Attachment 6

RISK ASSESSMENT - MICRONUTRIENTS[1]
APPLICATION A470– FORMULATED BEVERAGES

CONTENTS

[1] For the purpose of this report, the term 'micronutrients' is used for vitamins and minerals.

Summary and Conclusions

Risk Assessment – Micronutrients

A risk assessment has been conducted in relation to the addition of certain vitamins and minerals to formulated beverages at a level of 25 % of the recommended dietary intake (RDI) per 600 ml serve. These include: vitamin A, β-carotene, thiamin, riboflavin, niacin, folate, vitamin B_6, B_{12}, D, E, biotin, pantothenic acid, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, selenium and zinc. The applicant has requested vitamin C to be added at 100% of the RDI per 600 ml serve. After the Draft Assessment Report the Applicant has amended the Application, resulting in a request in a reduction of the number of vitamins and minerals to be added to formulated beverages. This Attachment still contains all the vitamins and minerals originally requested by the Applicant.

The results of the risk assessment are provided below and are summarised in **Table 1**.

Micronutrients without risk for the general population

For the following micronutrients, it is concluded that addition to formulated beverages at a level of 25% of the RDI per 600 ml (100% of RDI per 600 ml for vitamin C) would raise no public health and safety concerns for any sector of the population: β-carotene, thiamin, riboflavin, niacin, folate, vitamin B_6, B_{12}, C, D, E, pantothenic acid, calcium, magnesium, phosphorus and selenium.

Micronutrients with some risk for sensitive subpopulations

For the following micronutrients, it is concluded that while the general population is without risk, there may be a risk for certain sectors of the population:

Copper:

Individuals with Wilson's disease, Indian childhood cirrhosis or idiopathic copper toxicosis may respond adversely to copper in formulated beverages at a level of 0.75 mg per 600 ml.

Iodine:

Individuals with thyroid disorders or a long history of iodine deficiency may respond adversely to iodine in formulated beverages at a level of 37.5 μg per 600 ml.

Iron:

Individuals who are homozygous for hereditary haemochromatosis are susceptible to iron overload, even at normal dietary iron intakes, and are generally advised to avoid iron-supplements and highly iron fortified foods. As the majority of individuals with this condition are not diagnosed until sufficient iron has accumulated to produce adverse effects, the addition of iron to formulated beverages at a level of 3 mg per 600 ml serve may be a concern to these individuals.

Micronutrients with some risk for specific age groups

For the following micronutrients, there are potential risks for specific age groups if they were permitted to be added to formulated beverages:

Vitamin A:

The dietary modelling results suggest that young children consuming formulated beverages may have excess intakes of retinol for several years and therefore be at risk of hepatoxicity. For all other age groups and life-stages, there is no appreciable risk posed by excess intake of retinol. There are potential safety concerns for children up to the age of 3 years, and maybe up to 6 years, with the addition of retinol to formulated beverages at a level of 187.5 μg in a 600 ml serve.

Manganese:

An upper level of intake (UL) could not be established because of limitations with the human data and considerable uncertainty with the animal toxicity studies. The available data suggests that the margin between the intake level producing adverse effects in humans and animals and the estimated intake from food is very small. Based on the severity of the potential adverse effect (neurotoxicity), additional oral exposure to manganese beyond the levels normally present in food and beverages could pose a public health and safety risk. Therefore, there are potential safety concerns with the addition of manganese to formulated beverages at a level of 1.25 mg in a 600 ml serve.

Zinc:

Dietary modelling indicated that children up to 8 years of age, who are consumers of a diet high in zinc, are predicted to exceed the UL for zinc. For adolescents up to the age of 18 years, who are consumers of a diet high in zinc, the intake is predicted to be 80% of the UL of zinc. Chronic zinc toxicity is associated with symptoms of copper deficiency. These adverse effects include anaemia, neutropaenia and impaired immune response. Furthermore, the potential contribution from other sources (e.g. dietary supplements) has not been taken into consideration in the dietary intake assessment. The intakes of zinc may therefore be underestimated for children and adolescents up to the age of 18 years and, for this group, formulated beverages at a level of 3 mg per 600 ml serve pose a public health and safety risk.

Micronutrients with insufficient data to assess risk

For the following micronutrients there was insufficient data to characterise the potential risk:

Biotin and Chromium:

Due to insufficient data on potential adverse effects and only limited food composition data it was not possible to establish an UL for biotin and chromium or to undertake a complete dietary intake assessment. In the absence of sufficient information, it is currently not possible to evaluate the safety of the addition of biotin and chromium to formulated beverages.

Molybdenum:

An UL has been established based on reproductive effects in rats. While some food composition data are available for molybdenum, it is insufficient to undertake a complete dietary intake assessment at this present time. In the absence of sufficient information, it is not currently possible to evaluate the safety of the addition of molybdenum to formulated beverages.

Assessment of permitted forms

For pantothenic acid, biotin, chromium, manganese, molybdenum and selenium, currently there are no forms permitted in Standard 1.1.1 – Preliminary Provisions – Application, Interpretation and General Prohibitions. The requested permitted forms for pantothenic acid, copper and selenium have been included in evaluations of the toxicity of the micronutrients assessed, and are considered to be acceptable as permitted forms.

Table 1: Risk Assessment of High Micronutrient intake

	UL (adults)	Intake from total diet / Suppl	Adverse effect which is the basis for an UL	Vulnerable groups identified	Dietary Intake Assessment	Risk Characterisation	Proposed to be added to formulated beverage
Vitamin A, retinol form, μg/day	3000	Total diet	Teratogenicity, hepatoxicity	young children	up to 3 years exceed UL	Potential safety concerns for children up to 8 years	No
β-Carotene, food	N/A	diet	no safety concerns with β-carotene from the diet	-		No safety concerns	**Yes**
β-Carotene, supplements	No UL established	Suppl	Insufficient data to set a UL			No safety concerns	
Thiamin	N/A	-	No indication of adverse effects	-	Not needed	No safety concerns	**Yes**
Riboflavin	N/A		No indication of adverse effects	-	Not needed	No safety concerns	**Yes**
Niacin, nicotinic acid, mg/day	10	Suppl	Flushing	-	Intake below UL in all age groups, except 2-8 years	adverse effects for nicotinic acid not relevant for children	**Yes**
Niacin, nicotinamide, mg/day	900	Total diet	No adverse effects at UL	-	Intake below UL in all age groups	No safety concerns with nicotinamide,	
Folate, (as folic acid), mg/day	1.0	Suppl	Progressing neurological symptoms in vitamin B_{12} deficient patients	-	Intake below UL in all age groups	No safety concerns	**Yes**
Vitamin B_6, mg/day	25	Total diet	Neuropathy	-	Intake below UL in all age groups	No safety concerns	**Yes**
Vitamin B_{12}	N/A	-	No indication of adverse effects	-	Not needed	No safety concerns	**Yes**

	UL (adults)	Intake from total diet / Suppl	Adverse effect which is the basis for an UL	Vulnerable groups identified	Dietary Intake Assessment	Risk Characterisation	Proposed to be added to formulated beverage
Vitamin C	no UL established	Total diet	Insufficient data to set a UL, low toxicity at high doses, guidance level of 1000 mg/day	-	Not needed	No safety concerns	**Yes**
Vitamin D, µg/day	50	Total diet	Serum calcium levels	-	Intake below UL in all age groups	No safety concerns	**Yes**
Vitamin E, mg/day	300	Total diet	Blood clotting related to vitamin K deficiency	-	Intake below UL in all age groups	No safety concerns	**Yes**
Biotin	No UL established	-	Insufficient data to set a UL	unknown	No data available	Not possible to perform risk characterisation	No
Pantothenic acid	N/A	-	No indication of adverse effects		Not needed	No safety concerns	**Yes**
Calcium, mg/day	2500	Total diet	No adverse effects at UL, at higher doses kidney stones, milk-alkali syndrome,	-	Intake below UL	No safety concerns	**Yes**
Chromium	No UL established		Insufficient data to set a UL	unknown	No data available	Not possible to perform risk characterisation	No
Copper, mg/day	10	Total diet	Hepatoxicity	Wilson's disease, Indian childhood cirrhosis or idiopathic copper toxicosis	Intake below or at UL in all age groups	No safety concerns	**Yes**, but risk management to be considered

	UL (adults)	Intake from total diet / Suppl	Adverse effect which is the basis for an UL	Vulnerable groups identified	Dietary Intake Assessment	Risk Characterisation	Proposed to be added to formulated beverage
Iodine, µg/day	1100	Total diet	Elevated TSH levels	individuals with thyroid disorders or a long history of iodine deficiency	Intake below UL in all age groups (except 2-3 years old, no safety concern)	No safety concerns	**Yes,** but risk management to be considered
Iron, mg/day	No UL established	-	Insufficient data to set a UL, high iron stores in older adults	individuals who are homozygous for hereditary haemochromatosis	Intake in all age groups are below levels with potential adverse effects	No safety concerns	**Yes,** but risk management to be considered
Magnesium, mg/day	350	Suppl	Osmotic diarrhoea		Intake below UL in all age groups (except 2-3 years old)	UL based on a mild reversible effect, and modelling assumes worst case scenario, therefore, not of concern for young children	**Yes**
Manganese, mg/day	No UL established		Neurotoxicity, not possible to establish an UL for total intake, but risk of adverse effects above current intake	All individuals		Risks of adverse effects at levels above currently in the diet	No
Molybdenum, µg/day	600	Total diet	Reproductive effects	Unknown	No data available	Not possible to perform risk characterisation	No
Phosphorus, mg/day	4000	Total diet	Serum inorganic phosphorus levels	-	Intake below UL in all age groups	No safety concerns	**Yes**
Selenium, mg/day	0.40	Total diet	Brittle nails and hair pathology, adverse effects nervous system	-	Intake below UL in all age groups	No safety concerns	**Yes**

	UL (adults)	Intake from total diet / Suppl	Adverse effect which is the basis for an UL	Vulnerable groups identified	Dietary Intake Assessment	Risk Characterisation	Proposed to be added to formulated beverage
Zinc, mg/day	40	Total diet	Reduced copper status	Children and adolescents	2-8 years exceed UL 9-18 years approx 80% UL	Potential safety concerns up to 18 years, because of other potential sources of intake	No

N/A = not applicable

Introduction

A risk assessment has been conducted to identify potential public health and safety risks associated with the addition of certain vitamins and minerals to formulated beverages at a level of 25 % of the recommended daily intake (RDI). These include: vitamin A, β-carotene, thiamin, riboflavin, niacin, folate, vitamin B_6, B_{12}, D, E, biotin, pantothenic acid, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, selenium and zinc. The applicant has requested vitamin C to be added at 100% of the RDI. In this Attachment, the hazard identification and characterisation, dietary intake assessment and the risk characterisation for each micronutrient are presented. After the Draft Assessment Report the Applicant has amended the Application, resulting in a request in a reduction of the number of vitamins and minerals to be added to formulated beverages. This Attachment still contains all the vitamins and minerals originally requested by the Applicant.

Hazard Identification and Characterisation

Upper Level of Intake (UL)

The Upper Level of Intake (UL) has been defined by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) as: *a quantitative level of total intake at which, or below, no harm is expected to occur assuming nutrient adequacy is met* (FAO/WHO, 2004).

ULs have been established for the general population for vitamins and minerals by a number of countries as well as by an expert FAO and WHO working group (FAO/WHO, 2002). Australia and New Zealand have currently no established upper limits for the general population for vitamins and minerals, however, the National Health and Medical Research Council (NHMRC) is currently in the process of developing Nutrient Reference Values for Australia and New Zealand, which include ULs (NHMRC, 2004).

The ULs established by the United Kingdom (UK Expert Group on Vitamins and Minerals, 2003), the United States (US Institute of Medicine, 2000a; US Institute of Medicine, 2000b; US Institute of Medicine, 2000c; US Institute of Medicine, 2001a; US Institute of Medicine, 2001b) the European Union (European Commission Health & Consumer Protection Directorate-General, 2000a) and FAO/WHO (FAO/WHO, 2002) were compared and considered for their thoroughness and appropriateness for Australian and New Zealand populations.

The UL is derived by dividing the no observed adverse effect level (NOAEL) by the uncertainty factor (UF). UFs are empirical values applied to take into account uncertainties in the data. For example, an UF may need to be applied when extrapolating from results in experimental animals to humans or when extrapolating results from selected individuals to another group. These factors allow for differences in sensitivity between individuals and between species that may result from differences in absorption, metabolism, or biological effect of the substance under consideration. UFs may also be applied to account for uncertainties due to data base deficiencies (e.g. absence of a NOAEL requiring extrapolation from a low observed adverse effect level (LOAEL)), a poor data base, studies with small numbers of subjects, or because of the nature of a particular adverse effect.

ULs are derived for different life-stage groups using relevant data. In the absence of data for a particular life-stage group, extrapolations are made from the UL for other groups on the basis of known difference in body size, physiology, metabolism, absorption and excretion of a nutrient. When data are not available for children and adolescents, extrapolations are made on the basis of body weight using the reference weights from the draft Nutrient Reference Values for Australia and New Zealand (NHMRC, 2004), see table 2.

Table 2: Reference body weights[2]

Age	Reference weight, kg
1-3 years	13
4-8 years	22
9-13 years	40
14-18 years	61
Adult	69

For the safety assessment of vitamins and minerals, it has been assumed that the products could be used for a long-term period. Therefore, in the safety assessment, ULs are based preferentially on long-term effects.

As the vitamins and minerals are intended to be added to formulated beverages and would not be incorporated into a food matrix, it has been assumed that when ULs for certain micronutrients are set for supplement use only, these ULs are relevant for the risk assessment.

Permitted forms

For the following micronutrients, no permitted forms are specified in Standard 1.1.1 – Preliminary Provisions – Application, Interpretation and General Prohibitions: pantothenic acid, biotin, chromium, copper, manganese, molybdenum and selenium. Therefore, the Applicant requested the forms specified in table 3 for these micronutrients. An assessment of the permitted form was undertaken, when the risk characterisation did not indicate safety concerns with including the specified micronutrient in formulated beverages.

Table 3: Permitted forms requested by the Applicant

Micronutrient	Permitted form requested
Pantothenic acid	Calcium pantothenate Dexpanthenol
Biotin	d-Biotin
Chromium	Chromium sulphate Chromic chloride
Copper	Copper gluconate Cupric sulphate Cupric citrate Cupric carbonate

[2] The reference body weights used in this table for 14-18 years old and adults are the average of male and female body weights as specified by the NHMRC.

Micronutrient	Permitted form requested
Manganese	Manganese chloride Manganese gluconate Manganese sulphate Manganese carbonate Manganese citrate
Molybdenum	Sodium molybdate VI dehydrate
Selenium	Seleno methionine Sodium selenate Sodium selenite

Dietary Intake Assessment

A dietary intake assessment was conducted to determine the impact of consuming formulated beverages on nutrient intakes and to assess the potential risk to public health and safety. This Attachment focuses on the results of the dietary modelling relating to the safety assessments of the nutrients. However, further details on how the dietary intake assessments were conducted can be found at **Attachment 7 – Dietary modelling methodologies for micronutrient intake assessments**, which details information on methodologies used for conducting the intake assessments for nutrients, including the data sources, assumptions made and limitations of the dietary modelling. The results for the intake assessment for inadequacy and health benefits can be found in **Attachment 5 – Nutrition Assessment**.

The food consumption data used for the intake assessment were individual dietary records from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS. The 1995 NNS from Australia surveyed 13 858 people aged 2 years and above, and the 1997 New Zealand NNS surveyed 4 636 people aged 15 years and above. Both of the NNSs used a 24-hour food recall methodology. Approximately 10% percent of respondents to the Australian NNS and approximately 15% of people from the New Zealand NNS completed a second 24-hour recall. These second day data were used to adjust the majority of the nutrient intake estimates across two days, providing a better estimate of daily nutrient intakes across a longer period of time. See Attachment 7 for more details. For some nutrients, the second day adjustment was unable to be calculated for a number of reasons, including that the NNS data were not statistically robust enough to enable the adjustment to be done. Use of second day adjustments has been highlighted below in the dietary intake discussion for each of the nutrients, where relevant.

Mean nutrient intakes based on food consumption for day 1 only (from the 24-hour recall) will not differ much compared to mean intakes that are based on a longer period of time such as when second day adjustments are used using the second day 24-hour recall. However, estimated nutrient intakes based on day 1 food consumption only, will be overestimates at the 95th percentile. Adjusting the nutrient intakes by using the second day food consumption data will bring in the tails of the intake distribution resulting in a lower, more realistic, 95th percentile intake (Rutishauser I, 2000).

Nutrient concentrations for formulated beverages used in the dietary intake assessments were derived from the application. Nutrient concentrations for all other foods were those from the relevant 1995 Australian or 1997 New Zealand NNSs. There were some nutrients which were included in the New Zealand NNS however were not included in the Australian NNS.

For these nutrients (vitamin B_6, vitamin B_{12}, vitamin D, vitamin E, manganese and copper), the concentrations from the New Zealand NNS were used and matched to the most appropriate foods in the Australian NNS. For nutrients that were not included in either of the NNSs (iodine and selenium), survey and analytical data were used. No intakes from dietary supplements were included in the assessments. However, for some nutrients, only the supplemental uses were relevant for the safety assessment based on how the ULs were established, therefore, only fortified foods were included in the intake assessment.

The nutrients have been assessed for safety at 'baseline' and for 'Scenario 2'. Baseline intakes are nutrient intakes based on 1995 food composition data and assuming formulated beverages are not consumed. Scenario 2 assesses the impact on nutrient intakes assuming formulated beverages are consumed containing the requested levels of nutrients. Scenario 2 is a substitution scenario that assessed what will happen to nutrient intakes when people take specified beverages out of their diet, and replace them with formulated beverages. The food groups substituted were cordials (excluding those made up from powder), carbonated drinks, fruit juices, fruit juice drinks, sports drinks and bottled water. For Scenario 2 it is assumed that people drink the same amount of the formulated beverages as all of the beverages specified they replace, and do not follow any recommended serve size that may be specified on the label of formulated beverages.

In order to determine if the level of intake of the nutrients is likely to be a public health and safety concern, the estimated dietary intakes were compared to a UL where one was set.

Various age groups were assessed, depending on the UL set for a particular nutrient. The 1997 New Zealand NNS only included respondents aged 15 years and above. The raw data from the 2002 New Zealand National Children's Nutrition Survey are not in DIAMOND to allow nutrient intakes to be calculated for Scenario 2. However, the publication from the children's survey summarising the results provided baseline nutrient intakes for age groups between 5 and 14 years (Ministry of Health, 2003). The results from this publication have been included for nutrients where available.

Estimated intakes of nutrients and the percentage of the relevant ULs for baseline and Scenario 2 are shown below.

Vitamins and Minerals

Vitamin A

Hazard identification and characterisation

Chemistry

The term *vitamin A* describes a group of lipid soluble compounds related metabolically to all-trans-retinol. In the diet vitamin A is found in products of animal origin, as retinyl esters. The retinol esters, together with their metabolites, and synthetic derivatives that exhibit the same properties, are called retinoids. Some carotenoids can be cleaved into retinol, via an enzymatic process, which occurs mainly in the small intestine, and is readily saturated. The toxicity of carotenoids differ from that of retinoids, and the risk of high intakes of carotenoids are not linked to the adverse effects of retinoids.

Function

Vitamin A is a micronutrient essential to most mammalian species. Vitamin A is essential to the processes of vision, reproduction, embryonic development, morphogenesis, growth and cellular differentiation. With the exception of the visual process, most processes are related to the control of gene expression, with vitamin A metabolites, such as retinoic acid, acting as nuclear receptor-ligands.

Sources of vitamin A

Foods rich in pre-formed vitamin A (retinol, retinyl esters) include dairy products, fortified margarine, liver and fish oils.

Absorption, distribution, metabolism and excretion

Approximately 80% of dietary pre-formed vitamin A is absorbed but this may be reduced if diets are low in fat or individuals are suffering from fat malabsorption syndrome. Aqueous dispersions and emulsions achieve higher plasma levels, at a faster rate, with lower faecal losses, than oily solutions. Dietary retinyl ester is released from food by proteolytic digestion and hydrolysed to retinol in the gut.

The retinol is taken up into enterocytes, undergoes re-esterification and is incorporated into chylomicra, which are released into the circulation via the lymph. Following the breakdown of chylomicra by serum lipases, the retinyl esters are released, taken up by hepatocytes and re-hydrolysed. The resulting retinol is transferred to the stellate (fat storing) cells and stored in the form of long-chain fatty esters. Approximately 90% of the body's vitamin A is stored in the liver this way. The availability of hepatic stores of vitamin A may be decreased if protein status is low.

