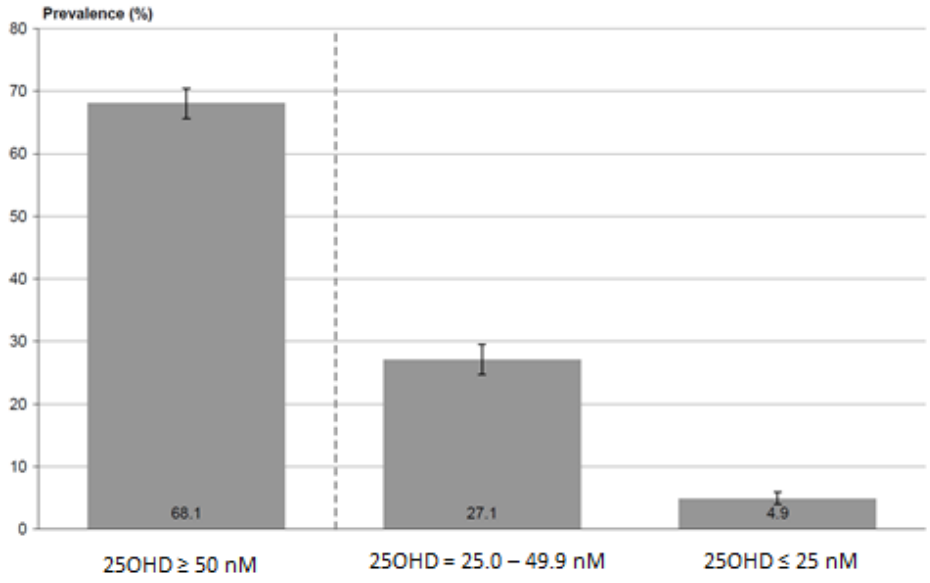

Figure A5: Prevalence of vitamin D status in New Zealand adults (ages 15 years and above)

Source: Figure 1 in 2008–09 New Zealand Adult Nutrition Survey: Vitamin D Status of New Zealand Adults (Ministry of Health 2012a).