Plasma retinol is usually maintained under tight homeostatic control and concentrations do not alter significantly unless hepatic stores are severely depleted. If hepatic storage capacity is exceeded, plasma levels of retinyl ester increase, but plasma levels of retinol itself do not. Mobilised retinol is transported in plasma bound to retinol-binding protein and transthyretin. Uptake into extra-hepatic tissues occurs via a receptor-mediated process. Once inside the cell, retinol undergoes a complex series of metabolic oxidations, isomerisations and conjugations, most of which are reversible. Several enzymes are involved in these reactions, including cytochromes P450. Cellular binding proteins direct the reactions. Other intracellular binding proteins facilitate transport of specific vitamin A metabolites, such as retinoic acid, into the nucleus of the cell, where they interact with the retinoid nuclear receptors (RARs and RXRs) and participate in the control of gene expression for differentiation and growth.

Oxidised products are excreted in the urine or conjugated with glucuronic acid and excreted in the urine or bile.

Toxicity

There are substantial data on the adverse effects of high vitamin A intakes. Acute toxicity is characterised by nausea, vomiting, headache, increased cerebrospinal fluid pressure, vertigo, blurred vision, muscular in-coordination, and bulging fontanel in infants.

These are usually transient effects involving single or short-term large doses of greater than or equal to 150,000 μg retinol equivalents (RE)[3] in adults and proportionately less in children.

The clinical picture for chronic hypervitaminosis A is varied and non-specific and may include central nervous system effects, liver abnormalities, bone and skin changes, and other adverse effects. Chronic toxicity is usually associated with ingestion of large doses, greater than or equal to 30,000 μg RE/day for months or years. Both acute and chronic vitamin A toxicity are associated with increased plasma retinyl ester concentrations. For the purpose of deriving an upper limit, three primary adverse effects of chronic vitamin A intake were recognised: 1) reduced bone mineral density, 2) teratogenicity, and 3) hepatoxicity. High β-carotene intake has not been shown to cause hypervitaminosis A. Therefore, only adverse effects of preformed vitamin A or retinol were investigated.

Reduced bone mineral density

Chronic, excessive vitamin A intake has been shown to lead to bone mineral loss in animals, making such a consequence in humans biologically plausible. Most human case reports are not well described and epidemiological studies are inadequate in design. However, some studies provide interpretable evidence relating changes in bone mineral density and risk of hip fracture with variation in dietary intake of preformed vitamin A.

A report (Melhus *et al*., 1998) found that the risk for hip fracture in Swedish women is doubled for retinol intake greater than 1500 μg RE/day as compared to intakes less than 480 μg RE/day. Based on univariate analysis, the relative risk at intakes of 500-1000 μg/day, 1000-1500 μg/day and >1500 μg/day, compared with individuals with intakes 500 μg/day, were 0.93 (0.61-1.41), 1.27 (0.80-2.02) and 1.95 (1.15-2.11) respectively. The intake was from dietary sources and therefore it is possible that the effects detected may have arisen from unrecognised confounding; however the mechanistic data on the actions of retinoic acid on bone metabolism are consistent with the reported relationship. An intake of 1500 μg RE/day is close to the population reference intake (600 μg RE/day for women in Europe) and lower than the actual intakes for a substantial proportion of the population.

A similar dose response relationship was reported (Feskanich *et al*., 2002) in data from a large cohort of women in the US, studied over a period of 18 years. The cohort was divided into quintiles for total vitamin A intake (<1250, 1250-1699, 1700-2249. 2250-2999, >3000 μg RE daily) and also for retinol intake (<500, 500-849. 850-1299. 1300-1999, >2000). Significant trends were apparent between relative risk and the intakes from food and supplements of total vitamin A and also retinol. A significant increase in relative risk was reported using a multivariate analysis for the two highest quintiles of retinol intakes (1300-1999, >2000 μg RE/day) compared with the lowest quintile (<500 μg RE/day). The trend analyses for retinol from food and supplements ($P \leq 0.001$) compared with food only ($P=0.05$) indicates an important contribution from supplements and this would be less likely to be affected by dietary confounding than the data from the study of Melhus et al. (1998).

[3] Vitamin A can be expressed on a weight basis as Retinol Equivalents (1 μg RE = 1 μg retinol). This takes into account the vitamin A potency of various esters.

Both of these major epidemiology studies indicate an increased risk of bone fracture over an intake range similar to that normally consumed from food and supplements. The findings on bone density and the risk of fracture were reported at lower daily intakes than other adverse effects. However, the currently available data are not considered to provide sufficient evidence of causality, and are not appropriate for establishing an UL according the US and EU. Furthermore, these adverse effects would only be relevant to elderly people, which are not the target group for formulated beverages.

Teratogenicity

The teratogenic effects of retinoic acids, the active oxidised metabolites of vitamin A, have been known for a long time and documented both in animals and in humans. Children exposed *in utero* to isotretinoin (13CRA) exhibit a pattern of congenital malformations, known as 'the retinoic acid syndrome', which include defects of the craniofacies (small or absent external ears and auditory canals, cleft palate, micrognathia, low set ears, of the central nervous system (micro- or anopthalmia, cerebellar or cortical defects, microcephaly), of the thymus and of the cardiovascular system (transposition of the heart vessels, aortic arch hypoplasia, ventricular septal defects). The incidence of these defects was 25 times higher in the exposed children, and was greater when neuropsychological dysfunctions were assessed. Most of these anatomical defects appear to be associated with alterations in the migration of cells from the neural crest. The gestational period at which exposure occurred is of critical importance in the generation of these effects. In humans the critical period seems to be between the second and the fifth week of pregnancy, although it is generally stated that caution should be taken from the very beginning and up to the 60th day of pregnancy. Some animal studies indicate that a high vitamin A dose would have a similar teratogenic potential whether there was adequate storage levels of vitamin A in the liver or whether there was vitamin A deficiency.

No association has been found in the majority of case-control studies between daily doses of vitamin A of 3000 μg retinoid equivalents (RE) or less and foetal malformation.

A prospective study (Rothman *et al*., 1995) was large enough to stratify the population according to the vitamin A intake. Moreover, the origin of the vitamin A intake (supplement or food) was available for all subjects. The authors found that for women taking more than 4500 μg RE of total vitamin A per day (from food and supplement) there was a 3.5 times higher prevalence of children born with cranial-neural-crest defects, compared to children of mothers ingesting less than 1500 μg RE/day.

When the analysis was restricted to the supplemental intake of vitamin A only, the prevalence of children with defects was 4.8 times higher for mothers ingesting more than 3000 μg RE/day than for those ingesting 1500 μg RE/day. The authors fitted a regression curve to their data, which indicated a rise in the ratio of prevalence of birth defects associated to the cranial-neural crest at doses greater than 3000 μg RE/day of vitamin A (food and supplement). The conclusions of the study remained the same when several potential confounding factors were considered.

An uncertainty factor is not considered necessary because this analysis is quite conservative and because the data from other studies indicated that the true threshold for an effect could be higher than this value.

Based on these studies an UL of 3000 μg RE/day was accepted by both the EU (European Commission Health & Consumer Protection Directorate-General, 2002c) and US (US Institute of Medicine, 2001b).

Hepatotoxicity

In humans, the available data clearly suggest that the occurrence of symptoms of hepatotoxicity depends both on the vitamin A dose taken on a regular bases, and on the duration of this intake. The most extensive report, included 41 cases, but reliable intake information was available on only 29 patients who had a mean daily intake of 28,770 μg RE (range, 6,000-120,000 μg RE). The duration on high intake averaged 7.17 ± 1.21 years (range 0.2-15 years). Interestingly, these authors reported that the most severely affected subjects, i.e. those with cirrhosis (n=13) had consumed significantly more vitamin A, both daily and in total, than the patients without cirrhosis. The lowest continuous daily consumption in patients with cirrhosis was 7500 μg RE/day taken over 6 years. A similar case (7500 μg RE/day for 6 years) has been reported more recently in which progressive liver failure led to death of the patient. Cases of hepatotoxicity have not been reported below 7500 μg RE/day, and it can be hypothesised that this value might be the upper threshold of the storage capabilities of the liver. It is not known if a dose lower than 7500 μg RE/day could induce hepatotoxicity if taken for more than 6 years, but such low intakes may not have been considered by physicians when they attempted to identify the cause of their patient's liver disease.

Differential sensitivity to vitamin A-induced hepatotoxicity has been considered by several authors. On a weight basis, it does not seem that children (more than one year old) are more sensitive than adults. In elderly people (64-88 years old) plasma retinyl esters and retinol values were correlated to their supplemental vitamin A intakes (up to 14,100 μg RE/day for 5 years), but not to liver function tests.

Evaluation

Vitamin A	**UL in adults, μg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2001b)	3000	Total diet	teratology	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	1500	Total diet	Bone fracture and teratology	human
EU (European Commission Health & Consumer Protection Directorate-General, 2002c)	3000	Total diet	teratology and hepatotoxicity	human

* Guidance level; the UK did not derive an UL, because of uncertainty regarding the effects on incidence of bone fracture at low levels and the potential teratogenic effects, both of which may occur with the known dietary intakes of vitamin A.

Teratogenicity is considered a relevant end point, because of the severe and irreversible nature of this form of toxicity. Based on the available studies an UL of 3000 μg RE/day is considered appropriate.

Although teratogenicity is only relevant to women of childbearing age, the UL of 3000 μg/day is appropriate for men, and for infants and children after correction for differences in body weight, because hepatoxicity was observed at 7500 μg/day. Using an uncertainty factor of 2.5 would result in an UL of 3000 μg/day. This UL does not apply for pro-vitamin A forms.

Based on the data from the US and EU evaluation, the ULs for **Vitamin A** for all age categories are:

1-3 years	**600 μg RE/day**
4-8 years	**900 μg RE/day**
9-13 years	**1700 μg RE/day**
14-18 years	**2800 μg RE/day**
Adult	**3000 μg RE/day**

Dietary intake

Estimated intakes of vitamin A have been calculated for retinol (Table 4) and for beta-carotene (Table 5) at baseline and for Scenario 2.

The requested concentration of vitamin A in a 600 ml reference quantity is 187.5 μg of retinol equivalents.

Estimated intakes for retinol were able to be adjusted for the majority of the population groups assessed apart from teenagers 14-18 years for Australia and 19 years and above for both Australia and New Zealand. Where second day adjustments could be made in DIAMOND, these were presented as the estimated intakes, as they provide a better indication of longer term nutrient intakes. Where second day adjustments could not be made, this was due to limited sample numbers in certain age groups and the distribution of intakes which meant the calculations could not be made. The estimated intakes for retinol for the population groups with unadjusted intakes will be higher than those for similar age groups that have adjusted intakes at the 95th percentile. The estimated intakes for younger children in New Zealand 5-14 years taken from the summary report are adjusted based on second day data (Ministry of Health, 2003).

Assuming formulated beverages were consumed, intakes of retinol increased around 100 μg/day (around 10-20% of the UL) from baseline across all age groups assessed.

All estimated mean intakes of retinol were below the ULs at baseline and assuming retinol is consumed in formulated beverages (Scenario 2). At the 95th percentile intake of retinol, only the estimated intakes for Australian children aged 2-3 years at baseline and from 2-8 years at scenario 2 exceeded the UL.

Table 4: Estimated dietary intakes of retinol, before and after formulated beverages are introduced into the diet, and percent of the UL

	Mean intake µg/day (%UL)		95th percentile intake µg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	423 (70)	506 (85)	674 (110)	757 (130)
4-8 years, Aus	430 (50)	528 (60)	693 (75)	808 (90)
5-6 years, NZ	^286 (**)	NA	#433 (**)	NA
7-10 years, NZ	^326 (**)	NA	#443 (**)	NA
9-13 years, Aus	607 (35)	738 (45)	741 (45)	870 (50)
11-14 years, NZ	^368 (**)	NA	#509 (**)	NA
14-18 years, Aus†	611 (20)	777 (30)	1466 (50)	1766 (65)
15-18 years, NZ	446 (15)	562 (20)	578 (20)	716 (25)
≥19 years, Aus†	579 (20)	652 (20)	1142 (40)	1292 (45)
≥19 years, NZ†	522 (15)	569 (20)	940 (30)	1047 (35)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.
† Not adjusted for second day nutrient intakes.

Risk characterisation

The dietary modelling predicts that at the 95th percentile of intake children aged 2-3 years will exceed their UL if retinol is added to formulated beverages at a level of 187.5 µg/600 ml serve (130% UL for 2-3 year olds). The adverse effect on which the UL for this age group was based was hepatoxicity. The LOAEL in adults for this adverse effect was 7500 µg/day for a period of 6 years. When this level is expressed on a body weight basis this would be approximately 1400 µg/day for 2-3 year olds. While the estimated intake is below this calculated LOAEL, uncertainty exists as to whether children might be more susceptible to hepatoxicity following high retinol intake. Furthermore, as the LOAEL represents an actual level measured in adults, uncertainty exists as to whether, and if so at what level, hepatoxicity could occur at levels below this measured LOAEL. An uncertainty factor of 2.5 was therefore applied, since there are no adverse effects reported at this level. It is not clear whether an uncertainty factor of 2.5 is sufficient in the case of children who may be more susceptible to hepatotoxicity following high retinol intake.

It could be assumed that children above the age of 3 years could sustain these high intakes of retinol for up to 6 years which may put them at increased risk of hepatoxicity. This assumption is based on the 95th percentile for the 4-8 years age group which is estimated to be 90% of the UL when formulated beverages are consumed, meaning 5% of this population group have higher intakes.

In conclusion, there are potential safety concerns for children up to the age of 3 years, and maybe up to 6 years, with the addition of retinol to formulated beverages at a level of 187.5 μg in a 600 ml serve. For all other age groups and life-stages, there is no appreciable risk posed by excess intake of retinol.

β-Carotene

Hazard identification and characterisation

Chemistry

β-carotene ($C_{40}H_{56}$) is a member of the carotenoids family of isoprenoid compounds, which are characterised by their polyunsaturated nature and antioxidant properties. The compound can exist in different geometrical forms (as cis- or trans- isomers); the majority of naturally occurring β-carotene, as well as virtually all of the compound prepared by chemical synthesis, is the all-trans isomer.

Function

Some dietary carotenoids serve as an important source of vitamin A, which is the major known function of carotenoids in humans. Its importance in any individual depends upon the level of preformed vitamin A in the diet.

Sources of carotenoids

β-carotene is synthesised in plants and microorganisms, but not in animals. The main food sources of β-carotene are yellow and green (leafy) vegetables and yellow fruits. Commercially available β-carotene is either synthetic or derived from palm oil, algae or fungi, and is widely used as a yellow colouring agent in foods and drinks.

Absorption, distribution, metabolism and excretion

Dietary fat and bile salts facilitate absorption in the upper small intestine, which occurs via incorporation into multilamellar lipid micelles. It has been estimated that, in humans, between 10 and 90% of the total β-carotene consumed in the diet is absorbed, with absorption decreasing as intake increases. Availability from food products is lower than that of a water-dispersed formulation, due to the need for disruption (by pepsin and proteolytic enzymes and by cooking), of the matrix of fibre, polysaccharide and protein. Bioavailability is reduced in very low fat diets.

A proportion of absorbed β-carotene is converted to retinol within intestinal mucosal cells. Unaltered β-carotene is transported via the lymph to the plasma where it is associated with lipoproteins. Tissue uptake and distribution are not well characterised. In the case of regular high intake, long-term accumulation occurs preferentially in adipose tissues. Serum levels of β-carotene have been reported to be low in smokers, in individuals with a high alcohol intake, and in those with HIV infection. Low β-carotene status may be associated with conditions of impaired lipid absorption such as jaundice, liver cirrhosis and cystic fibrosis. β-Carotene is mainly converted to retinol (vitamin A) in the cytosol of intestinal mucosal cells.

Experiments in rats have shown that the levels of β-carotene and of preformed vitamin A regulate the process. *In vitro* studies have shown that other β-carotene derivatives may also occur, but their biological activity, and whether they are synthesised *in vivo*, is unknown.

Carotenoid absorption and metabolism vary considerably between animal species. No single species provides a good model for studying all aspects of the biokinetics and metabolism of β-carotene in humans. The rat is particularly unsuitable, due to the high efficiency of conversion to vitamin A, such that significant levels of unaltered β-carotene are absorbed only when very high doses are given, for prolonged periods of time. The pre-ruminant calf, the ferret and the Mongolian gerbil are suggested to be more useful models, although it is apparent that there are many differences in carotenoids absorption, distribution and metabolism between these animals and humans.

Absorbed β-carotene is secreted into the bile and excreted in the faeces. It is also excreted in the sweat.

Toxicity

Animal studies

In animal studies, no adverse effects of high-dose oral β-carotene supplementation have been observed in several standard toxicological studies in various experimental animals (rat, mice, rabbits). These studies included acute studies up to 5000 mg/kg bw, chronic toxicity / carcinogenicity studies up to 1000 mg/kg bw/day for life in rats or mice, teratogenicity and reproductive toxicity studies. β-carotene shows no genotoxicity *in vitro* or at high doses *in vivo* and was not carcinogenic in experimental rodent studies.

However, β-carotene supplementation for 6 months (2.4 mg/kg bw/day, with or without exposure of the animals to cigarette smoke) was associated with the development of squamous cell metaplasia in the lungs of ferrets. The assessed histopathological endpoint, squamous metaplasia, may not be directly related to carcinogenesis, but this study did reveal interestingly related molecular/biochemical changes in the lungs of the animals tested.

Human studies

In humans, doses of 20-180 mg/day β-carotene have been used to treat patients with erythropoietic photoporphyria, with no evidence of toxicity and without the development of abnormally elevated blood vitamin A.

Hypercarotenaemia (high levels of β-carotene in the blood) is generally considered to be a benign condition. It is often related to unusually high intake of carotene-rich foods. Hypercarotenodermia (yellowing of the skin, particularly the palms, soles of the feet, chin, behind the ears, over the knuckles and on the abdomen and buttocks) is a physical manifestation of β-carotene excess, which is caused by accumulation of the substance in fatty tissues, particularly subcutaneous fat. Although β-carotene is a precursor of vitamin A, excess intake has not been associated with vitamin A toxicity in humans, possibly because the conversion is tightly controlled.

The promise shown by β-carotene and other putative biological antioxidants as prospective agents for cancer prevention led to the instigation of numerous small scale supplementation studies and, of particular importance, a small number of large-scale, primary prevention trials in humans, involving supplementation with β-carotene, alone or in combination with other vitamins and/or minerals.

Some major prevention studies (Greenberg *et al*., 1990; McLarty, 1992; Blot *et al*., 1993; Li *et al*., 1993; Greenberg *et al*., 1994) did not show any adverse effects on increased tumours, but this might have been through their design.

The Alpha-Tocopherol/Beta-Carotene trial in Finland (ATBC study group, 1994) involved 29,133 male smokers (age 50-59) with a smoking history averaging one pack/day for 36 years. The 2x2 factorial design evaluated 20 mg β-carotene and/or 50 IU alpha-tocopherol (vitamin E) daily for 6.5 years. These doses represent a 10-fold and 5-fold excess over the median intake of β-carotene and α-tocopherol, respectively, in this population. After 2 years of treatment, median serum β-carotene levels had increased 17.5-fold in the β-carotene treatment groups. Participants receiving β-carotene alone or in combination, had significantly higher lung cancer incidence (Relative Risk (RR) 1.18; 95% Confidence interval (CI) 1.03-1.36) and higher mortality (RR 1.08; CI 1.01-1.16) than subjects receiving placebo. The excess lung cancer incidence was not apparent in the initial 18 months, but the incidence curves significantly diverged thereafter. Subsequent subgroup analysis (see (Albanes *et al*., 1996) revealed a higher risk in heavy smokers (20 or more cigarettes/day) (RR 1.25, CI 1.07-1.46) than in light smokers (5-19 cigarettes/day) (RR 0.97, CI 0.76-1.23). Associations with alcohol intake and with non-small-cell histology were also noted. The risk was confined to the heavier drinkers (more than 11 g ethanol per day).

Interestingly, in agreement with earlier observational studies, both dietary intake and serum β-carotene levels at baseline (before treatment) were found to be inversely related to risk of lung cancer during the trial (Albanes *et al.,* 1996).

The β-Carotene And Retinol Efficacy Trial (CARET) study ((Omenn *et al*., 1996b), see also (Omenn *et al*., 1996a; Omenn, 1998) successfully randomised 18,314 participants in the USA. 30 mg β-carotene and 25,000 IU vitamin A (retinyl palmitate) were administered daily to 14,254 smokers and former smokers (45% female) aged 50-59 at enrolment, and to 4,060 asbestos-exposed males (age 45-74). After five years of study the median serum β-carotene levels in the active treatment group was increased by 12-fold (170 ng/ml *versus* 2100 ng/ml). A total of 388 new cases of lung cancer were diagnosed during the 73,135 person-years of follow- up (mean 4.0 years). The active treatment group had a RR of lung cancer of 1.28 (CI 1.04-1.57), compared with the placebo group. The differences (significant from 24 months of treatment onwards) were greater as the intervention progressed. There were no statistically significant differences in the risks of other types of cancers. In the active group the RR of death from any cause was 1.17, of death from lung cancer, 1.46, and of death from cardiovascular disease, 1.26. As in a further analysis from ATBC published in the same issue (Albanes *et al.,* 1996), there was an association (less clear trend than in ATBC study) of the excess lung cancer incidence between treatment groups with the highest quartile of alcohol intake, but no association with baseline serum β-carotene concentrations.

In the CARET study it is not possible to distinguish the β-carotene effects from those of the vitamin A, since the two compounds were administered in combination.

The Physicians Health Study was to test the effect of aspirin on cardiovascular disease incidence (Steering Committee of the Physicians' Health Study Research Group, 1989). β-carotene was added in a 2x2 design, using 50 mg β-carotene on alternate days. 22,071 male physicians were followed for a mean of 12.5 years. Those assigned to receive β-carotene had significantly higher serum concentrations than those given placebo (2240 nmol/l vs. 560 nmol/l) (4-fold). It has to be noted that this increase is lower compared with that obtained in the two previously considered trials, a situation that could be related to higher basal levels in the PHYS population and/or to a lower bioavailability of β-carotene compared with the other trials. In this healthy population, with 50% never-smokers and only 11% current smokers, 170 lung cancers were accumulated over the follow up period. The relative risks were 1.02 (CI 0.93-1.11) for overall mortality, 0.98 (CI 0.91-1.06) for all malignant neoplasms, and 0.93 for lung cancer.

In summary there was no effect of ß-carotene supplementation on total cancer, on total mortality, or on heart disease. Neither was an effect on lung cancer observed, but due to the lower number of cases, the power of the statistical analysis underlying this conclusion is rather weak.

Mechanisms

In light of the adverse findings in human intervention trials, in which β-carotene supplementation was associated with a promotional effect on lung tumourigenesis in smokers, studies in animals have been carried out to elucidate potential mechanisms by which these effects may have occurred. The EU has proposed three mechanisms in the evaluation, which are related to effects in the same target tissue, the lungs, where the adverse effects have been observed in humans. The first mechanism proposes that β-carotene has a co-carcinogenic effect through a P450 enzyme related activities. The second mechanism proposes altered retinoid signalling: a mechanism to enhance lung tumourigenesis after high doses of β-carotene supplementation in smokers. The last mechanism proposes a pro-oxidant activity of β-carotene at high levels.

Dose response assessment

No dose-response relationship for β-carotene effects is available from the intervention trials in humans, as single doses were used in each study, and the conditions were different across studies.

The study in ferrets also used a single daily dose. Further studies in ferrets using a range of different β-carotene doses and a wider range of selected parameters would be appropriate to assist in future toxicological evaluation.

It can be presumed that the effects of β-carotene are dependent on the specific source of exposure, and that differences will not be unexpected with different matrices or different formulations containing β-carotene, depending on the composition of accompanying antioxidants and of other components, and also depending on the relative proportion of isomers of β-carotene.

Evaluation

β-carotene	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2000c)	no UL	suppl	Insufficient data	human
UK (UK Expert Group on Vitamins and Minerals, 2003)	7	suppl	Lung tumours in smokers	human
EU (European Commission Health & Consumer Protection Directorate-General, 2000b)	no UL	suppl	Insufficient data	human

β-Carotene is of low toxicity in both animals and man, and prior to the publication of a number of intervention studies was thought to be without adverse effect, other than a yellowing of the skin, which occurred after sustained high intake. However, supplementation of smokers and subjects previously exposed to asbestos has been associated with an increased risk of lung cancer.

The US and EU stated that the existing evidence from human trials indicated that supplemental β-carotene (20 mg/day or more) is contraindicated for use in current, heavy smokers. However, there is insufficient scientific basis to set a precise figure for an UL of isolated β-carotene, as no dose-response relationship for β-carotene effects is available either from the intervention trials in humans or from appropriate animal models. Moreover, it is not possible to be more specific in distinguishing different isomeric forms of β-carotene or specific formulations.

The UK has set an UL. There is no evidence that β-carotene supplementation has any effect on non-smokers. As a matter of prudence the UK has set an UL for supplementation based on the ATBC-study. The LOAEL from this study was 20 mg/day. Applying an uncertainty factor of 3, to extrapolate from a LOAEL to a NOAEL, results in a UL for supplementation of 7 mg/day. This UL applies to supplements only, as there is no evidence to suggest that current levels of β-carotene intake from food result in adverse effects.

Based on the data considered in the US, EU, and UK evaluation, there is **insufficient evidence to establish an UL for β-carotene for supplemental use.** However**, an UL for β-carotene from food or food additives does not need to be established**, based on no indication of adverse effects. Furthermore, 7 mg β-carotene per day is a conservative estimate for a guidance level for supplemental use.

Dietary intake

Dietary modelling has been conducted for all forms of β-carotene in food only, without making a distinction between natural β-carotene and β-carotene added to food as food additives or from food fortification. Dietary modelling results are shown in Table 5.

The concentration of β-carotene requested to be added to formulated beverages by the Applicant was in the form of retinol equivalents (RE). For dietary modelling purposes, estimated intakes of β-carotene were expressed as micrograms per day (Table 5).

Therefore, the requested concentrations of β-carotene in the formulated beverages had to be converted to a concentration in micrograms for dietary modelling purposes. The conversion was made using a factor of 12, (as per the Food Standards Code). Therefore, the requested concentration of vitamin A to be added to formulated beverages was 187.5 µg RE/600 ml, or 31.3 µg/100g, resulting in a β-carotene concentration used in the dietary modelling of 376 µg/100g.

Estimated intakes for β-carotene were adjusted using second day data from the NNSs.

Estimated intakes of β-carotene increased with the consumption of formulated beverages, between 30 and 100 µg/day from baseline intakes, depending on the population group assessed. There was no UL for β-carotene to compare the estimated intakes.

Risk characterisation

The intake level from all food sources is much lower than the level at which increased lung tumours in smokers were observed in prevention studies when β-carotene supplementation was given.

In conclusion, the addition of β-carotene to formulated beverages at a level of 187.5 µg retinol equivalents per 600 ml serve poses no appreciable public health and safety risk.

Table 5: Estimated dietary intakes of beta carotene, before and after formulated beverages are introduced into the diet

	Mean intake µg/day		95th percentile intake µg/day	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	1902	1934	4684	4725
4-8 years, Aus	2030	2077	5267	5282
5-6 years, NZ	^1488	NA	#2508	NA
7-10 years, NZ	^1848	NA	#3612	NA
9-13 years, Aus	2559	2627	4510	4689
11-14 years, NZ	^1992	NA	#3204	NA
14-18 years, Aus	3119	3211	5158	5338
15-18 years, NZ	2882	2982	4080	4345
≥19 years, Aus	3548	4432	6548	7821
≥19 years, NZ	3544	3591	6508	6562

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
N/A = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Thiamin

Hazard identification and characterisation

Chemistry

Thiamin (vitamin B_1) is a relatively heat- and acid-stable, water-soluble compound, containing a pyrimidine and a thiazole nucleus linked by a methylene bridge. Derivatives of thiamin include the mono-, pyro- and triphosphate forms and the synthetic hydrochloride and slightly less water-soluble mononitrate salt. Synthetic non water-soluble derivatives of thiamin are available but these are not used in food supplements.

Function

Thiamin pyrophosphate (TPP) is a co-enzyme in several enzymatic reactions. TPP may also have a non-co-enzymic function during stimulation of neuronal cells and other excitable tissues, such as skeletal muscle.

Sources of thiamin

Foods providing rich sources of thiamin include unrefined grain products, meat products, vegetables, dairy products, legumes, fruits and eggs. In Australia, but not in New Zealand, there is mandatory fortification of flour used for making bread. Bread flour must contain no less than 6.4 mg/kg of thiamin (Standard 2.1.1 – Cereals and Cereal Products).

Absorption, distribution, metabolism and excretion

Thiamin present in food is efficiently absorbed. However, water-soluble supplements, such as thiamin hydrochloride and thiamin mononitrate, are poorly absorbed due to saturation of transport mechanisms. At physiological concentrations, intestinal uptake occurs mainly via a carrier-mediated transport mechanism. However, this process is saturable and at higher concentrations, uptake is predominately by slower passive diffusion.

In the blood and tissues, thiamin is present as the free form and mon-, di- (pyro) and triphosphorylated forms, which are interconvertible. Free and phosphorylated forms are transported within the erythrocytes, but plasma and cerebrospinal fluid contain only the free and monophosphorylated forms. Within the tissues most thiamin present is converted to the pyrophosphate form. Liver contains the highest concentration of thiamin. Catabolic metabolism amounts to approximately 1 mg/day, and most of this occurs in the liver.

Thiamin metabolites and thiamin in excess of requirements are excreted in the urine. The level of unchanged thiamin in the urine increases as intake increases.

Toxicity

In humans, orally ingested thiamin has a long history of use as an oral supplement for the treatment or prophylaxis of thiamin deficiencies without reported adverse effects. Due to its therapeutic action in some frequently observed clinical syndromes (such as chronic alcoholism), thiamin hydrochloride has been advised and used over a long period of time. There are no reports of adverse effects of oral thiamine, even at dosages of several hundred milligrams a day.

After parenteral administration, a small number of individuals may show an allergic response to lower doses, but reports of these lower dose-related events are rare.

The animal database is also very limited. The oral LD_{50} in mice is 3-15 mg/kg bw. A lethal dose of thiamin in rodents is preceded by CNS effects such as shock, muscle tremor, convulsions, respiratory disturbance and collapse, symptoms that are similar to acute thiamin toxicity in humans.

Due to the lack of oral dose-response studies, no LOAEL and NOAEL can be established in both human and animal studies.

Evaluation

Thiamin	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	N/A	food	no adverse effects	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	100	supplemental	no specific effect	human
EU (European Commission Health & Consumer Protection Directorate-General, 2001c)	N/A	total diet	no adverse effect	human

N/A not applicable
* Guidance level, for water-soluble forms of thiamine only

Based on the data from the US and EU evaluations, it can be concluded that orally ingested thiamin has a very low toxicity. This may be because at intake levels higher than 5 mg absorption rapidly declines and, because absorbed thiamin is actively excreted in the urine.

Based on the data considered in the US and EU evaluation, **thiamin** has a very low oral toxicity, and therefore an **UL does not need to be established**.

Dietary Intake

No dietary intake estimates were calculated for thiamin, as it was determined to have very low oral toxicity, and no ULs have been established, as outlined above.

Risk characterisation

No UL has been established for thiamin, based on the lack of adverse effects even at high doses. Therefore, no dietary modelling was required.

In conclusion, the addition of thiamin to formulated beverages at a level of 0.275 mg per 600 ml serve poses no appreciable public health and safety risk.

Riboflavin

Hazard identification and characterisation

Chemistry

Riboflavin is a water-soluble vitamin of the B group (vitamin B_2). It is stable to mineral acids in the dark at 27°C. Decomposition occurs in both acidic and alkaline solutions.

Function

Clinically, riboflavin promotes normal growth and assists in the synthesis of steroids, red blood cells, and glycogen. Flavin adenine dinucleotide (FAD) also play a role in oxidation-reduction reactions, interacting with a group of enzymes known as flavoproteins. Riboflavin helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system. It supports the activity of antioxidants and is involved in the production of adrenaline by the adrenal glands. It is thought that riboflavin also aids the body in absorbing iron, since it is common for iron deficiency to accompany a deficiency in riboflavin.

Sources of riboflavin

Riboflavin is widely distributed in foodstuffs and all plant and animal cells contain it, but there are very few rich sources. Only yeast and liver contain more than 2 mg/100 g. Other good sources are milk, egg white, fish roe kidney and leafy vegetables.

Absorption, distribution, metabolism and excretion

Riboflavin is readily absorbed from the small intestine, primarily by a specialised transport mechanism involving phosphorylation of the vitamin to flavin mononucleotide (FMN). Passive diffusion plays only a minor role at levels ingested in the diet. Riboflavin has been shown to undergo active secretion into, and saturable reabsorption from, the kidney tubules in rat, dog and human.

Riboflavin is distributed to all tissues. It is present in red blood cells, and appears to bind to a subfraction of immunoglobulins in plasma. Very little riboflavin is stored. Free riboflavin is transformed in the liver to form flavin coenzymes, (FAD and FMN), which are utilised as electron transfer factors in enzymatic reductions.

When riboflavin is ingested in amounts approximately equivalent to the minimal daily requirement, only about 10-20% appears in the urine. As the intake is increased above minimal requirements, larger proportions are excreted unchanged. Riboflavin is also found in faeces, sometimes in quantities exceeding that ingested. This probably represents the riboflavin synthesised by intestinal microorganisms, which is not absorbed.

Toxicity

In animals oral riboflavin administration is of low toxicity, which can probably be explained by the limited capacity of the intestinal absorption mechanism.

Some evidence of adverse effects associated with the group of flavins is based on *in vitro* studies showing involvement in the formation of active oxygen species and in the axonal degeneration on intense exposure to ultraviolet and visible light.

The absorption of riboflavin reduces as the level of administration increases to high-level doses. Data on adverse effects from high oral riboflavin intake are insufficient to establish an UL. Given the lack of any demonstrated functional disorders or adverse structural effects in humans following excessive oral riboflavin intake and considering the reduced intestinal absorption following high dose exposure, the relevance of the mild effects shown in *in vitro* studies to human health is questionable.

Available data from 3-month human studies and from pharmacokinetics studies do not show adverse effects after oral administration. The minor gastrointestinal disorders, in some individuals are not clearly related to the riboflavin intake.

Evaluation

Riboflavin	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	N/A	food	no adverse effects	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	40	Suppl	no specific effect	human
EU(European Commission Health & Consumer Protection Directorate-General, 2000g)	N/A	total diet	no adverse effects	human

N/A not applicable
* Guidance level,

Although limited, none of the available studies has reported significant adverse effects in humans following excess riboflavin consumption from food or supplements. Therefore, based on the present database it is not possible to derive an UL for riboflavin. The limited evidence available from clinical studies indicates that current levels of intake of riboflavin from all sources do not represent a risk to human health.

Based on the data considered in the US and EU evaluations, **riboflavin** has a very low toxicity and therefore **an UL does not need to be established**.

Dietary Intake

No dietary intake estimates were calculated for riboflavin, as it was determined to have very low toxicity, and no ULs have been established, as outlined above.

Risk characterisation

No UL has been established for riboflavin, based on the lack of adverse effects even at high doses. Therefore, no dietary modelling was required.

In conclusion, the addition of riboflavin to formulated beverages at a level of 0.425 mg per 600 ml serve poses no appreciable public health and safety risk.

Niacin

Hazard identification and characterisation

Chemistry

Niacin (vitamin B_3) is the generic term for nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (nicotinic acid amide), and the coenzyme forms of the vitamin. Nicotinamide is the active form, which functions as a constituent of two coenzymes, namely, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These coenzymes in their reduced states (NADH/NADPH) are the principal forms of niacin that exist in animal tissues.

Function

Niacin is not strictly speaking a vitamin because it is formed from the metabolism of tryptophan, and is not *per se* essential to the body, providing that there is an adequate supply of the essential amino acid tryptophan. In the form of the coenzymes NAD and NADP, niacin functions in many biological redox reactions.

Sources of niacin

Niacin is present in food largely as bound forms that require hydrolysis to release the free nicotinamide or nicotinic acid prior to absorption. In animal tissues niacin is present mainly as the coenzymes NAD and NADP.

Absorption, distribution, metabolism and excretion

In humans, niacin is rapidly absorbed from the stomach and intestine by a sodium carrier-mediated mechanism at low concentrations.

Niacin circulates in the plasma in the unbound form as both the acid and the amide. Each enters peripheral tissues by passive diffusion, followed by metabolic trapping by conversion to the pyridine dinucleotides, NAD(H) and NADP(H). Most is found as NAD(H) and the oxidised form NAD. The plasma half-life of nicotinic acid is relatively short, approximately one hour. Animal studies have shown that nicotinic acid rapidly disappears from the blood and is mainly concentrated in the liver, but also in adipose tissue and in the kidneys. The main metabolites in humans are N-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-5-carboxamide.

The pattern of niacin products excreted after ingestion of the vitamin depends largely on the amount and form of niacin ingested and on the niacin status of the individual. However, the two major excretion products in humans are N-methylnicotinamide and N-methyl-2-pyridone-5-carboxamide, with minor amounts of the unchanged vitamin, nicotinamide-N-oxide and 6-hydroxynicotinamide also being excreted.

Toxicity

The principal identification of hazards associated with excessive intakes of niacin have arisen from studies in which high doses of nicotinic acid have been used for its therapeutic effects in lowering blood cholesterol and blood hyperlipidaemias. A number of hazards have been reported to be associated with high doses of nicotinic acid. In addition, nicotinamide has been investigated as a method for reducing the risk of development of diabetes.

The toxicity of nicotinic acid and nicotinamide are discussed separately.

Nicotinic acid

Vasodilation is commonly seen in patients given high doses of nicotinic acid for the treatment of hyperlipidaemias. Very large single doses cause hypotension, although tolerance develops to this effect after several days of continued high dose intake. In general, flushing is a mild and transient effect, although in many clinical trials it has resulted in patients withdrawing from treatment. The flushing activity appears to be related to the presence of a carboxyl group on the pyridine nucleus since compounds lacking this function, including nicotinamide, are not associated with facial flushing. Flushing is associated with periods of rapid rises in blood concentrations, and sustained-release formulations were developed for the use of nicotinic acid in the treatment of hypercholesterolaemia, in order to minimise this side-effect. Flushing is produced via prostaglandin D_2 release. Theoretically if flushing occurred in the elderly, it could exacerbate mild postural hypotension, and could increase the risk of falls, which are a common cause of morbidity in the elderly. This risk relates to taking supplements containing nicotinic acid (not nicotinamide), especially if taken on an empty stomach.

At higher intakes of nicotinic acid over long periods of time, liver dysfunction has been reported. Symptoms such as elevated liver enzymes, elevated bilirubin levels and jaundice have been observed. Other adverse effects reported include hyperglycaemia and adverse ophthalmological effects such as blurred vision and cystoid macular oedema.

The more severe forms of toxicity of nicotinic acid occur principally at does of greater than 500 mg/day. The limiting adverse effect at lower dose is flushing, and this has been reported at much lower intakes than the other adverse effects. The most severe and potentially life-threatening adverse effects, such as hepatotoxicity, occur at doses one order of magnitude higher than have been reported for flushing. The dose of free nicotinic acid reported to produce flushing consistently in clinical studies is 50 mg/day. The available data indicated that flushing would be unlikely to occur repeatedly in subjects given less than 50 mg/day, but occasional flushing was reported at a dose of 30 mg of nicotinic acid daily.

Nicotinamide

Nicotinamide does not produce the flushing response that has been used as the basis for the UL for nicotinic acid. There has been only one reported case of hepatoxicity in a patient receiving high-dose nicotinamide (however, nicotinamide has not been subject to extensive clinical trials (at 3 g per day or more) for use as a hypolipidaemic agent).

No significant adverse effects have been reported in trials on the possible benefits of nicotinamide in patients with or at risk of diabetes, where doses up to the equivalent of 3 g/day, for periods up to 3 years, have been used.

The NOAEL from these studies is approximately 25 mg/kg bw/day. This value represents the lowest reported dose in a number of recent trials of high quality, many of which used sensitive biomarkers of hepatic function and glucose homeostasis, and included a range of age groups, with some subjects treated with up to 50 mg/kg bw/day.

Evaluation

Niacin	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	35	Suppl	flushing	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	17 nicotinic acid 500 nicotinamide	Suppl. Suppl.	flushing no adverse effects	human
EU (European Commission Health & Consumer Protection Directorate-General, 2002e)	10 nicotinic acid 900 nicotinamide	Suppl Total	flushing no adverse effects	human

* Guidance level

Nicotinic acid

The US has set an UL for niacin based on flushing for all forms. The US did not add a separate UL for nicotinamide based on the fact that nicotinamide is not associated with flushing. Both the UK and EU separated the effects for nicotinic acid and nicotinamide, because of the differences in adverse effects. The EU has established ULs, while the UK stated that there was insufficient data to establish a UL for both nicotinic acid and nicotinamide. FSANZ considers the EU approach to be the most appropriate.

The EU set a UL for nicotinic acid of 10 mg/day based on the available data indicating occasional flushing at 30 mg/day. An uncertainty factor of 3 was used to allow for the fact that a slight effect was reported, and that the study was performed in a small number of subjects, but taking into account the steep dose-response relationship. This results in an UL that is 300-fold below the dose frequently used clinically for the treatment of hypercholersterolemia (3 g/day) and which is associated with a high incidence of serious adverse reactions. The only reports of flushing associated with the ingestion of nicotinic acid with food have occurred following the addition of free nicotinic acid to food prior to consumption. Although flushing might be considered a minor health effect, it has been used as the basis for setting the UL for nicotinic acid, because of concerns about the possibility of a transient hypotensive episode, especially in the elderly, leading to an increased risk of falls.