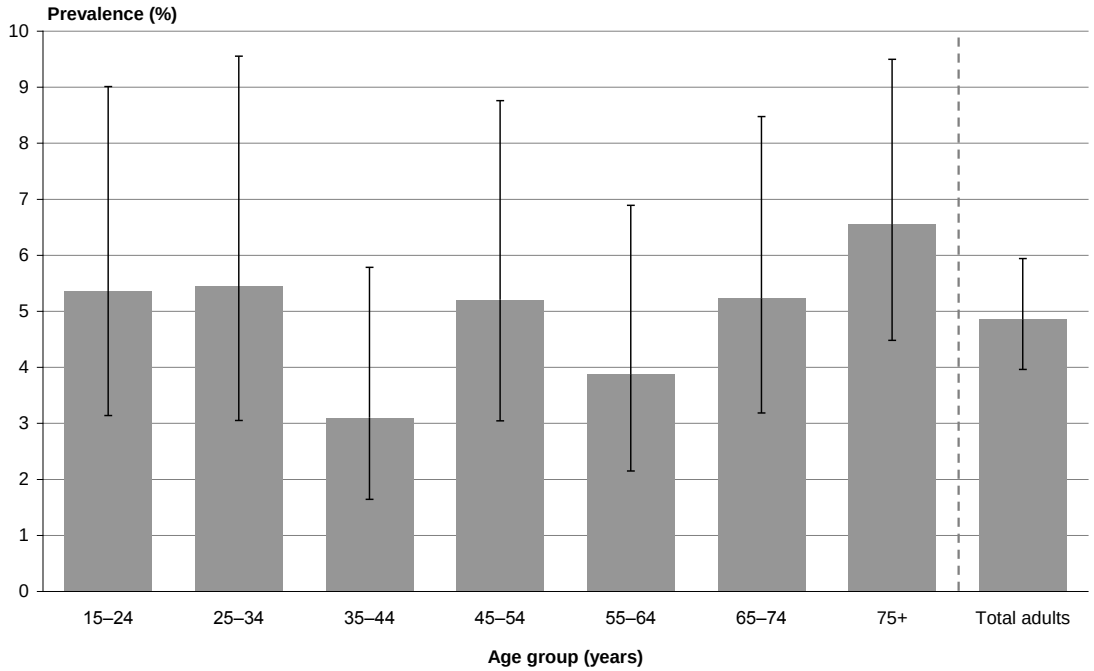

Figure A6: Prevalence of vitamin deficiency by age group

Source: Figure 2 in 2008–09 New Zealand Adult Nutrition Survey: Vitamin D Status of New Zealand Adults (Ministry of Health 2012a).

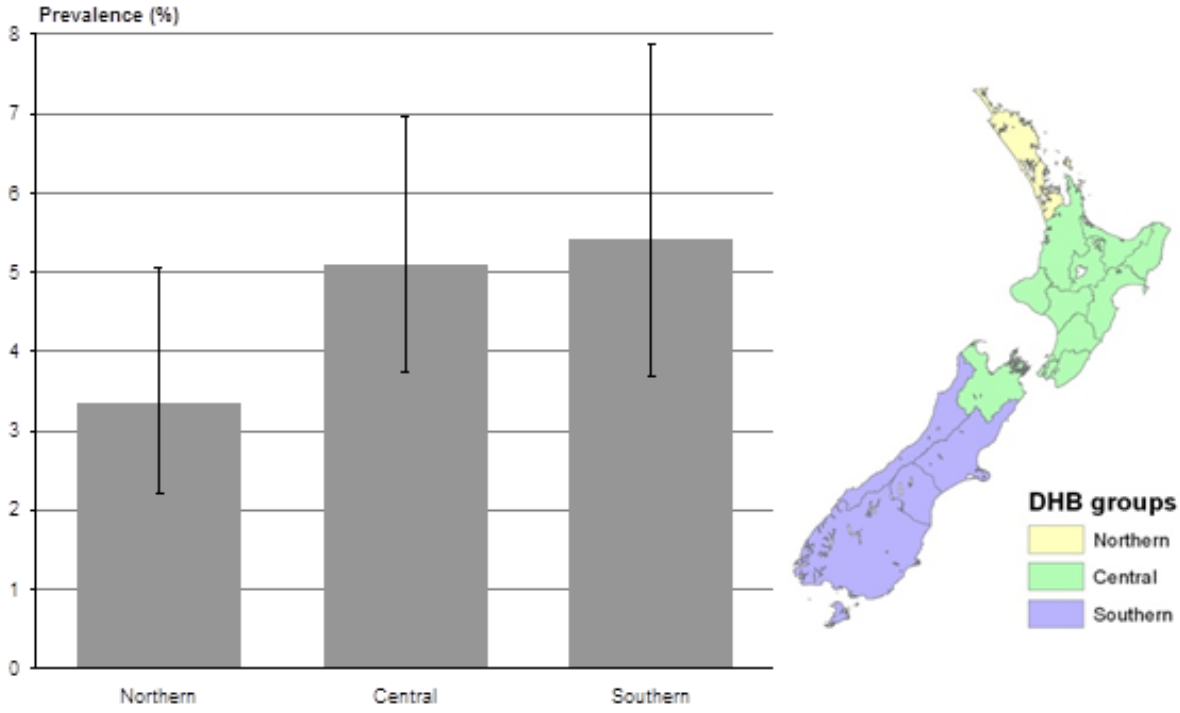

Figure A7: Prevalence of vitamin deficiency by region

Source: Figure 8 in 2008–09 New Zealand Adult Nutrition Survey: Vitamin D Status of New Zealand Adults (Ministry of Health 2012a).