The UL of 10 mg/day for free nicotinic acid is not applicable during pregnancy or lactation because of inadequate data relating to this critical life stage. The ULs for intake by children and adolescents have been derived on the basis of their body weights.

In summary, the ULs for **free nicotinic acid** for the various age groups are:

1-3 years	**2 mg/day**
4-8 years	**3 mg/day**
9-13 years	**6 mg/day**
14-18 years	**9 mg/day**
adults	**10 mg/day**

Nicotinamide

For nicotinamide a NOAEL of 1800 mg/day was established based on the absence of adverse effects in recent trials of high quality. An uncertainty factor of 2 has been used to allow for the fact that adults may eliminated nicotinamide more slowly than the study groups, many of which were children, and that data for children would not reflect the full extent of intersubject variability that could occur in an older population. The UL for nicotinamide is established at 900 mg/day for adults.

The UL of 900 for nicotinamide is not applicable during pregnancy or lactation because of inadequate data relating to this critical life stage. The ULs for intake by children and adolescents have been derived on the basis of their body weights

In summary, the ULs for **nicotinamide** for the various age groups are:

1-3 years	**150 mg/day**
4-8 years	**250 mg/day**
9-13 years	**500 mg/day**
14-18 years	**750 mg/day**
adults	**900 mg/day**

Dietary intake

Estimated dietary intakes of niacin were calculated for total dietary niacin from all foods in the diet, as well as for nicotinic acid from formulated beverages only, both expressed as niacin equivalents (NE).

The concentration of nicotinic acid requested to be added to formulated beverages was 2.5 mg NE/600 ml reference quantity.

Estimated intakes of niacin from all foods in the diet were adjusted using second day NNS data.

Standard 1.3.2 – Vitamins and Minerals in the Code currently permits niacin to be added to a small range of foods, including some cereal based products, yeast extracts and legume based products. The concentrations of niacin in foods were those determined for the 1995 Australian and 1997 New Zealand NNSs expressed as NE. From the data it was not possible to distinguish between niacin present as nicotinic acid or nicotinamide. The food name descriptors used in the NNSs did not allow foods that may have been fortified with niacin to be identified.

Estimated intakes of niacin from all dietary sources increased by around 1 mg NE/day for all population groups assessed when formulated beverages are consumed. Estimated intakes of niacin from all foods in the diet were not compared to a UL, as the UL is for free nicotinic acid added to foods.

Table 6: Estimated dietary intakes of total niacin from all foods (as NE), before and after formulated beverages are introduced into the diet

	Mean intake mg NE/day		95th percentile intake mg NE/day	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	23.8	24.6	32.5	33.2
4-8 years, Aus	27.8	28.9	40.6	42.5
5-6 years, NZ	^23.9	NA	#30.3	NA
7-10 years, NZ	^28.0	NA	#38.5	NA
9-13 years, Aus	35.5	36.9	54.7	55.8
11-14 years, NZ	^32.8	NA	#43.2	NA
14-18 years, Aus	41.9	43.7	70.6	72.7
15-18 years, NZ	36.9	37.9	56.7	58.2
≥19 years, Aus	41.3	42.2	68.1	69.2
≥19 years, NZ	35.4	35.9	56.3	57.2

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Intakes of niacin as free nicotinic acid were estimated from added sources only in the diet. Baseline intakes could not be estimated, as the form of niacin in foods reported as consumed in the NNSs could not be determined. Therefore, it was assumed that no foods were fortified with nicotinic acid for the baseline estimate of intake.

For scenario 2 where formulated beverages are consumed in place of other beverages in the diet, estimated intakes (adjusted using second day NNS data) did not exceed the UL for free nicotinic acid for any population group at the estimated mean intake, and only exceeded the UL at the 95th percentile intake for children aged 2 to 8 years.

Table 7: Estimated dietary intakes of nicotinic acid (as NE) from formulated beverages only, after formulated beverages are introduced into the diet, and percent of the UL

	Mean intake mg NE/day (%UL)	95th percentile intake mg NE/day (%UL)
Age group	**Scenario 2***	**Scenario 2***
2-3 years, Aus	1.00 (50)	2.86 (140)
4-8 years, Aus	1.31 (45)	3.17 (110)
5-6 years, NZ	NA	NA
7-10 years, NZ	NA	NA
9-13 years, Aus	1.63 (25)	3.70 (60)
11-14 years, NZ	NA	NA
14-18 years, Aus	1.90 (20)	5.08 (55)
15-18 years, NZ	1.35 (15)	3.62 (40)
≥19 years, Aus	0.97 (10)	3.19 (30)
≥19 years, NZ	0.65 (6)	2.30 (25)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

The hazard characterisation identified that it was appropriate to set different ULs for nicotinic acid and nicotinamide. Both forms are permitted forms for niacin in the Code (Standard 1.1.1 – Preliminary Provisions – Application, Interpretation and General Prohibitions). Therefore two different types of modelling were performed. In the first model, total niacin intake was calculated based on all foods in the diet at baseline and for Scenario 2, without making a distinction between nicotinic acid and nicotinamide. This modelling was performed because the UL for nicotinamide is based on total intake from all foods. The second model assumed at baseline that there are no foods on the market fortified with nicotinic acid since this form is currently not permitted to be added to beverages in the Code (Standard 1.3.2 – Vitamins and Minerals), and the UL for nicotinic acid relates only to the free form of nicotinic acid.

The dietary modelling for total intakes of niacin from the whole diet indicated that if nicotinamide were added as the permitted form to formulated beverages there are no safety concerns, since no adverse effects have been observed at much higher levels of intake (NOAEL was 1800 mg/day vs. intake levels of 30-70 mg/day for various age-groups).

The addition of nicotinamide to formulated beverages at a level of 2.5 mg in a 600 ml serve poses no appreciable risk to public health and safety.

For estimated intakes of niacin from added sources in formulated beverages only, assuming that the permitted form would be nicotinic acid children at the 95th percentile intake, aged 2-8 years exceeded the UL for nicotinic acid (140% UL and 105% UL for age groups 2-3 and 4-8 years, respectively).

The UL for free nicotinic acid was derived from data on flushing, following administration of a single oral dose given in solution added to tomato juice and consumed with a meal. For children the level was based on a body weight basis. Flushing is not reported as being associated with the bound forms of nicotinic acid present in food. Very large single doses of nicotinic acid cause hypotension, although tolerance develops to this effect after several days of continued high dose intake. The adverse effects are considered mild and reversible (flushing) and have been based on the possibility that the flushing detected at higher doses in young subjects could result in transient hypotensive episodes later in life when elderly. Theoretically if flushing occurred in the elderly, it could exacerbate mild postural hypotension, and could increase the risk of falls, which are a common cause of morbidity in the elderly.

The relevance of flushing as an adverse effect in children is however questionable.

The addition of nicotinic acid to formulated beverages at a level of 2.5 mg in a 600 ml serve might pose a small risk for children, resulting in flushing. This particular adverse effect however is considered to be of minor significance for children.

In conclusion, the addition of niacin (all permitted forms) to formulated beverages at a level of 2.5 mg in a 600 ml serve poses no appreciable public health and safety risk.

Folate

Hazard identification and characterisation

Chemistry

Folate is a water-soluble vitamin. The term *folate* is used generically to refer to the various forms of the vitamin, both naturally-occurring and synthetic, and its active derivatives (Department of Health, 2000). Naturally-occurring folate generally contains more than one, typically five to seven, glutamate moieties attached to pteroic acid (polyglutamate). Folic acid (pteroylmonoglutamic acid) is the most common form of synthetic folate and contains a single glutamate moiety attached to pteroic acid. Folic acid is the most stable form of folate and is most often used in vitamin supplements and in fortified foods.

Function

Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine.

Sources of folate

Natural forms of folate are found in a wide variety of foods including green leafy vegetables, cereals, fruits, grains, legumes, yeast extract, and liver. Dietary forms are broken down to monoglutamates during storages processing and cooking. The synthetic pharmaceutical form used for food fortification and in supplements is folic acid, as this compound is more stable in comparison to other forms of the vitamin.

Absorption, distribution, metabolism and excretion

The majority of dietary folate is absorbed within the proximal region of the small intestine by active, carrier-dependent mechanisms, and also by passive diffusion. Polyglutamate forms are first hydrolysed to monoglutamates by conjugase enzymes within the enterocyte brush border. Ingested folic acid is enzymatically reduced and methylated within the intestinal lumen and enterocytes, although ingestion of high concentrations results in the direct appearance of the compound, unmodified, in the plasma.

Naturally-occurring food folate has been found to be only approximately 50% bioavailable. Folic acid supplements taken on an empty stomach have been shown to be 100% bioavailable, while folic acid added to food, or supplements taken with food are approximately 85% bioavailable.

Absorbed folate is excreted into the bile and undergoes enterohepatic circulation and reabsorption. The liver is also the main storage site, containing approximately half of the total body folate. The majority of plasma folate is present as 5-methyl-tetrahydrofolates (THF)-monoglutamate. Within cells, folate is retained in the cytoplasm by polyglutamation. 5-Methyl-THF is not a good substrate for polyglutamation, and must be first converted, via a vitamin B_{12}-dependent reaction, to THF. Alternatively, folic acid can be converted to polyglutamate (i.e. metabolically active) forms via a vitamin B_{12}-independent pathway.

Folate is excreted in the urine, either as the metabolically active form or as breakdown products, and in the faeces.

Toxicity

From the available data it can be concluded that (synthetic) folic acid used in supplements can cause adverse effects at high dose levels, whereas no adverse effects have been reported with the consumption of excess folate from foods.

Folic acid may lead to reversal of the haematological symptoms of vitamin B_{12} deficiency, potentially allowing the neuropathy associated with vitamin B_{12} deficiency to develop untreated. Vitamin B_{12} deficiency is most prevalent in older people. A serious adverse effect known in humans is the potential of progression of the neurological symptoms associated with vitamin B_{12} deficiency. Masking of the vitamin B_{12} deficiency in PA patients occurs with high frequencies and consistently with daily intakes of 5 mg; however, insufficient data are available for evaluation of dose levels between 1-5 mg. Both the US and EU considered the level of 5 mg per day as the LOEL.

Folic acid is generally considered safe when used therapeutically. Adverse effects may, potentially occur in specific groups, such as individuals being treated with drugs that interact with folic acid metabolism. Women who take folate supplements at up to 4 mg/day in order to reduce the risk of neural tube defect in the foetus do not report adverse effects.

Evaluation

Folate	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	1.0 (as folic acid)	Suppl	progressing neurological symptoms in vitamin B_{12} deficient patients	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	1.0 (as folic acid)	Suppl	masking vitamin B_{12} deficiency	human
EU (European Commission Health & Consumer Protection Directorate-General, 2000c)	1.0 (as folic acid)	Suppl	progressing neurological symptoms in vitamin B_{12} deficient patients	human

* Guidance level

Both the US and EU concluded that the progression of the neurological symptoms due to folic acid supplementation should be considered as the most serious adverse effect. Masking of the haematological signs in pernicious anaemia patients was considered a diagnostic problem that could be circumvented by using more specific tests to identify cases of undiagnosed B_{12} deficiency. Both the US and EU set a LOAEL of 5 mg folic acid and used an uncertainty factor of 5 because no NOAEL could be derived resulting in an UL of 1 mg of folic acid. No data are available to suggest that other life-stage groups have increased susceptibility to adverse effects of high folic acid intake. Therefore, the UL is also applicable for pregnant or lactating women. The UL of 1000 μg/day for adults was adjusted for children and adolescents on the basis of relative body weight and values have been rounded down.

Based on the data considered in the US and EU evaluations, the ULs for **folic acid from fortified foods or supplements** for the various age groups are as follows:

1-3 years:	**300 µg/day**
4-8 years:	**400 µg/day**
9-13 years	**600 µg/day**
14-18 years	**800 µg/day**
19 and older	**1000 µg/day**

Dietary intake

Dietary modelling has been performed for folic acid only for the risk assessment, where for the baseline situation, it has been assumed that only breakfast cereals are fortified with folic acid.

Food composition data in the NNS were for total dietary folate for each food, not from added sources only so could not be used in this risk assessment. Therefore, a dataset was constructed assigning folic acid concentrations to breakfast cereals to enable an intake of folic acid from added sources to be estimated for the baseline and with formulated beverages included in Scenario 2. The requested concentration of folic acid to be added to formulated beverages was 50 µg/600 ml reference quantity.

Estimated intakes for folic acid increase around 30 µg/day with the consumption of formulated beverages. Estimated intakes do not exceed the UL for any population group assessed.

Table 8: Estimated dietary intakes of folic acid from fortified foods only, before and after formulated beverages are introduced into the diet, and percent of the UL

	Mean intake µg/day (%UL)		95th percentile intake µg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	43.7 (15)	64.8 (20)	94.0 (30)	125.9 (40)
4-8 years, Aus	52.8 (15)	79.7 (20)	133.6 (35)	167.5 (40)
5-6 years, NZ	NA	NA	NA	NA
9-13 years, Aus	69.1 (10)	102.9 (15)	208.1 (35)	246.3 (40)
7-10 years, NZ	NA	NA	NA	NA
11-14 years, NZ	NA	NA	NA	NA
14-18 years, Aus	66.5 (8)	106.8 (15)	228.1 (30)	272.0 (35)
15-18 years, NZ	37.1 (5)	65.4 (8)	146.7 (20)	173.4 (20)
≥19 years, Aus	44.8 (4)	64.9 (6)	156.6 (15)	187.9 (20)
≥19 years, NZ	35.4 (4)	48.8 (5)	146.7 (15)	148.0 (15)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
NA = not assessed, because the New Zealand 2002 CNS did not include folic acid in isolation of folate.

Risk characterisation

Folate supplementation is recommended at a dose of 400 µg per day for women of childbearing age, and this needs to be taken into consideration when assessing the risk of high intake of folic acid.

For the adult population the 95th percentile intake was approximately 150 μg/day of folic acid for both Australia and New Zealand populations from fortified foods, including formulated beverages. In children, consumption of fortified foods would result in 95th percentile intakes there were 35-40% of the UL for the various age groups.

If it is assumed that all women of childbearing age took folic acid supplementation at the recommended dose of 400 μg per day, this would still not result in the UL for folic acid being exceeded. For children, folic acid supplementation is not considered to be relevant, since they are not a target group.

Therefore, the addition of folic acid to formulated beverages at a level of 50 μg in a 600 ml serve poses no public health and safety risk assuming only breakfast cereals are fortified.

Vitamin B_6 (pyridoxine)

Hazard identification and characterisation

Chemistry

Vitamin B_6 comprises a group of six related compounds, pyridoxal, pyridoxine, pyridoxamine and their respective 5'-phosphates. Pyridoxal 5'-phosphate is a coenzyme for more than 100 enzymes involved in amino acid metabolism, including aminotransferases, decarboxylases, racemases, and dehydratases.

Function

The cofactor forms of pyridoxine are pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate. Pyridoxal phosphate is involved as a cofactor particularly in the metabolic transformation of amino acids, including decarboxylation, transamination and racemisation. Vitamin B_6 is a cofactor in the conversion of tryptophan to 5-hydroxytryptamine and of methionine to cysteine. Pyridoxine can modify the action of steroid hormones in vivo by interacting with steroidreceptor complexes. Pyridoxine is essential for the manufacture of prostaglandins and for the formation of red blood cells.

Pyridoxine is involved in cellular replication and antibody production. An adequate supply of pyridoxine is necessary for the function of the nervous system. The vitamin is involved in the biosynthesis of several neurotransmitters, including serotonin, gamma amino-butyric acid (GABA), dopamine and noradrenaline and so has a role in the regulation of mental processes and mood. It is also involved in sodium-potassium balance, histamine metabolism, the conversion of tryptophan to niacin, absorption of vitamin B_{12} and the production of hydrochloric acid in the gastrointestinal tract.

Clinical signs of deficiencies include retarded growth, acrodynia, alopecia, skeletal changes and anaemia, while changes in neurotransmitters such as dopamine, serotonin, noradrenalin, tryptamine, tyramine, histamine, GABA and taurine, affect brain function and can lead to seizures and convulsions.

Sources of vitamin B_6

Pyridoxine is found in chicken (4.2 mg/kg), fish, liver, kidney, pork, eggs (1.1 mg/kg), milk, wheat germ (11.5 mg/kg) and brewer's yeast (25 mg/kg). Other sources include brown rice (5.5 mg/kg), soybeans (6.3 mg/kg), oats, whole-wheat grains, peanuts and walnuts (7.3 mg/kg). Long-term storage, canning, roasting or stewing of meat and food processing techniques can destroy pyridoxine. Boiling reduces the pyridoxine content of food because of losses into the water. In Australia and New Zealand various foods can be voluntary fortified with vitamin B_6 at levels of 0.11-0.5 mg per reference dose (Standard 1.3.2 – Vitamins and minerals).

Absorption, distribution, metabolism and excretion

The phosphate forms of vitamin B_6 in food are dephosphorylated in the intestinal lumen, and pyridoxine, pyridoxal and pyridoxamine are taken up from the small intestine by an energy dependent process. All three are converted to pyridoxal phosphate in the tissues.

A proportion of the vitamin B_6 present in plant-based foods is biologically unavailable because it is present as pyridoxine glycosides that are not hydrolysed by intestinal enzymes. These glycosides may be absorbed, but do not act as a coenzyme in the body and are excreted unchanged in the urine.

All three forms of vitamin B_6 (pyridoxine, pyridoxal and pyridoxamine) are readily absorbed in the small intestine. The extent of absorption is decreased following gastric resection or in patients with malabsorption syndrome. Excess pyridoxine is excreted in the urine, and an adequate daily intake is therefore essential.

Pyridoxine in food is converted to active forms in the liver, a process which requires zinc and riboflavin. Vitamin B_6 is stored in the liver, with about 50% also being present in muscle, bound to glycogen phosphorylase. Pyridoxine is also stored in the brain. The total body storage for adults is between 6 and 27 mg. Pyridoxine in the form of pyridoxal crosses the placenta, with foetal plasma concentrations being five times the level found in maternal plasma. The three forms of vitamin B_6 are present in body tissues, mainly as 5-phosphorylated derivatives of pyridoxal and pyridoxamine. The half-life of pyridoxine is 15-20 days, and it is not significantly bound to plasma proteins.

Pyridoxine, pyridoxal and pyridoxamine are all largely metabolised in the liver through phosphorylation by pyridoxal kinase. Pyridoxine phosphate is oxidised to the active coenzyme form, pyridoxal-5-phosphate, by an enzyme found mainly in liver. Pyridoxal-5-phosphate interconverts with pyridoxamine-5-phosphate through enzymatic transamination. The phosphorylated forms are hydrolysed by phosphatases. Pyridoxal is oxidised in the liver to pyridoxic acid.

Pyridoxic acid, the main excretory metabolite, is eliminated via the urine.

Toxicity

High doses of vitamin B_6 have been used for the treatment of premenstrual syndrome, depression, Down's syndrome, hyperkinesis, autism, neurosis, Hodgkin's disease and Parkinson's disease.

The principal toxicity of concern associated with excessive intakes of vitamin B_6 is neuronal damage, and sensory and motor effects. The initial observations were from studies in experimental animals, but more recent studies using human volunteers and patients, as well as case reports, have shown that the effects can also be produced also in humans. The effect occurs after consumption of high doses and/or long duration. Generally the symptoms are reversible once the exposure is stopped but in some cases involving high doses, the effects are irreversible. Progressive sensory ataxia occurs, presenting initially as unstable gait and numb feet, then numbness in the hands, followed by profound impairment of position sense and vibration sense in the distal limbs. The senses of touch, temperature and pain are less affected.

The available dose-response data in humans are difficult to analyse because many of the publications relate to case reports and true incidence data are not available. It is generally accepted that 500 mg of pyridoxine daily represents a potentially toxic dose for adults. The data for doses between 100 mg/day and 500 mg/day are less clear, largely because they relate to case reports or observations in groups of patients, that were not subject to a proper double-blind, placebo-controlled evaluation. The various studies show clear effects at 500 mg/day or more, a low incidence of effects at 200 mg/day in one study (if taken for up to 2 years) and the possibility of effects at about 100 mg/day (if consumed for about 3 years). In consequence a clear NOAEL has not been established and an intake of 100 mg/day cannot be excluded as a possible effect level for long-term intake.

Evaluation

Vitamin B_6	**UL in adults, mg/day**	**Total diet / Suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	100	Total diet	neuropathy	human
UK (UK Expert Group on Vitamins and Minerals, 2003)	10	Suppl	histological changes in nerves	dogs
EU (European Commission Health & Consumer Protection Directorate-General, 2000h)	25	Total diet	neurological effects	human

The US has set the UL for vitamin B_6 at 100 mg/day, based on neuropathy in human studies. A NOAEL of 200 mg/day could be identified by the critical evaluation of two studies, one where 70 patients with diabetic neuropathy or carpal tunnel syndrome were treated with 100 to 150 mg/day of pyridoxine- some for up to 5 years. In this study no sensory neuropathy was detected. In the second study 24 patients were treated for carpal tunnel syndrome with pyridoxine at doses of 100 to 300 mg/day for 4 months. A NOAEL of 200 mg/day represents the average of 100 and 300 mg/day. Other studies supported a NOAEL of 200 mg/day. An uncertainty factor of 2 was selected based on the limitations of the data, and therefore the UL in the US is set at 100 mg/day.

The UK stated that the human data are inadequate to establish an UL, since the effect levels are unclear and the studies at low levels of intake are of limited quality. Therefore the UL is based on animal data, in which histological changes were apparent in the nerves of dogs treated with 50 mg/kg bw/day for 100-112 days.

Clinical signs of toxicity were not apparent in this group but were observed in the high dose group, which received 200 mg/kg bw/day. Using uncertainty factors of 300 (consisting of 3 for LOAEL to NOAEL extrapolation of a histopathological change, 10 for inter-species and 10 for inter-individual variation) a safe UL of 0.17 mg/kg bw/day can be derived. This relates to supplemental pyridoxine because the basal pyridoxine content of the diet in the key study is unknown. This UL is equivalent to 10 mg/day in a 60 kg adult.

The EU derived the UL from a study where vitamin B_6 intake and clinical signs were monitored in women attending a private clinic specialising in the treatment of premenstrual tension. Based on the apparent inverse relationship between dosage and duration of intake, a significant difference in duration of intake (average 2.9 years), but not dosage in women with 'neurological effects' while taking low doses is exactly the relationship that would be predicted. An UL has been calculated by dividing the average intakes in this study of approximately 100 mg per day (the mean intake was 117 mg/day and the median was <100 mg/day) by a factor of 2, because the intake corresponds to a possible effect level for long-term intake, and by a second factor of 2 to allow for deficiencies in the database. A larger uncertainty factor was not considered necessary, because the data were for a sub-group with high plasma concentrations, and because the resulting UL of 25 mg per day has not been associated with adverse effects in any of the large number of published studies. Therefore the upper limit in the EU is 25 mg/day.

As the UL of both the EU and US were based on human data, using total dietary intake, they are considered more relevant than the UL derived by the UK. The UL from the EU report is considered the most relevant, because it was derived from longer-term studies in humans as compared to the US UL, and the apparent inverse relationship between dose and time of treatment.

Based on the data from the EU evaluation, the ULs for **vitamin B_6** for all age categories are:

1-3 years	**7 mg/day**
4-8 years	**10 mg/day**
9-13 years	**15 mg/day**
14-18 years	**20 mg/day**
Adult	**25 mg/day**

Dietary intake

Estimated dietary intakes were calculated for vitamin B_6 from all foods in the diet, and have been adjusted using second day intakes from the NNSs. Estimated intakes at baseline and for Scenario 2 are shown in Table 9.

Vitamin B_6 was not included in the 1995 Australian NNS. Therefore, in order to estimate intakes for the Australian population, the concentration data from the New Zealand NNS were matched to the most appropriate Australian food code, and these values were used to estimate dietary intakes for the Australian population groups.

The concentration of vitamin B_6 requested to be added to formulated beverages was 0.4 mg pyridoxine/600 ml reference quantity.

Estimated intakes of vitamin B_6 increased by around 0.5 mg/day or less when formulated beverages are consumed, across all population groups assessed. Estimated mean intakes are lower for Scenario 2 when it is assumed formulated beverages are consumed, compared to baseline for 15-18 year olds from New Zealand. This would be due to consumers substituting an formulated beverage for a beverage or beverages that were higher in vitamin B_6 content than the formulated beverage.

Estimated intakes do not exceed the UL for any population group assessed.

Table 9: Estimated dietary intakes of Vitamin B_6, before and after formulated beverages are introduced into the diet, and percent of the UL

	Mean intake mg/day (%UL)		95th percentile intake mg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	1.2 (15)	1.3 (20)	1.7 (25)	1.9 (30)
4-8 years, Aus	1.2 (10)	1.4 (15)	1.9 (20)	2.2 (20)
5-6 years, NZ	^1.2 (**)	NA	#1.6 (**)	NA
7-10 years, NZ	^1.3 (**)	NA	#1.8 (**)	NA
9-13 years, Aus	1.6 (10)	1.8 (10)	2.4 (15)	2.7 (20)
11-14 years, NZ	^1.5 (**)	NA	#2.1 (**)	NA
14-18 years, Aus	1.7 (9)	2.1 (10)	3.2 (15)	3.7 (20)
15-18 years, NZ	1.6 (8)	1.5 (7)	2.1 (10)	2.3 (10)
≥19 years, Aus	1.6 (6)	1.7 (7)	2.8 (10)	3.0 (10)
≥19 years, NZ	1.5 (6)	1.4 (6)	2.3 (9)	2.3 (9)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Toxicological evaluation and dietary intake data indicate that no age-groups are likely to approach the UL-set for vitamin B_6, either at the mean level of intake or at the 95th percentile of intake when included in formulated beverages (7%UL and 10%UL for Australia and 6%UL and 10%UL for New Zealand, for mean and 95th percentile intake in adults, respectively). The group exposed to the highest level as a percentage of the UL were children aged 2-3 years. In this age group, at the 95th percentile intake, the intake of vitamin B_6 was estimated to be equivalent to 30% of the UL.

It is concluded that addition of vitamin B_6 to formulated beverages at a level of 0.4 mg pyridoxine in a 600 ml serve poses no appreciable public health and safety risk.

Vitamin B_{12}

Hazard identification and characterisation

Chemistry

Vitamin B12 (cobalamin, Cbl) is a water-soluble vitamin and a member of a family of related molecules known as corrinoids which contain a corrin nucleus made up of a tetrapyrrolic ring structure. The centre of the tetrapyrrolic ring nucleus contains a cobalt ion that can be attached to methyl, deoxyadenosyl-, hydroxo- or cyano- groups.

Function

Vitamin B_{12} plays a specific role in amino acid metabolism, i.e. in methylation reactions together with folate, in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA in succinyl CoA.

Sources of Vitamin B_{12}

Major dietary sources of vitamin B12, mainly in the forms of methyl, deoxyadenosyl- and hydroxocobalamin, include meat, particularly liver and fish. Hydroxocobalamin and, in particular, cyanocobalamin are synthetic forms used in the fortification of food.

Absorption, distribution, metabolism and excretion

Vitamin B_{12} requires intrinsic factor (IF), secreted mainly from the gastric parietal cells, to ensure adequate absorption at normal dietary intake levels. Thus the absorption of physiological doses of vitamin B_{12} is limited to approximately 0.0015 – 0.002 mg/dose or meal, due to saturation of the uptake system. Regardless of dose, approximately 1.2% of vitamin B12 is absorbed by passive diffusion and consequently this process becomes quantitatively important at pharmacological levels of exposure. Protein binding in certain foods may reduce the bioavailability of the vitamin, particularly in individuals with impaired gastric acid and/or digestive enzyme secretion. The different forms of crystalline cobalamin appear to be absorbed or retained to different extents, depending on the dose. Differences are most apparent at low doses.

Ingested vitamin B_{12} is released from the food matrix by the action of digestive enzymes and gastric acid and becomes bound to salivary haptocorrin-binding proteins. As the pH rises further along the gut, and under the influence of pancreatic enzymes, vitamin B_{12} is released from the salivary haptocorrin and becomes complexed with intrinsic factor (IF). The cobalamin-IF complex binds to a specific cell wall receptor of the ileal enterocyte and is internalised by endocytosis. Once inside the cell, the IF is degraded and the liberated vitamin is converted to the methyl or the deoxyadenosyl form, is bound to transcobalamin II (TC II) binding protein and then exported into the portal blood. In the general circulation, most cobalamin is bound to transcobalamin I (TC I) but the majority of cobalamin available for uptake into the tissues is that bound to TC II.

Vitamin B_{12} is distributed into the liver, bone marrow and virtually all other tissues, including the placenta and breast milk of nursing mothers. The liver is the predominant storage site for vitamin B_{12}.

Uptake into cells occurs through receptor mediated endocytosis involving specific TC II cell wall receptors. Once inside the tissues/cells, the complex is degraded by the lysosomes, and the released cobalamin is metabolised either to methyl-cobalamin in the cytosol, where it binds to methionine synthase, or to deoxyadenosyl-cobalamin in the mitochondria, where it binds to methylmalonyl CoA mutase.

Excretion occurs mainly via the faeces and urine, but also through the shedding of skin cells. Excretion is very slow, with significant enterohepatic cycling.

Toxicity

No adverse effects have been associated with excess vitamin B_{12} intake from food or supplements in healthy individuals. Vitamin B_{12} has a history of safe long-term use as a therapeutic agent given in high dosage per os, or via intramuscular injections, for treatment of disorders associated with impaired vitamin B_{12} absorption, such as in gastrectomy and malabsorption.

No systematic toxicological studies have been reported for vitamin B12. There are no reports attributing carcinogenic, mutagenic or teratogenic potential to cyanocobalamin. In one study a tumour promoting effect was reported in a rat model, but this study is not considered relevant for safety assessment in humans.

There are also no adverse effects known for vitamin B_{12} from foods, or from supplements in amounts far in excess of needs. Some studies suggested acne formation after high parenteral doses of hydroxocobalamin, but not with cyanocobalamin, or after a combination of vitamins A, B_6 and B_{12} given orally.

Oral and parenteral supplementation with dosages between 1-5 mg every fortnight or month have been given for long periods, up to at least 5 years, to patients with compromised vitamin B_{12} absorption, without any identified adverse effects. It should be noted, however, that these studies were not designed to identify adverse effects.

Evaluation

Vitamin B_{12}	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	N/A		No adverse effects	
UK (UK Expert Group on Vitamins and Minerals, 2003)*	2.0	Suppl	No adverse effects	Humans
EU (European Commission Health & Consumer Protection Directorate-General, 2000f)	N/A		No adverse effects	

N/A not applicable
* Guidance level

There are no clearly defined adverse effects produced by vitamin B_{12} that can be used to define a LOAEL or NOAEL, and which can be used as a basis for deriving an UL.

When high doses are given orally only a small percentage of vitamin B_{12} can be absorbed from the gastrointestinal tract, which may explain the apparent low toxicity.

Based on the data considered in the US and EU evaluation, **vitamin B_{12}** has a very low oral toxicity, and therefore an **UL does not need to be established**.

Dietary intake

No dietary intake estimates were calculated for thiamin, as it was determined to have very low oral toxicity, and no ULs have been established, as outlined above.

Risk characterisation

No UL has been established for vitamin B_{12}, based on the lack of adverse effects even at high doses. Therefore, no dietary modelling was required.

In conclusion, the addition of vitamin B_{12} to formulated beverages at a level of 0.5 μg per 600 ml serve poses no appreciable public health and safety risk.

Vitamin C

Hazard identification and characterisation

Chemistry

Vitamin C is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidised derivative L-dehydroascorbic acid.

Function

Vitamin C is a strong reducing agent and as an antioxidant is involved in prevention of the damaging effects of free radicals. Vitamin C is involved in the synthesis of collagen, neurotransmitters and carnitine; it is an enzyme co-factor and also increases the gastrointestinal absorption of non-haem iron.

Sources of vitamin C

Food of plant origin, particularly citrus and soft fruits and leafy green vegetables, are major sources of vitamin C. Kidney and liver are good animal-derived sources of vitamin C.

Absorption, distribution, metabolism and excretion

Gastrointestinal absorption of vitamin C is efficient and occurs in the small intestine via a saturable active transport mechanism. Absorption efficiency of low oral doses of vitamin C (4-64 mg) may be as high as 98%, but decreases with increasing doses of the vitamin.

Ascorbic acid is widely distributed in all tissues of the body, with higher levels found in the adrenal glands, pituitary and retina, and lower levels in kidney and muscle tissue.

Vitamin C is oxidised to dehydroascorbic acid, which is hydrolysed to diketogulonic acid and then oxidised to oxalic and threonic acid. Some oxidation to carbon dioxide occurs at high doses.

Unmetabolised vitamin C and vitamin C metabolites, such as oxalate, are largely excreted in urine. Approximately 3% of a 60 mg oral dose is excreted in the faeces. More of the vitamin is excreted unchanged at higher levels of vitamin C intake.

Toxicity

The vitamin is of low acute toxicity as indicated by the limited data available from studies in animals and humans. Despite the extensive use of high doses of vitamin C in some vitamin supplements, there have been few controlled studies that specifically investigated adverse effects. Overall, acute gastrointestinal intolerance (e.g., abdominal distension, flatulence, diarrhoea, transient colic) is the most clearly defined adverse effect at high intakes, but there are limited data on the dose-response relationship for adults or for groups such as children or the elderly.

The US evaluation considered a 3 g/day intake as the LOAEL, based on human data which suggest that an intake of vitamin C greater than 3 g/day is likely to cause osmotic diarrhoea in many individuals, although some reports involving a few individuals suggest this may occur at 3 g/day.

While there is uncertainty whether high intakes of vitamin C increase renal excretion of oxalate which could increase the risk of renal stones, an increased risk of kidney stones was not found in individuals with habitual intakes of 1.5 g/day.

Evaluation

Vitamin C	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000c)	2000	Suppl	osmotic diarrhoea and gastrointestinal	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	1000	Suppl.	gastrointestinal	human
EU (The Scientific Panel on Dietetic Products, 2004)	-		insufficient data	
WHO/FAO (FAO/WHO, 2002)	1000*	total	gastrointestinal	human

* Guidance level

Available human data suggest that supplemental daily doses of vitamin C up to about 1 g in addition to normal dietary intakes are not associated with adverse gastrointestinal effects, but that acute gastrointestinal effects may occur at higher intakes (3-4 g/day). While there is uncertainty whether high intakes of vitamin C increase renal excretion of oxalate, which could increase the risk of renal stones, an increased risk of kidney stones was not found in individuals with habitual intakes of 1.5 g/day. The absorption of vitamin C is saturated at high doses, and therefore intakes above 1 g/day would be associated with negligible increased uptake and tissue levels, but an increased risk of adverse gastrointestinal effects.

There are no data on the gastrointestinal absorption or tolerability of esterified forms of vitamin C, such as ascorbyl palmitate, but such esters might be expected to show similar properties, and therefore this conclusion applies to these forms as well as ascorbic acid and its salts.

The US set a UL for vitamin C based on osmotic diarrhoea. The US used an uncertainty factor of 1.5 to extrapolate from LOAEL to NOAEL. Thus the 3 g/day intake is considered a LOAEL, and a NOAEL of 2 g/day is estimated for adult humans. Because the database has no other significant sources of uncertainty and because of the mild, reversible nature of osmotic diarrhoea caused by high vitamin C intakes, no further uncertainty factors were considered necessary.

The evaluation from the FAO/WHO was very limited and based on osmotic diarrhoea.

Gastrointestinal effects are the most common adverse effects but these are associated with acute, high doses of vitamin C given over a short period of time.

Based on the data considered in the US, UK and EU evaluation, **vitamin C** has a very low oral toxicity, and therefore an **UL does not need to be established**. For guidance purposes, a dose of 1000 mg/day, in addition to normal dietary intakes, would not be expected to have any significant adverse effects.

Dietary intake

Intakes of vitamin C were estimated at baseline and for scenario 2 assuming formulated beverages are consumed. Results are shown in Table 11. The estimated intakes have been adjusted based on second day nutrient intakes from the NNSs.

The concentration of vitamin C requested to be added to formulated beverages was 40 mg/600 ml reference quantity.

Intakes of vitamin C increased by around 10 to 15 mg/day when formulated beverages are consumed, depending on the population group assessed. Estimated intakes were not compared to ULs as none were established for vitamin C.

Table 11: Estimated dietary intakes of Vitamin C, before and after formulated beverages are introduced into the diet

	Mean intake mg/day (%UL)		95th percentile intake mg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	105	113	215	197
4-6 years, Aus	106	115	216	195
5-6 years, NZ	^104	NA	#156	NA
7-10 years, Aus	108	124	245	246
7-10 years, NZ	^113	NA	#159	NA
11-14 years, Aus	114	133	235	253
11-14 years, NZ	^117	NA	#193	NA
15-18 years, Aus	131	156	273	315
15-18 years, NZ	123	142	225	252
≥19 years, Aus	124	138	251	269
≥19 years, NZ	111	119	213	224

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Dietary intake of vitamin C for high consumers at baseline was estimated to be 210-250 mg per day for adults, and at 220-270 mg per day for adults when formulated beverages are consumed. These intake of vitamin C are significantly lower than the guidance level of 1000 mg / day.

In conclusion, the addition of vitamin C to formulated beverages at a level of 40 mg per 600 ml serve poses no appreciable public health and safety risk.

Vitamin D

Hazard identification and characterisation

Chemistry

Vitamin D refers to a group of fat-soluble seco-steroid compounds. Two nutritionally significant compounds are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Both vitamins are metabolised in the liver and kidney to an active steroid hormone.

Function

The principal function of vitamin D ($1,25(OH)_2D$) in the body is to maintain intracellular and extracellular calcium concentrations within a physiologically acceptable range. The vitamin accomplishes this goal through the action of $1,25(OH)_2D$ on regulating calcium and phosphorus metabolism in the kidney, small intestine and bone. In the kidney, $1,25(OH)_2D$ regulates calcium transport in the proximal tubule; in the small intestine, it regulates calcium and phosphate uptake from the gut. $1,25(OH)_2D$ is also involved in the maintenance of plasma calcium levels via bone resorption and formation. $1,25(OH)_2D$ regulates the synthesis of parathyroid hormone (PTH) by a negative feedback mechanism.

Sources of vitamin D

Throughout the world, the major source of vitamin D for humans is the exposure of the skin to sunlight. During sun exposure, the ultraviolet B photons with energies between 290 and 315 nm are absorbed by cutaneous 7-dehydrocholesterol to form the split (seco) sterol previtamin D_3. Upon prolonged UV exposure a regulation mechanism is operating in that both previtamin D_3 and vitamin D_3 can be photolysed to inert compounds. Hence, sunlight alone apparently cannot cause overt toxicity due to overproduction of vitamin D. Some studies indicate that the degree of pigmentation of the skin also has an impact on the amount of vitamin D synthesised as melanin absorbs UV B photons: the darker the skin, the less is produced. Skin thickness decreases linearly with age from the age of 20 years and there is a marked decrease in the precursor 7-dehydrocholesterol in the skin and less vitamin D production.

Vitamin D is found in only a few foodstuffs, with fatty fish and fish oils, liver, milk and eggs being the main natural sources. In Australia and New Zealand various products can be fortified with vitamin D (10 to 25% of the recommended daily intake) according to Standard 1.3.2 – Vitamins and Minerals.

Furthermore, in Standard 2.4.2 – Edible oil spreads, table edible oil spreads and table margarine, must contain no less than 55 μg/kg of vitamin D. However this subclause does not apply to table edible oil spreads and table margarine produced in, or imported into, New Zealand.

Absorption, Distribution, Metabolism and Excretion

Vitamin D is absorbed from the small intestine as bile salt-dependent micelles and circulated in the body via the lymph. Absorption of polar derivatives, such as 25(OH)D, is more efficient and less dependent on bile salts. These polar derivatives are generally not present in any significant amount in food or food supplements, although small amounts of 25(OH)D are found in meat and breast milk.

The importance of the chemical form of vitamin D, i.e. vitamin D_2 or D_3 with a lower biological efficiency of vitamin D_2, should be noted. In addition the vehicle used (fat or emulsion) could influence bioavailability. Vitamin D from cod liver oil emulsified in milk is about three times as potent as vitamin D given in cod liver oil or propylene glycol.

There is substantial storage of vitamin D in adipose tissue. Vitamin D is metabolised to the steroid hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), a process which is promoted by parathyroid hormone (PTH). The first step of activation takes place by hydroxylation at position C-25, mainly in the liver. The product, 25-hydroxyvitamin D (25(OH)D), is transported to the kidneys, where 1α-hydroxylation takes place and the active form of vitamin D is formed. This reaction is regulated by parathyroid hormone (PTH), which is secreted in response to low plasma calcium levels.

The 25-hydroxylation of vitamin D is poorly regulated, i.e. the capacity of the 25-hydroxylase in the liver is high. The levels of 25(OH)D increase in proportion to vitamin D intake, and for this reason, plasma 25(OH)D levels are commonly used as indicator of vitamin D status.