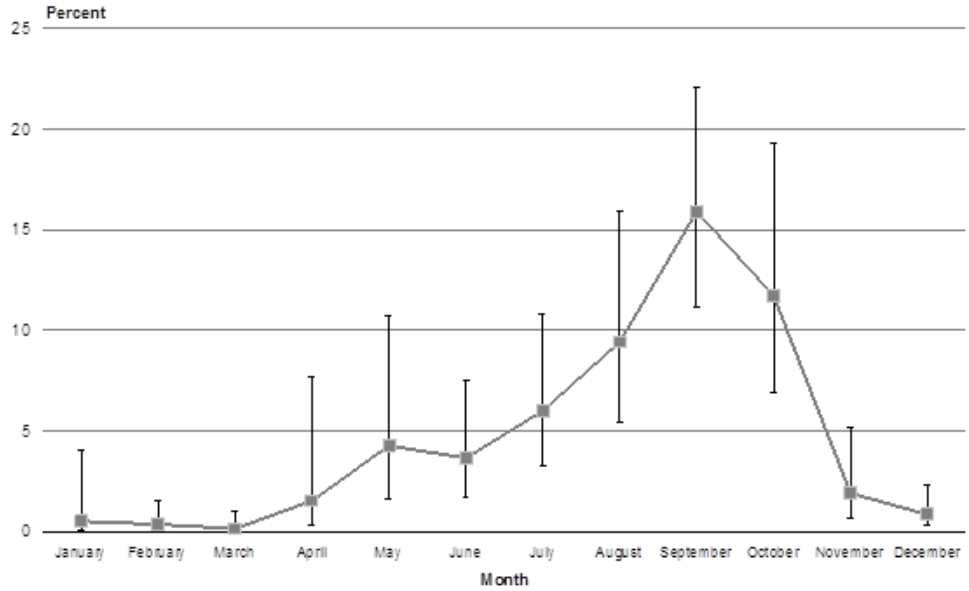

Figure A8: Prevalence of vitamin deficiency by month

Source: 2008–09 New Zealand Adult Nutrition Survey: Vitamin D Status of New Zealand Adults (Ministry of Health 2012a).

2002 New Zealand National Children's Nutrition Survey

The 2002 NZ NCNS also included serum 25OHD measurements (Rockell et al. 2005). A national sample of school children (n=1585) was used with an over-sampling of Maori and Pacific children to allow ethnic-specific analysis to be conducted. Serum 25OHD was determined using the DiaSorin radio-immunoassay method that measured total 25OHD concentration and this is a different assay than that used for the Australian and New Zealand adult surveys. Standard reference materials were not available for this study although randomly selected samples were assayed in a second laboratory using the same method and there was no statistically significant variation between the two laboratory analyses.

Table A1 Mean serum 25OHD and proportion below two cut-off values, 2002 New Zealand Children's Survey*

Population group	Age (years)	Mean serum 25OHD (nM)	Proportion (%)	
			25OHD < 17.5 nM	25OHD < 37.5 nM
All children	5–14	50	4	31
All boys	5–14	52	3	27
	5–6	57	1	19
	7–10	53	3	24
	11–14	50	4	33
All girls	5–14	47	4	36
	5–6	48	1	29
	7–10	51	2	31
	11–14	42	7	43
All Maori	5–14	43	5	41
Maori boys	5–14	47	4	36
	5–6	48	4	26
	7–10	47	4	38
	11–14	45	4	40

Population group	Age (years)	Mean serum 25OHD (nM)	Proportion (%)	
			25OHD < 17.5 nM	25OHD < 37.5 nM
Maori girls	5–14	40	7	46
	5–6	42	4	45
	7–10	46	2	33
	11–14	35	12	58
All Pacific	5–14	36	8	59
Pacific boys	5–14	38	7	53
	5–6	42	6	49
	7–10	40	6	50
	11–14	36	10	61
Pacific girls	5–14	34	9	64
	5–6	39	3	47
	7–10	34	8	66
	11–14	32	13	71
All children	5–14			
Winter		43	4	42
Summer		58	1	14
South Island		48	2	27
North Island		50	3	29

* Source: Adapted from Table 2 and Table 4 in Rockell et al. (2005). Data were adjusted for survey weighting.

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