There is a consensus that serum 25(OH)D concentration is a correct functional indicator of vitamin D status. A level of 25(OH)D below 27.5 nmol/L is considered to be consistent with vitamin D deficiency in infants, neonates and young children. Little information is available about the level of 25(OH)D needed to maintain normal calcium metabolism and peak bone mass in adolescents and middle aged adults. For elderly there is increasing evidence of a greater requirement of vitamin D to maximise bone mineralization. Less certain and more controversial is the optimal serum concentration of 25(OH)D.

Vitamin D is principally excreted in the bile. It is also metabolised to water-soluble metabolites, such as calcitroic acid, and excreted in the urine.

Toxicity

The principal critical effect of hypervitaminosis D/vitamin D toxicity is hypercalcaemia. It has, however, been reported that patients with hypervitaminosis D (increased level of 25(OH)D >130 nmol/L), hypercalciuria and a depressed PTH status can be normo-calcaemic. Thus, hypercalciuria apparently is an earlier phenomenon than hypercalcaemia that could predispose to kidney stone formation.

There is limited evidence that suggests that direct effects of high concentrations of vitamin D may be expressed in various organ systems, including kidney, bone, central nervous system, and cardiovascular system.

The most frequently noted clinical manifestations of hypervitaminosis D are anorexia, weight loss, weakness, fatigue, disorientation, vomiting and constipation. Hypercalcaemia may also lead to growth retardation in children, irritability, asthenia, persisting fever, polyuria and polydipsia, dehydration, hypertension and functional renal insufficiency. Long-term toxicity with persistent hypercalcaemia may cause excess calcium precipitates as extra-skeletal calcium in soft tissues, particularly in the renal parenchyma, urinary tracts, vascular walls, muscles and tendons.

The vitamin D intake associated with exceeding the upper reference value of 25(OH)D in serum would vary greatly in the population. It is, for instance, dependent on the exposure to sunlight and sensitivity to vitamin D. The importance of the chemical form of vitamin D, i.e. vitamin D_2 or D_3 with a lower biological efficiency of vitamin D_2, should be noted. In addition the vehicle used (fat or emulsion) could influence bioavailability. Vitamin D from cod liver oil emulsified in milk is about three times as potent as vitamin D given in cod liver oil or propylene glycol. For some individuals an intake of 250 µg vitamin D would not exceed of this value while in others this could occur. Data indicate that the upper reference value of serum 25(OH)D at 150 nmol/L or 200 nmol/L is exceeded by 5% of the population at an approximate vitamin D intakes of about 80 or 100 µg/day, respectively. These levels of 25(OH)D in serum can be considered NOAELs with respect to increased risks of hypercalciuria and hypercalcaemia, respectively. On the other hand, two studies reported that the upper reference serum concentration of 25(OH)D was not exceeded upon supplementation with 100 µg cholecalciferol (vitamin D_3)/day.

Taking into account all the information the risk of hypercalciuria/hypercalcaemia probably starts to increase in some parts of the population at an intake above 100 µg vitamin D/day. The risk of exceeding the upper reference concentration of 25(OH)D in serum will also increase. A dose of 100 µg vitamin D/day and a serum level of 200 nmol 25(OH)D/L are considered a NOAEL.

Children

In infants the regulation of 1α-hydroxylase and the normal feedback suppression by $1,25(OH)_2D$ on the kidney enzyme seem to work less well compared to adults.

The upper reference level for 25(OH)D for infants is similar to that of adults and the approach used for adults by setting the UL at an oral dose of vitamin D not associated with exceeding the upper reference level (i.e. 130-150 nmol/L) could in theory be done.

A problem is that there are very few data on doses of vitamin D above the recommended intake and corresponding concentrations of 25(OH) in serum.

A small and old study considering hypercalcaemia indicated a NOAEL of 45 μg vitamin D/day for infants. However, in two more recent and larger well-controlled studies in infants receiving 25 μg vitamin D_2/day in addition to breast milk or 32 μg vitamin D_2/day, hypercalcaemia was not observed. Based on these data, a NOAEL of 25 μg/day can be derived.

Vulnerable groups

The feedback mechanism of $1,25(OH)_2D$ synthesis seems to operate poorly, if at all, in tissues other than that of the renal tubule. In patients with sarcoidosis, $1,25(OH)_2D$ is believed to be synthesised in macrophages, which in these patients have an increased enzyme capacity, or other cells in the granulomas. Also the clearance of $1,25(OH)_2D$ may be decreased. Contrary to normal in these patients there is a positive correlation between 25(OH)D within reference levels and 1,25(OH)2D in serum. Even normocalcaemic patients with sarcoidosis have unregulated production of $1,25(OH)_2D$ in response to vitamin D. Also exposure to sunlight may increase the level of active metabolite.

In some lymphomas, typically B-cell lymphomas, there is an increased blood level of $1,25(OH)_2D$, which is probably synthesised by lymphocytes.

Excessive endogenous synthesis of $1,25(OH)_2D$ occurs in children with subcutaneous fat necrosis.

Vitamin D deficiency can mask primary hyperparathyroidism and this could account for the occasional cases of hypercalcaemia observed when large groups of elderly people are given vitamin D supplements.

Evaluation

Vitamin D	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000a)	0.050	total diet	serum calcium levels	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	0.025	suppl	serum calcium levels	human
EU (European Commission Health & Consumer Protection Directorate-General, 2002d)	0.050	total diet	serum calcium levels	human

* Guidance level

The EU evaluation was the most extensive evaluation available for vitamin D. The following paragraphs are from the EU evaluation.

The EU established a NOAEL of 100 μg/day for the adult population. An uncertainty factor of 2 is considered adequate to account for the inter-individual variation. An UL of 50 μg vitamin D/day is considered to offer adequate protection against the risk of hypercalciuria and hypercalcaemia.

The EU indicated that no data was available to suggest that other life-stage groups have increased susceptibility to adverse effects of high vitamin D intake. Given the minor impact of circulating vitamin D on calcium levels *in utero* and in breast-fed infants with maternal supplements of 25 and 50 μg vitamin D/day there does not seem to be an increased sensitivity during this period. Therefore the UL of 50 μg/day should be considered to apply also to pregnant and lactating women.

For infants, the lower values from studies with infants taking into account the higher biological activity and toxicity of vitamin D_3 and the other information provided above an UL of 25 μg vitamin D/day for infants 0-24 months of age is derived. It seems that susceptibility towards vitamin D changes with age. Using a cautious approach taking into consideration a lower weight in children up to 10 years the following upper limits are set: UL of 25 μg vitamin D/day for children from 2 up to and including 10 years of age and an UL of 50 μg/day for adolescents 11-17 years of age.

It should be noted that the intake of vitamin D via food would add to synthesis caused by exposure to sunlight. Depending on the amount of sunlight the risk of adverse effects at an intake at the UL would increase.

Based on the data considered in the EU evaluation, the UL for **vitamin D** for the various age groups are:

1-10 years	**25 μg/day**
11 and over	**50 μg/day**

Dietary intake

Intakes for vitamin D were estimated at baseline and for Scenario 2 assuming formulated beverages were consumed.

The concentration of vitamin D requested to be added to formulated beverages was 2.5 μg/600 ml reference quantity.

Vitamin D was not included in the 1995 Australian NNS, therefore, there were no concentration data available in DIAMOND for Vitamin D for Australia. Vitamin D was included in the New Zealand 1997 NNS, however, was not included in the New Zealand 2002 CNS. The concentrations of Vitamin D in food from the 1997 New Zealand NNS were matched to the most appropriate foods in the 1995 Australian NNS to enable an estimated intake to be calculated for Australia.

Estimated intakes for vitamin D were able to be adjusted for the majority of the population groups assessed apart from respondents aged 4-18 years for Australia. Where second day adjustments could be made, these were presented as the estimated intakes, as they provide a better indication of longer term nutrient intakes. Where second day adjustments could not be made, this was due to limited sample numbers in certain age groups and the distribution of intakes which meant the calculations could not be made. The estimated intakes for vitamin D for the population groups with unadjusted intakes will be higher at the 95th percentile than those for similar age groups that have adjusted intakes. The estimated intakes for younger children in New Zealand 5-14 years were not included in the 2002 New Zealand children's nutrition survey and therefore could not be include here (Ministry of Health, 2003).

Estimated intakes of vitamin D increased by around one to two micrograms per day with the consumption of formulated beverages. Estimated intakes did not exceed the UL for any population group assessed.

Table 12: Estimated dietary intakes of Vitamin D, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake µg/day (%UL)		95th percentile intake µg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	1.3 (5)	2.2 (9)	2.1 (8)	4.2 (15)
4-8 years, Aus^	1.5 (6)	2.8 (10)	3.6 (15)	6.7 (25)
5-6 years, NZ	NA	NA	NA	NA
7-10 years, NZ	NA	NA	NA	NA
9-13 years, Aus^	2.0 (4)	3.7 (7)	5.2 (10)	8.6 (15)
11-14 years, NZ	NA	NA	NA	NA
14-18 years, Aus^	2.1 (4)	4.3 (9)	5.4 (10)	10.9 (20)
15-18 years, NZ	2.3 (5)	2.3 (5)	4.1 (8)	4.1 (8)
≥19 years, Aus	2.0 (4)	3.0 (6)	3.8 (8)	6.1 (10)
≥19 years, NZ	2.4 (5)	2.4 (5)	3.9 (8)	4.0 (8)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
NA = not assessed, because Vitamin D was not included in the New Zealand 2002 CNS.
^ Not adjusted for second day intakes.

Risk characterisation

Toxicological evaluation and dietary intake data indicate that both children and adult Australian and New Zealand consumers are unlikely to approach the UL set for vitamin D, at the 95th percentile of intake either at baseline or when included in formulated beverages (10% UL and 8% UL for adults from Australia and New Zealand, respectively). The group with the highest level of intake as a percentage of the UL were children aged 4-8 years (25% UL), at a level of intake still well below the UL.

Therefore, dietary intake of vitamin D for all consumers is considered to be within the safe range of intake for both mean and high consumers.

In conclusion, the addition of vitamin D to formulated beverages at a level of 2.5 µg in a 600 ml serve poses no appreciable public health and safety risk.

Vitamin E

Hazard identification and characterisation

Chemistry
Vitamin E is the term used to describe a group of related fat-soluble tocochromanols, including eight naturally occurring components, which exhibit antioxidant activity and are nutritionally essential. The two major homologous series of tocochromanols, the tocopherols and tocotrienols, both have vitamin E activity in humans and animals and are synthesised by higher plants and cyanobacteria.

In all homologues, the basic structural unit is a chroman ring system (2-methyl-6-hydroxychroman) with an isoprenoid side chain of 16 C atoms. The compounds, including α-, β-, γ-, and δ-homologues, differ in number and position of the methyl substituents in the chroman ring. Tocopherols differ from their corresponding tocotrienols in having a saturated side chain. The presence of the phenolic hydroxyl group in the tocochromanols is important for their activity as antioxidants. At least one methyl group in the benzene ring is of primary importance. α-Tocopherol with three methyl groups is the most active of all homologues, followed by β-, γ-, and δ-tocopherol. The only forms retained in human plasma are the RRR-α-tocopherol and the 2R-stereoisomers, RSR-, RRS- and RSS-α-tocopherol; the various 2S-stereoisomers (SRR-, SSR-, SRS- and SSS-α-tocopherol) which form part of synthetic *all rac-α*-tocopherol are not maintained in plasma. The vitamin E activity is expressed as *RRR*-α-tocopherol equivalents, which accounts for about 90% of the activity in human tissue; the relative potency of α-, β-, γ-, and δ-tocopherol is reported to be approximately 100:50:25:1. The commercially available synthetic form is all rac-α-tocopheryl acetate with the activity of 0.67 x *RRR*-α-tocopherol. For practical purposes, 1 International Unit (I.U.) of vitamin E is referred to as 1 mg of all rac-α-tocopheryl acetate.

In this assessment the term vitamin E is related to α-tocopherol equivalents.

Function

The basic mode of action of tocopherols in human tissue is to prevent the oxidation of polyunsaturated fatty acids (PUFA) by trapping free radicals and donating hydrogen. It is effective in protecting the integrity of lipid and phospholipid in membranes and thus the requirement for vitamin E and the recommended intake is determined to a large extent by the intake of PUFAs. It has been shown that increasing the PUFA content of a diet low in α-tocopherol equivalents has adverse effects on tocopherol status.

Sources of vitamin E

The major food sources of vitamin E are vegetable oils, unprocessed cereal grains, and nuts with smaller amounts in fruits and vegetables and meats (mainly the fatty portion).

Absorption, distribution, metabolism and excretion

The bioavailability of vitamin E is related to the efficiency of absorption. Intestinal absorption of lipids and fat-soluble vitamins depends on pancreatic function, biliary secretion to form micelles with the hydrolysed fat, and transfer across intestinal membranes. Nearly all of the vitamin E absorbed across the intestinal mucosa is free tocopherol. *In vivo* and *in vitro* studies suggest that the rate of uptake of vitamin E is controlled by passive diffusion. Absorption of tocopherols is incomplete; the extent of absorption is dependent on intake and varies between 20-80%. The proportion absorbed decreases with increasing amount added to experimental diets; the average absorption is about 40-60% while pharmacological doses of 200 mg and more are absorbed to the extent of <10%. Cannulation studies indicate that there is no difference in absorption between α-tocopherol and α-tocopheryl acetate at physiological doses. At 95th percentiles of intake, (>400 IU/day) a higher degree of absorption was obtained with free tocopherol than tocopheryl esters.

About 90% of the free α-tocopherol is transported via the lymphatic system into the bloodstream, where it is distributed into lipoproteins on passage into the liver.

The main systemic transport system of tocopherols is the LDL-fraction (55-65%) followed by the HDL (24-27%) and VLDL (8-18%). There is very close correlation (r=0.925) between the total serum α-tocopherol and that portion carried by LDL.

In human metabolism, vitamin E is known to interact with other nutrients which are also involved in the pathways of oxidation processes. Vitamin C, selenium and zinc interact synergistically with vitamin E. Conversely, an iron overload is associated with a lowering of serum vitamin E levels.

At normal intake levels, vitamin E is conjugated with glucuronic acid and this conjugate is excreted (via bile) in the faeces. Up to 30-70% of vitamin E is excreted via this route with less than 1% being excreted in the urine. Some vitamin E may be eliminated via the skin.

Toxicity

Animal studies

Vitamin E has a very low acute oral toxicity.

Two long-term studies of up to 16 months and 2 years duration respectively have been conducted in rats (Yang and Desai, 1977; Wheldon *et al*., 1983). A LOAEL of 500 mg/kg body weight/day can be identified based on a critical evaluation by Wheldon et al (Wheldon *et al.,* 1983). They fed rac-α-tocopheryl acetate to Charles River CD strain rats at levels of 500, 1000, or 2000 mg/kg body weight/day for 104 weeks. Haemorrhages from the gut, the urinary tract, the orbit and meninges, and the claws were observed in male rats only by week 15 in the highest-dose group, by week 16 in the intermediate-dose group, and by week 18 in the low-dose group. Additional vitamin K supplementation (10 mg vitamin K_3/day) was initiated at week 24 and prothrombin times returned to normal by week 26. Although this was a chronic study, the correction of vitamin K levels at week 24 means that the combined vitamin E-vitamin K effect was evaluated only on a sub-chronic basis. The only other treatment-related effect of significance was the presence of vacuolated lipid staining macrophages in the liver.

Human studies

Vitamin E has low toxicity. At very high doses, however, vitamin E can produce signs indicative of antagonism with the function of other fat-soluble vitamins (vitamins A, D, K). Isolated reports of adverse effects in humans consuming up to 1000 IU of vitamin E per day include headache, fatigue, nausea, double vision, muscle weakness, mild creatinuria and gastrointestinal distress. A number of human supplementation studies on vitamin E are available.

The principal negative effect observed was on prothrombin time or other factors related to blood clotting. In several studies no effects were reported but in others there were effects on blood clotting and it was claimed that high doses of vitamin E only influenced blood clotting in cases of low vitamin K status. One of the reported adverse effects concerns decreased blood coagulation. Studies with healthy humans with vitamin E supplementation have shown that there are no changes in platelet aggregation or adhesion with daily vitamin E intake up to 800 mg α-tocopherol equivalents (1,200 IU).

The question of bleeding time was studied by Meydani et all (Meydani *et al*., 1998) who found no adverse effects, including the bleeding time, after a 4-month daily supplementation with 60, 200 or 800 IU (40, 134 or 537 mg α-tocopherol equivalents) vitamin E (88 healthy volunteers, aged >65 years divided between control and three dose groups, extensive measurements of parameters). The published reports concluded that vitamin E at high dietary intakes affects blood coagulation if vitamin K status is inadequate. High doses of α-tocopherol affected the vitamin K metabolism by reducing the cyclooxygenase pathway and therefore thromboxane synthesis, thus impairing the thromboxane-dependent blood coagulation and also decreasing the coagulation factor II and VII. It was suggested that high doses (800-1200 α-tocopherol equivalents) should be avoided for two weeks prior to and following surgery. In a critical comment on the high UL for vitamin E of 1000 mg/day derived by the US Food and Nutrition Board attention was drawn to the observation that the tendency to haemorrhage in aspirin users is increased by vitamin E (Liede *et al*., 1998).

The effects on blood clotting are not, however, the only adverse effects requiring consideration. Side effects reported in therapeutic use of vitamin E supplements include severe muscular weakness and fatigue induced in adults receiving daily doses of 720 mg α-tocopherol. These side effects were confirmed in a double-blind study on two healthy male subjects given the same dose of α-tocopherol and the symptoms were associated with a large increase in 24 hr urinary creatinine and elevated serum creatine phosphokinase.

When patients with porphyria cutanea tarda were given daily doses of 1.0 g α-tocopherol for 3 months there was a marked increase in 24 hour urinary androgens (androsterone, etiocholanolone plus dehydroepiandrosterone) from 3.5 to 4.6 mg/day while mean 24 hour pregnanediol fell from 2.2 to 0.5 mg/day. The authors concluded that the significance of these endocrine changes was uncertain but could be important for patients with endocrine sensitive tumours.

A group of 52 elderly patients (average age 72 years) showed an average increase in serum cholesterol of 74 mg/dL when given repeated daily doses of 300 mg α-tocopherol. Conversely, no such increase was seen in a small group of healthy men taking 588 mg α-tocopherol (800 I.U.) daily.

There are limited data relating to the effects of vitamin E on morbidity and mortality from chronic diseases. In the ATBC study (ATBC study group 1994) an increase was observed in the numbers of deaths from haemorrhagic stroke among male smokers. Although the number of haemorrhagic stroke cases with 50 mg α-tocopherol was 66 compared to 44 in the control group (total n = 29,133) no statistical significance was published. A more recent analysis of this study indicated that there was an increased risk of subarachnoidal haemorrhage in hypertensive men (RR 2.45; CI 1.08-5.55) and a significantly higher mortality. Gingival bleeding occurred more frequently in subjects who were also taking aspirin. In two other studies, the Secondary Prevention with Antioxidants of Cardiovascular Disease in endstage renal disease (SPACE) and the Primary Prevention Project Collaborative Group of the Primary Prevention Project, (Boaz *et al*., 2000; de Gaetano, 2001) there was a non-statistically significant increase in fatal haemorrhages.

Evaluation

Vitamin E	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2000c)	1000	Suppl	haemorrhagic toxicity	animals
UK (UK Expert Group on Vitamins and Minerals, 2003)	540	Suppl	blood clotting	human
EU (European Commission Health & Consumer Protection Directorate-General, 2003d)	300	Total	blood clotting	human

US (2000)

Based on considerations of causality, relevance, and the quality and completeness of the database, the US selected haemorrhagic effects as the critical endpoint on which to base the UL for vitamin E. The human data fail to demonstrate consistently a causal association between excess α-tocopherol intake in normal, apparently healthy individuals and any adverse health outcome. The human data demonstrating the safety of supplemental α-tocopherol have been accumulated primarily in small groups of individuals receiving supplemental doses of 3,200 mg/day of α-tocopherol or less (usually less than 2,000 mg/day) for relatively short periods of time (weeks to a few months). Thus, some caution must be exercised in judgments regarding the safety of supplemental doses of α-tocopherol over multiyear periods.

The haemorrhagic effects seen in experimental animals are encountered only with very high doses of α-tocopherol and can be corrected by administration of supplemental vitamin K. A LOAEL of 500 mg/kg body weight/day was derived from a rat study (Wheldon *et al.,* 1983). Although this was a chronic study, the correction of vitamin K levels at week 24 means that the combined vitamin E-vitamin K effect was evaluated only on a subchronic basis. An uncertainty factor of 36 was used (2 LOAEL-NOAEL; 2 subchronic-chronic; 3 interspecies; 3 intraspecies).

With this uncertainty factor the US established a UL of 1,000 mg/day.

EU (2003)

The establishment of a NOAEL depends on the interpretation of asymptomatic effects on clinical biochemical parameters reported in some human studies and supported by similar effects in experimental animals. No NOAEL could be established from the chronic toxicity studies in the rat with respect to blood clotting and liver histology. The Committee decided that the critical effect is on blood clotting and that the study by Meydani et al (Meydani *et al.,* 1998) provided the best basis for an evaluation of the UL. The NOAEL established in this study was 540 mg/day. The Committee concluded that an uncertainty factor of 2 would adequately cover inter-individual differences in sensitivity. Therefore, EU established a UL for vitamin E of 270 mg/day for adults, rounded to 300 mg/day.

UK (2003)

In the trials by Gillilan *et al.* (Gillilan *et al*., 1977) and Meydani *et al.* (Meydani *et al.,* 1998) the biochemical and physiological effects of vitamin E were investigated in some detail and the findings indicate that supplemental doses of 800 to 1600 IU/day are without apparent adverse effect. The results were derived from small groups that may not be representative, thus an additional uncertainty factor could be applied to account for interindividual variation. However, the results of the larger CHAOS trial (2002 patients with atherosclerosis, 3-981 days of treatment with 800 IU/day for first 546 patients and 400 IU for remainder; (Stephens *et al*., 1996) support the view that 800 IU/day supplemental vitamin E would not result in any adverse effects and, taking the three studies together, no further uncertainty factors are necessary.

UK recommended an UL of 800 IU/day (540 mg *d*-α-tocopherol equivalents/day) supplemental vitamin E . This is equivalent to 9.0 mg/kg bw/day for a 60 kg adult. Assuming an intake of 18 mg/day from food, a total intake of 560 mg *d*-α-tocopherol equivalents/day would not be expected to result in any adverse effect. This is equivalent to 12.4 mg/kg bw/day.

Evaluation by FSANZ

The US evaluation is now somewhat dated since there are now additional clinical studies with larger patient groups and for longer duration available. Without going back to the original studies, it is not possible to comment on the reasons for establishing an uncertainty factor of 36.

The evaluation of both EU and UK are based on human studies. Both EU and UK decided that the Meydani study (Meydani *et al.,* 1998)was the most relevant clinical study, because it looked at an extensive range of relevant safety parameters. The NOAEL in this study is 540 mg/day. The disadvantage of this study is that the number of subjects was low (17-19 subjects/group) and the duration was only 4 months.

The EU in their evaluation has chosen a UF of two because they considered it would adequately cover inter-individual differences in sensitivity. A larger uncertainty factor was not considered necessary because data from a number of other, albeit older and less well-controlled studies showed no adverse effects at considerably higher intakes.

The UK did not consider a UF necessary because the CHAOS trial supports the view that 800 IU/day would not result in any adverse effect. This was slightly unusual since the UK report itself indicated that the CHAOS trial was limited in its capacity to detect adverse effects.

FSANZ is of the opinion that a UF of 2 is warranted because, on the basis of the available data, there is still some uncertainty associated with the level of 540 mg/day being safe for the whole population. The epidemiological studies indicated that severe adverse effects would not occur at the doses as administrated, but the studies were not designed specifically for assessing the safety assessment of vitamin E and thus more subtle effects would not be identified. The critical adverse effect indicated by the US, UK and EU reports is blood coagulation - this would not be assessed adequately in the prevention studies examined.

In conclusion, on the basis of the available data, FSANZ has established an UL of 300 mg/day for vitamin E, similar to the EU. There are no data specifically relating to children and adolescents. The UL for children and adolescents is derived by scaling the adult upper limit on the basis of body weight.

In summary, the UL for **vitamin E (as α-tocopherol equivalents)** for the various age groups are:

1-3 years	**70 mg/day**
4-8 years	**100 mg/day**
9-13 years	**180 mg/day**
14-18 years	**250 mg/day**
adult	**300 mg/day**

Dietary intake

Intakes of vitamin E were calculated at baseline, and assuming formulated beverages were consumed in Scenario 2. Estimated intakes were adjusted based on second day intake data from the NNSs.

The concentration of vitamin E requested to be added to formulated beverages was 2.5 mg/600 ml reference quantity.

Concentrations of vitamin E for Australia were not available in the 1995 NNS, therefore, concentrations from the 1997 New Zealand NNS were matched to the most relevant food in the Australian NNS to allow dietary modelling to be conducted.

Estimated intakes for vitamin E increased from baseline by between 1 and 3 mg/day, depending on the population group assessed. Estimated mean intakes are lower for Scenario 2 when it is assumed formulated beverages are consumed, compared to baseline for New Zealanders aged 19 years and over. This would be due to consumers substituting a formulated beverage for a beverage or beverages that were higher in vitamin E content than the formulated beverage. Estimated intakes of vitamin E did not exceed the UL for any population group assessed.

Table 13: Estimated dietary intakes of Vitamin E, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake mg/day (%UL)		**95th percentile intake mg/day (%UL)**	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	6.0 (9)	6.9 (10)	9.2 (15)	10.7 (15)
4-8 years, Aus	7.0 (7)	8.3 (8)	10.8 (10)	12.6 (15)
5-6 years, NZ	^6.6 (**)	NA	#6.3 (**)	NA
7-10 years, NZ	^7.5 (**)	NA	#7.3 (**)	NA
9-13 years, Aus	9.1 (5)	10.8 (6)	13.8 (8)	16.1 (9)
11-14 years, NZ	^9.4 (**)	NA	#8.9 (**)	NA
14-18 years, Aus	9.5 (4)	11.5 (5)	15.4 (6)	18.7 (7)
15-18 years, NZ	10.2 (4)	9.9 (4)	15.6 (6)	15.3 (6)
≥19 years, Aus	9.6 (3)	10.6 (4)	16.1 (5)	17.6 (6)
≥19 years, NZ	10.0 (3)	9.5 (3)	15.9 (5)	15.5 (5)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Toxicological evaluation and dietary intake data indicate that both children and adult Australian and New Zealand consumers, are unlikely to approach the UL for vitamin E, at the 95th percentile of dietary intake, either at baseline or when included in formulated beverages (15% UL for children 2-3 years and 6% UL and 5% UL for adults from Australia and New Zealand, respectively). Therefore, dietary intake of vitamin E for all consumers is considered to be within the safe range of intake for both mean and high consumers.

In conclusion, the addition of vitamin E to formulated beverages at a level of 2.5 mg alpha-tocopherol equivalents in a 600 ml serve poses no appreciable public health and safety risk.

Biotin

Hazard identification and characterisation

Chemistry

D-Biotin (biotin, coenzyme R, vitamin H) is a water-soluble vitamin. It has a bicyclic ring structure. One ring contains an ureido group and the other contains a heterocyclic sulphur atom and a valeric acid side-group.

Function

Biotin acts as an essential cofactor for the acetyl-CoA, propionyl-CoA, ß-methylcrotonyl-CoA and pyruvate carboxylase enzymes, which are important in the synthesis of fatty acids, the catabolism of branched-chain amino acids and the gluconeogenic pathway. Biotin may also have a role in the regulation of gene expression arising from its interaction with nuclear histone proteins.

Sources of biotin

Biotin is widely distributed in natural foodstuffs but at very low levels compared to other water-soluble vitamins. Foods relatively rich in biotin include egg yolk, liver, kidney, muscle and organ meats, and some vegetables.

Absorption, distribution, metabolism and excretion

Biotin uptake from the small intestine occurs by a carrier-mediated process that operates with a high carrier affinity and also by slow passive diffusion. The carrier is driven by an electron-neutral sodium (Na+) gradient, has a high structural specificity and is regulated by the availability of biotin, with upregulation of the number of transporter molecules when biotin is deficient.

The colon is also capable of absorbing biotin via a similar transport mechanism. Approximately 80% of biotin in plasma is in the free form and the remainder is either reversibly or covalently bound to plasma proteins. The existence of a specific biotin carrier protein in plasma is a subject of debate. Factors determining the bioavailability of biotin present in the diet are uncertain.

There are few data concerning the bioavailability of crystalline biotin supplements, but a recent study has suggested that doses as high as 22 mg may be completely absorbed. The nutritional significance of biotin synthesis by bacteria present in the lower gut is a subject of controversy.

Uptake into tissues occurs by specific transport mechanisms dependent upon Na+ gradients. Transplacental transport is thought to involve the active accumulation of biotin within the placenta followed by its passive release into the foetal compartment. Biotin is metabolically trapped within the tissues by its incorporation into carboxylase enzymes. In the normal turnover of cellular proteins, carboxylase enzymes are broken down to biocytin or oligopeptides containing lysyl-linked biotin. Biotin may be released for recycling by the hydrolytic action of biotinidase. Liberated biotin may be reclaimed in the kidney against a concentration gradient. Biotin not incorporated into carboxylase enzymes may be metabolised oxidatively at the sulphur present in the heterocyclic ring and/or at the valeric acid side chain.

Biotin metabolites are not active as vitamins and are excreted in the urine. Very little biotin is thought to undergo biliary excretion and the substantial amounts of biotin that appear in the faeces are derived from colonic bacteria.

Toxicity

The US considered the data on adverse effects from high biotin intake not sufficient for a quantitative risk assessment and a UL could not be derived. Several studies involving high biotin intakes reported no adverse effects. No adverse effects have been reported after intravenous administration of 50 mg of biotin to haemodialysis patients. Also no adverse effects have been reported in mother and infant after administration of 120 mg/day of biotin during the ninth month of pregnancy. Some case studies with 10 mg/day of biotin also did not report any adverse effects.

The animal toxicity database for biotin is very limited, especially when given by the oral route.

Evaluation

Biotin	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000b)	No UL		Insufficient data	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	0.9	Suppl	Insufficient data	human
EU (European Commission Health & Consumer Protection Directorate-General, 2001a)	No UL		Insufficient data	human

* Guidance level

No upper daily limit for biotin is set in either the EU (European Commission Health & Consumer Protection Directorate-General, 2001a) or the US (US Institute of Medicine, 2000b), based on the lack of data and no reported adverse effects in humans and animals. .

The EU characterised the risk of human toxicity from the usual dietary intake of biotin and from biotin supplements to be low according to available data. There are insufficient data to draw any conclusions concerning the safety of very high-level supplements.

Although no numerical UL can be established, existing evidence from observational studies indicates that current levels of intake from biotin in the EU from all sources do not represent a health risk for the general population

Based on the data considered in the US and EU evaluation, the available data on **biotin is too limited to set an UL.**

Dietary intake

There were no food composition data available to enable a comprehensive dietary intake assessment to be conducted for biotin. Whilst there are small amounts of data available, these data were either not from Australian or New Zealand sources, were not extensive enough across the whole diet, or were not in the correct format or had not been assessed for accuracy.

Risk characterisation

Due to insufficient data it was not possible to establish an UL for biotin. However this does not mean that there are hazards associated with high intakes of biotin. For biotin only limited food composition data are available therefore it is not currently possible to undertake a complete dietary intake assessment for biotin.

In the absence of sufficient information on potential adverse effects and food composition data it is currently not possible to evaluate the safety of the addition of biotin to formulated beverages.

Pantothenic acid

Hazard identification and characterisation

Chemistry

Pantothenic acid consists of a pantoic acid moiety amide-linked to a ß-alanine subunit. Pantetheine consists of pantothenic acid linked to a ß-mercaptoethylamine group. In living systems, the compound is a component of coenzyme A (CoA), which is composed of 4'-phosphopantetheine linked to adenosine 5'-monophosphate, modified by a 3'-hydroxyl phosphate. 4'-Phosphopantetheine is also found covalently linked to various proteins, particularly those involved in fatty acid metabolism.

Function

Pantothenate, usually in the form of CoA-containing species (e.g. acetyl CoA, succinyl CoA), fulfils multiple roles in cellular metabolism and in the synthesis of many essential molecules.

Sources of pantothenic acid

Pantothenic acid is widely distributed among foods, especially high concentrations are found in yeast and organ meat (liver, kidney), eggs, milk, whole grain cereals and vegetables. In most foods it is present in bound form (as CoA), requiring enzymatic treatment for analysis of total contents.

Absorption, distribution, metabolism and excretion

Pantothenic acid is readily absorbed throughout the gastrointestinal tract. Ingested CoA is hydrolysed within the intestinal lumen, via the formation of dephospho-CoA, phosphopantetheine and pantetheine, to pantothenic acid. Uptake of these latter two compounds into intestinal tissues has been demonstrated, and subsequently the enzyme, pantetheinase, can hydrolyse pantetheine to pantothenic acid. Uptake into intestinal cells occurs both by a sodium-dependent active transport mechanism and by passive diffusion. Limited data are available regarding the bioavailability of dietary pantothenic acid. One study found that pantothenic acid in natural foods was approximately 50% bioavailable compared with calcium pantothenate given in a formula diet, as assessed by subsequent urinary excretion of the vitamin.

Absorbed pantothenic acid is transported to body tissues via the blood, primarily as bound forms within erythrocytes. Plasma levels do not correlate well with dietary intake. The majority of tissues import pantothenic acid via an active sodium co-transport mechanism. Analysis of rat tissues has shown high concentrations of pantothenic acid in the heart and kidneys. CoA is synthesised from pantothenic acid within cells, with the first, and apparently rate-limiting, step catalysed by pantothenate kinase.

Catabolism of CoA leads to the formation of pantothenate, which is excreted in the urine. Excretion levels correlate well with dietary intake.

Toxicity

The available toxicological data on pantothenic acid are limited. However, case reports and some earlier, uncontrolled human studies suggested a lack of acute or chronic toxic effects of pantothenic acid compounds (calcium or sodium pantothenate, panthenol) at very high doses (approximately 10,000 mg/day, in some cases for a number of years). However, doses at such levels have been associated with diarrhoea and gastrointestinal disturbances. In more recent, controlled studies, no side effects have been reported with pantothenic acid supplementation at levels up to approximately 2000 mg/day, for periods varying from several days to several weeks. These studies were generally designed to assess the potential benefits of pantothenic acid supplementation in specific subgroups, for example patients suffering joint disease.

Data regarding the toxicity of pantothenic acid and its commonly used pharmaceutical forms in experimental animals are also limited. However, doses of 500 and 2000 mg/kg bw/day in rats and 200-250 mg/kg bw/day in dogs and monkeys, given in the diet for periods of six months, were not associated with adverse effects.

Evaluation

Pantothenic acid	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2000b)	N/A		Insufficient data, no adverse effects	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	200	Suppl	Insufficient data-	human
EU (European Commission Health & Consumer Protection Directorate-General, 2002b)	N/A		Insufficient data, no adverse effects	human

N/A not applicable
* Guidance level

No UL for pantothenic acid is set in either the EU (Scientific Committee on Food, 2003) or the US (United States Institute of Medicine, 2000a), based on the lack of data and no reported adverse effects in humans and animals.

Based on the data considered in the US and EU evaluation, **pantothenic acid** has a very low oral toxicity, and therefore an **UL does not need to be established**.

Permitted forms

The Applicant requested the following forms to be permitted for pantothenic acid: calcium pantothenate and dexpanthenol. Both forms are already permitted in Standard 2.9.1 – Infant Formula Products.

Within the assessment of pantothenic acid toxicity by the EU the following was stated on the various forms of pantothenic acid. *Pantothenic acid (MW 219.23) is the only occurring natural form. Free pantothenic acid and its sodium salt are chemically unstable, and therefore the usual pharmacological preparation is the calcium salt (calcium pantothenate). The alcohol, panthenol, is a synthetic form which can be oxidised in vivo to pantothenic acid*.

Dexpanthenol is a synonym of panthenol (The Merck Index, 2001).

In conclusion, the available evidence indicates that both calcium pantothenate and dexpanthenol are appropriate as permitted forms for pantothenic acid.

Dietary intake

No dietary intake estimates were calculated for pantothenic acid, as it was determined to have very low oral toxicity, and no ULs have been established, as outlined above.

Risk characterisation

No UL is established for pantothenic acid, based on the lack of adverse effects even at high doses. Therefore, no dietary modelling was required.

In conclusion, the addition of pantothenic acid to formulated beverages at a level of 1.25 mg per 600 ml serve poses no appreciable public health and safety risk.

Calcium

Hazard identification and characterisation

Chemistry

Calcium is an alkaline earth metal belonging to Group II of the periodic table. It is a divalent cation with an atomic weight of 40. Calcium shows a single oxidation state of +2. It is the fifth most abundant element in the human body.

Function

In the vertebrate skeleton, calcium provides rigidity in the form of calcium phosphate, embedded in collagen fibrils. Calcium is also a key component in the maintenance of cell structure. Membrane rigidity, viscosity and permeability are partly dependent on local calcium concentrations. Calcium fulfils important physiological roles as a cofactor for many enzymes, as an important component of the blood clotting mechanism and through an active role as an intracellular signal. This signalling controls events such as cell aggregation, muscle contraction and cell movement, secretion, transformation and cell division, as well as muscle protein degradation.

Sources of calcium

Calcium must be ingested with the diet in sufficient amounts to allow for calcium deposition during bone growth and modelling and to compensate for obligatory intestinal, daecal and dermal losses during the life-time.

Foods vary widely in calcium content. The best sources are milk and milk products, from which about 32% is absorbable. Some plants are good sources of well-absorbable calcium, e.g. brassica, almonds, dried apricots. However, some vegetables contain considerable amounts of calcium, which is poorly absorbed because of a high content in oxalate (rhubarb, spinach) and which forms sparingly soluble calcium oxalate. Drinking water and mineral waters can also be good sources of absorbable calcium.

Within populations and population groups dietary calcium intakes show a great variability related to varying dietary habits.

In Australia and New Zealand various products can be voluntary fortified with calcium, such as breakfast cereals, certain milk products and analogues for milk products at 25 to 50% pf the recommended daily intake according to Standard 1.3.2 – Vitamins and Minerals.

Furthermore, FSANZ is currently assessing the extension of voluntary fortification of certain food groups with calcium in Application A424 – Fortification of Foods with Calcium and A500 – Addition of Calcium to Cereal Based Beverages.

Absorption, distribution, metabolism and excretion

About 25 - 50% of dietary calcium is absorbed and delivered to the exchangeable calcium pool. Most of the calcium in food is in the form of complexes with other dietary constituents, which must be broken down and the calcium released in a soluble and ionised form before it can be absorbed.

Calcium crosses the intestinal mucosa by both active and passive transport mechanisms. The active transport mechanism is a saturable, transcellular process which involves the calcium-binding protein, calbindin. Calbindin is regulated by the hormonal form of vitamin D (1,25-$(OH)_2D_3$). The passive transport mechanism is a nonsaturable, paracellular process which is not affected by calcium status or parathyroid hormone. The efficiency of calcium absorption increases when calcium intakes are low and decreases when calcium intakes are high.
Two major factors affect the efficiency of calcium absorption. Firstly, interactions with other dietary constituents can affect calcium absorption. Secondly, absorption is regulated by physiological factors, including hormones. Compounds enhancing calcium absorption include fibre, lactose, vitamin D. Dietary factors antagonising calcium absorption include vitamin D deficiency, calcium-phosphorus imbalance, phytic acid, oxalic acid, dietary fibre and excessive fat.

Fractional calcium absorption is highest (about 60%) in breastfed infants. Net calcium absorption, defined as intake minus faecal excretion in percent of intake, is lower in infants fed cows' milk formula, decreases in young childhood, shows a rise in puberty, decreases to 15 to 20% in young adults and declines gradually thereafter. Calcium absorption is increased in pregnant and lactating women compared to non-pregnant women.

The calcium content of the human body is 25 to 30 g at birth (0.8% of the body weight) and between 900 and 1300 g in adult men (up to 1.7% of body weight). Of this, 1% is located in the serum, lymph and other fluids and the remaining 99% is located in the bone (as hydroxyapatite) and teeth. The cellular regulation of calcium concentration is also important. The concentration of ionised calcium in serum is closely regulated to within 10% of approximately 2.5 mmol/l.. Calcium is present in blood in three different forms: as free Ca^{2+} ions, bound to protein (about 45%), and complexed to citrate, phosphate, sulphate and carbonate (about 10%).

Distribution of the free ionised calcium is dependent upon interactions between three major hormones, PTH, calcitonin and vitamin D. Additionally, other hormones affect calcium metabolism including oestrogen, testosterone, glucocorticoids, thyroid hormones, growth hormone and insulin.

The majority of absorbed calcium is stored in the skeleton. Excess absorbed calcium is excreted in urine, faeces, and to a lesser extent, sweat. Calcium balance is positive in healthy children, adolescents and young adults before bone growth and modelling cease, provided that they have an adequate calcium intake.

Renal calcium excretion is the result of glomerular filtration (about 8 to 10 g calcium per day in adults) and tubular reabsorption (normal over 98% of the filtered load), which is primarily passive in the proximal tubules and for 20% active in the distal part of the convoluted tubules and connecting tubules.

Active transport is under the control of parathyroid hormone, calcitonin and $1,25(OH)_2D$. Renal excretion is not strongly related to dietary calcium intake in healthy persons.

Toxicity

This section of the assessment is mainly based on the EU assessment report (European Commission Health & Consumer Protection Directorate-General, 2003a).

Acute hypercalcaemia can impair renal function by causing vasoconstriction with consequent decreases in both the renal blood flow and glomerular filtration rate. Hypercalcaemia increases absorption of bicarbonate in the proximal tubule, thus predisposing the patient to metabolic alkalosis. Chronic hypercalcaemia, hyperphosphataemia and metabolic alkalosis promote irreversible renal calcification.

Calcium levels in the body are under control of genetic and hormonal factors. Therefore an excessive accumulation of calcium in blood or tissue solely through excessive calcium consumption should not occur in the absence of diseases such as bone cancer, hyperthyroidism, and hyperparathyroidism or in the absence of excessive vitamin D intake. Adverse effects which have been reported due to high calcium intakes include the so-called milk-alkali syndrome, the formation of kidney stones in persons with a propensity for nephrolithiasis, hypercalciuria and for hyperabsorption of calcium, and interference with the absorption of other minerals

Kidney function

Some peri-menopausal women with total calcium intakes between 2 and 3 g/day may show a tendency for compromised glomerular function as indicated by increases in serum creatinine. No such effect was observed in another study with women receiving comparable calcium amounts. This finding should be investigated systematically before it is attributed to calcium.

Milk-alkali syndrome

Manifestation of the milk-alkali syndrome through the combined intake of calcium both from food and especially from supplements and of absorbable alkalinising substances is facilitated by renal insufficiency, alkalosis and dehydration due to vomiting and anorexia and/or the use of thiazide diuretics, which increase renal tubular calcium reabsorption. All reported cases of milk-alkali syndrome in association with the prolonged or acute ingestion of calcium supplements used calcium carbonate as the nutrient source. In these reports the supplemental calcium intakes were reported as between 1.0 and 23 g/day. Their dietary calcium intakes are often not known. The US (1997) has taken the approximate median of 4.8 g of reported calcium supplements as the LOAEL for total calcium intake, applied an uncertainty factor of 2 and defined an UL of 2.5 g calcium/day. The EU considered this LOAEL inappropriate. Seven low-supplement users are reported not to have an additional high dietary calcium intake (>0.9 g/day). It is questionable if it is justified to derive a LOAEL for the total dietary calcium intake from data on effects of alkalinising substances plus calcium.

The use of calcium carbonate supplements in doses up to 2000 mg/day, and thereby achieving total daily calcium intakes up to more than 3000 mg/day, for preventive purposes in presumably healthy subjects, has not provoked the development of the milk-alkali syndrome, whereas the administration of large amounts (11.2 g calcium/day) of calcium carbonate in addition to large amounts of milk (1.8 g calcium/day) over 7 days to 20 gastric/duodenal ulcer patients resulted in reversible hypercalcaemia (2.8 mmol/L) in nine patients and renal insufficiency in all. The control group of 20 patients with gastric/duodenal ulcers who received aluminium hydroxide and milk for the same duration did not develop these abnormalities.

Cases of milk-alkali syndrome have been reported with long-standing calcium intakes in the range of 2 to 2.5 g/day with chronic high intakes of antacids and of low supplemental calcium intakes (1g/day) in addition to unknown dietary intakes plus sodium bicarbonate. These observations seem to indicate that the harmful calcium dose can be lower than 3 g/day if taken together with alkali.

The EU concluded that on the basis of the available evidence, a calcium dose, which by itself might cause milk-alkali syndrome, could not be identified.

Kidney stones

The quantitative relationship between calcium intake, both from the diet and from supplements, and hypercalciuria as a risk factor for nephrolithiasis is far from clear. Also, it is dependent on other dietary factors, especially sodium intake. From epidemiologic studies it appears that dietary calcium intakes in the range of recent recommendations have a favourable effect in the prevention of kidney stone formation and that lower intakes increase the risk. From the available data no conclusion is possible on a detrimental calcium dose in individuals with idiopathic hypercalciuria (up to 6% of the population). From the study in patients with kidney stones and idiopathic hypercalciuria it can be deduced that a sodium restricted diet with a normal recommended calcium content of 1200 mg/day does not raise urinary calcium excretion but reduces it.

In conclusion, both observational studies on the relationship between total calcium intake and kidney stone incidence and interventional studies with calcium supplements do not allow definition of a calcium intake on a population basis which promotes kidney stone formation.

Interaction with minerals

The studies of acute effects of single calcium supplements at various doses and from various sources on iron and zinc absorption cannot be converted into general statements on a dose dependent negative effect of total daily dietary calcium intake, because the timing of the supplement and other interfering factors of the diet have to be taken into account.

The EU concluded that single-dose experiments demonstrate interference of both dietary and supplemental calcium with the absorption of other minerals. This effect is not demonstrable in long-term observational and interventional studies at dietary calcium intakes in the range of recommended intakes and at supplemental calcium of up to 2000 mg/day in adults and up to 1200 mg/day in one study with infants.

Vulnerable groups

Persons at risk from developing milk-alkali syndrome include those using drugs such as thiazide and those with renal failure. These groups should be identified and monitored for alkalosis and hypercalcaemia when using calcium supplements. This would be particularly important for patients with renal failure who already receive calcium carbonate therapy to control serum phosphorous levels.

Patients with absorptive or renal hypercalciuria, primary hyperparathyroidism and sarcoidosis may have a higher risk of renal stone formation following calcium supplementation.

It has been proposed that there may be an individual hypersensitivity to developing hypercalcaemia. This is because only a limited number of individuals develop the metabolic complications involved in MAS, and excessive calcium intake alone is not enough to induce hypercalcaemia.

Evaluation

Calcium	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000a)	2,500	Total	Milk-Alkali syndrome	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	1,500	Suppl	gastrointestinal	human
EU (European Commission Health & Consumer Protection Directorate-General, 2003a)	2,500	Total	evidence from different interventional studies	human

* Guidance level

FSANZ considers the EU assessment as the most comprehensive and complete and therefore the ULs are based on the EU assessment.

Adults

The UL for calcium is derived from different interventional studies of long duration in adults, some of which were placebo-controlled in which total daily calcium intakes of 2500 mg from both diet and supplements were tolerated without adverse effects. Because of the abundance of data the application of an uncertainty factor was considered unnecessary. An UL of 2500 mg of calcium per day for calcium intake from all sources is proposed. There are no data to suggest an increased susceptibility for pregnant and lactating women.

Children and adolescents

No adverse effects of calcium citrate-malate supplements (500 to 1000 mg calcium over 1.5 to 3 years) and of extra dairy foods or foods fortified with milk extracts (700 to 820 mg calcium extra over one year) were reported in 217 children between 6 and 14 and 6.6 and 11 years, respectively in comparison to unsupplemented controls.

These data are considered insufficient to derive an UL for children and adolescents. The EU decided that it was inappropriate to base the UL for calcium for this age group on the UL for adults of 2500 mg calcium/day, with correction for differences in basal metabolic rate using scaling according to body surface area (body weight$^{0.75}$). For calcium deposition in bone during the growth period proportionality to lean body mass cannot be assumed. Therefore, age-dependent ULs for children and adolescents cannot be proposed.

The EU concluded in their risk characterisation that, although there are no data to set a numerical UL for children and adolescents no appreciable risk has been identified even with current extreme levels of calcium intake in this age group.

In summary, the UL for **calcium** for the various age groups are:

1-18 years	**not necessary to set UL, no risks identified in children**
adults	**2500 mg/day**

Dietary intake

Intakes of calcium were estimated at baseline and when formulated beverages are consumed. Results are in Table 14. Estimated intakes were adjusted based on second day intake data from the NNSs.

The concentration of calcium requested to be added to formulated beverages was 200 mg / 600 ml reference quantity.

Estimated intakes increased from baseline by around 100 mg/per day when formulated beverages were consumed across the population groups assessed. The UL was not exceeded for adults in Australia or New Zealand.

Table 14: Estimated dietary intakes of calcium, before and after formulated beverages are introduced into the diet, and percent of UL for adults

	Mean intake MG/DAY (%UL)		95th percentile intake mg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	806	886	1257	1328
4-6 years, Aus	769	866	1253	1359
5-6 years, NZ	^675	NA	#960	NA
7-10 years, Aus	867	992	1440	1533
7-10 years, NZ	^730	NA	#1005	NA
11-14 years, Aus	927	1058	1633	1698
11-14 years, NZ	^839	NA	#1166	NA
15-18 years, Aus	963	1131	1928	2157
15-18 years, NZ	865	968	1604	1703
≥19 years, Aus	831 (35)	913 (35)	1555 (60)	1681 (65)
≥19 years, NZ	793 (30)	841 (35)	1397 (55)	1466 (60)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Toxicological evaluation and dietary intake data indicate that adult Australian and New Zealand consumers are unlikely to approach the UL for calcium, either at the mean or 95th percentile of dietary intake, at baseline or including formulated beverages (40% UL and 70% UL for Australia and 35% UL and 60% UL for New Zealand, respectively). Therefore, the estimated dietary intake of calcium for adults is considered to be within the safe range of intake for both mean and high consumers.

If intakes for children and adolescents are compared to ULs for adults, 95th percentile consumers in the highest intake group (15-18 year old Australians, 2157 mg/day) have an intake below the UL. High intake of calcium in adolescents would most likely be beneficial for bone health.
In conclusion, the addition of calcium to formulated beverages at a level of 200 mg in a 600 ml serve poses no appreciable public health and safety risk, assuming baseline levels of fortification in other foods and not taking supplement use into account.

Chromium

Hazard identification and characterisation

Chemistry

Chromium is a metallic element that can exist in a variety of oxidation states; oxidation states other than 0, +2, +3 and +6 are uncommon. Biologically, trivalent (III) and hexavalent (VI) chromium are most important. Chromium in foods or supplements are in the trivalent form.

Function

Trivalent chromium has been shown to potentiate insulin action and thereby influences carbohydrate, lipid and protein metabolism.

Sources of chromium

Chromium in foods or supplements is in the trivalent form. Processed meats, whole grain products, pulses and spices are the better sources of chromium, but chromium levels are low in staple foods.

Absorption, distribution, metabolism and excretion

Intestinal absorption of trivalent chromium is low (0.5-2.0%). The mechanism of absorption has not been clearly defined, but it appears to involve processes other than passive diffusion.

Absorbed trivalent chromium does not enter blood cells, but binds to plasma proteins such as transferrin and is transported to the liver. In contrast, hexavalent chromium does penetrate red blood cells, where it is reduced by glutathione to trivalent chromium, which binds to haemoglobin. Excess hexavalent chromium is taken up into the kidneys, spleen, liver, lungs and bone.

Ingested trivalent chromium remains largely unabsorbed and is excreted via the faeces. Absorbed chromium is mainly excreted via urine, with only small amounts being eliminated in perspiration and bile.

Toxicity

The data on oral chromium toxicity are limited. However, it is apparent that the toxicity of chromium varies depending on the valency state, with hexavalent (VI) chromium, being generally more toxic than trivalent (III) chromium. This assessment concentrates on the evaluation of trivalent chromium, as this is the form found in food and dietary supplements. Ingested trivalent chromium has a low level of toxicity, due partly to its poor absorption. Chromic acid at chronic doses of up to 750 mg chromium/kg bw/day given in food to adult animals for periods of up to 24 weeks was not associated with adverse effects. Absorption was not demonstrated in this study.

Chromium picolinate and chromium chloride were not associated with adverse effects at doses of 15 mg chromium/kg bw/day. Increased levels of tissue chromium indicated that absorption had occurred. Higher doses of chromium (approximately 100 mg/kg bw/day) are associated with reproductive and developmental effects, although these may be secondary to parental toxicity. In general, hexavalent chromium has given positive results in *in vitro* mutagenicity tests, whereas trivalent chromium compounds have been negative.

Limited data from human supplementation studies have indicated that doses up to 1 mg/day of trivalent chromium compounds in general were not associated with adverse effects, although it is unclear what adverse effects were evaluated. The human studies were conducted in a variety of small groups and investigated a range of different endpoints, so limited conclusions may be drawn from these.

Evaluation

Chromium	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2001b)	no UL	total diet	insufficient data	human
UK(UK Expert Group on Vitamins and Minerals, 2003)*	10	total diet	no adverse effects	animal
EU (European Commission Health & Consumer Protection Directorate-General, 2003c)#	no UL	total diet	insufficient data	human
WHO (WHO, 1996)	0.25	supplementation	no adverse effect	human

* Guidance level, applies for trivalent chromium only. Chromium picolinate is excluded from this guidance level.
# not applicable for chromium picolinate.

Both the EU and US concluded that the data were too limited to derive an UL.

Adequate human data on trivalent chromium is limited. No adverse side effects were reported in a number of supplementation trials, in which subjects received up to 1 mg chromium/day, mostly as picolinate for several months.

These trials, however, were mainly studies of efficacy and not designed to find potential toxic effects. The limited data from studies on subchronic, chronic, and reproductive toxicity on soluble trivalent chromium salts and the available human data do not give clear information on the dose response relationship. Therefore, an UL can not be derived.

The UK also concluded that overall there are insufficient data from human and animals studies to derive a safe UL for chromium. However, in their opinion a total daily intake of about 0.15 mg trivalent chromium per kg body weight and day (or 10 mg/person) would be expected to be without adverse health effects. This value was based (using a 100-fold margin of safety) on a 24-week rat study, which indicated that 15 mg trivalent chromium/kg bw/day is not associated with adverse effects.

Based on the data considered in the US and EU evaluation, there are **insufficient data to establish a UL** for **soluble chromium III salts**.

Dietary intake

There were no food composition data available to enable a comprehensive intake assessment to be conducted for chromium. Whilst there are small amounts of data available, these data were either not from Australian or New Zealand sources, were not extensive enough across the whole diet, were not in the correct format or had not been assessed for accuracy.

Risk characterisation

Due to insufficient data it is not possible to establish an UL for chromium, however this does not mean there are no hazards associated with high intakes of chromium. For chromium, limited food composition data are available therefore it is not possible to undertake a complete dietary intake assessment for chromium at the present time.

In the absence of sufficient information on potential adverse effects and food composition data it is currently not possible to evaluate the safety of the addition of chromium to formulated beverages.

Copper

Hazard identification and characterisation

Chemistry

Copper has two valency states, cuprous (copper I) and cupric (copper II). It occurs in nature mainly in the form of its oxide, Cu_2O and sometimes as the chloride, $CuCl_2$ which, in the presence of humidity and oxygen, is oxidised to the basic copper (II) chloride, Cu(OH)Cl. The most important copper compounds in the aquatic environment are cupric chloride, cuprous nitrate and cupric sulphate.

Function

Copper is an essential micronutrient normally subject to effective homeostatic control. It is involved in the function of several enzymes, including cytochrome c oxidase, amino acid oxidase, superoxide dismutase and monoamine oxidase.

Copper is thought to be required for infant growth, host defence mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism, myocardial contractility and brain development.

Sources of copper

The main dietary sources of copper are shellfish, fish, liver, meats, nuts and chocolate. A lower concentration is found in legumes, grains, human milks, and especially cows milk. In drinking water in both New Zealand and Australia the guidance level, based on aesthetic considerations is 1 mg/L, with typical concentrations of about 0.05 mg/L (Ministry of Health, 2000; NHMRC and NRMMC, 2004).

Administration, distribution, metabolism and excretion

In mammals, absorption of copper occurs primarily in the small intestine. The efficiency of absorption of the metal ion is high; values for apparent absorption by adult humans average between 55% and 75% and do not drop appreciably with age. Actual absorption rates for rats are lower at 30–50%. Data from animal studies as well as from human studies indicate that the actual proportion of ingested copper that is absorbed will increase if copper intake is low, and vice versa. Studies with rats have confirmed that this also holds true with extreme intakes of copper where copper intake ten times normal will result in a copper absorption as low as 10%. This data is indicative that copper levels in the body are under homeostatic control.

Copper is absorbed across the brush border in the cells of the intestinal mucosa and is then subsequently transferred across the basolateral membrane into the interstitial fluid and blood. The basolateral membrane is the site where competition for absorption between copper and other transition metal ions takes place. Abnormally high concentrations of zinc, and possibly also iron, directly or indirectly inhibit uptake and transfer of copper from the diet to the blood.

On entering the interstitial fluid and blood plasma from the intestinal cells, copper initially becomes bound to two proteins, albumin and transcuprein, in the portal blood and general circulation. These two proteins appear to be the primary components of the exchangeable plasma copper pool. Albumin is responsible for binding about 18% of the ionic copper in human plasma. The rest of the copper is bound to ceruloplasmin (~65%), transcuprein (~12%) and components of low molecular weight. Ceruloplasmin copper is not part of the exchangeable copper pool; copper is added during the synthesis of ceruloplasmin by the liver.

Most of the bound copper is then rapidly deposited in liver hepatocytes, with lesser amounts entering the kidney. Appreciable copper uptake by other tissues is only seen once ceruloplasmin, bearing newly absorbed copper, is secreted into the plasma.

Therefore, there appears to be two phases of copper distribution. The first phase, mediated primarily by transcuprein involves transport into the liver (and kidney) and the second phase, mediated by ceruloplasmin involves distribution of copper to the other tissues.

On entering the cells, copper normally finds its way readily to the sites where it is needed. Most of the copper appears to be active or in transit with little or no excess copper stored.

In most mammals, copper is excreted easily. The rate of excretion appears to be the main process for maintaining copper homeostasis.

Of the net copper that is absorbed and lost daily by human adults, only a tiny fraction enters the urine. The major excretory route appears to be the bile. Bile has the highest copper concentration of the body fluids and it has been estimated that about 2.5 mg of copper per day is secreted into the gastrointestinal tract. However, other fluids secreted in the gastrointestinal tract also contain copper, and together these contribute about another 2 mg per day. All but 0.5–1.0 mg of the total copper must be reabsorbed every day to maintain the status quo. Evidence indicates that most of the non–reabsorbed copper comes from bile and, therefore, it seems likely that it is the main route of net copper excretion. The biliary route may also be a more prominent route of copper excretion when large doses of copper enter the body acutely.

Toxicity

FSANZ[4] reviewed copper toxicity in 1999 as part of Proposal P157 – Metal Contaminants (ANZFA, 1999). Since then new evaluations have become available and therefore the safety of copper has been revisited.

The compartmentalisation and metabolism of copper is highly regulated through homeostatic mechanisms. Toxicity is likely to occur only when such homeostatic control within any particular compartment is overwhelmed and/or basic cellular defence or repair mechanisms are impaired. The essentiality and potential toxicity of copper in biological systems results from the chemical properties of the copper ion. Copper is fairly reactive and able to bind strongly to many types of electron rich structures. In excess, this property can cause a number of adverse reactions such as cellular injury due to the production of oxygen radicals, structural impairment of essential metal binding sites by the displacement of metal in receptors and transporter proteins, and functional impairment of DNA and other macromolecules through direct binding of copper.

Reviews of the toxicity studies in experimental animals indicate that these studies are not useful for setting an upper limit for humans. Very few of these studies used chronic exposure, only one or two doses were used, and the reporting of experimental details and results was incomplete. In addition, some studies used routs of exposure that are not relevant to human intake. Finally, animal species vary markedly in their sensitivity to copper; thus it is difficult to determine the most appropriate model in which to assess human toxicity to copper.

Acute copper toxicity is infrequent in humans, and is usually a consequence of ingesting contaminated foodstuff and beverages or from accidental or intentional ingestion of high quantities of copper salts. Case reports of single oral exposures to high levels of copper have been reported. Such exposures, including suicide attempts with $CuSO_4$, have occurred in youths and adults at doses ranging from 0.4 to 100g Cu.

Symptoms include vomiting, lethargy, acute haemolytic anaemia, renal and liver damage, neurotoxicity, increased blood pressure and respiratory rates. In some cases, coma and death followed.

[4] as the Australia New Zealand Food Standards Authority (ANZFA)

For the general population, the majority of reports of chronic copper toxicity relate to exposure through contaminated drinking water and these are usually confounded by lack of characterisation of the microbiological quality of the water supplies and limitations in reporting.

One study reported that recurrent episodes of gastrointestinal illness in certain members of a family could be attributed to a copper main. The median level in the incoming water was 3.07 mg/L; a single maximum level taken was 7.8 mg/L. No estimated dose was provided or able to be inferred from this data. No symptoms were observed in two other families of similar age and sex distribution exposed to lower levels (medians, 1.58 and 0.02 mg/L). Symptoms ceased with a change in the water source.

Another study described a case of micronodular cirrhosis and acute liver failure in a 26 year old male who consumed copper tablets at 30 mg/day for two years followed by 60 mg/day for an unspecified period before presenting with symptoms of liver failure. Laboratory investigations revealed normal serum copper levels (22.6 mmol/L) and serum ceruloplasmin (0.27 mmol/L) but very high urinary excretion of copper (207 mmol/24 hour) compared to normal (<1.2 µmol/24 hour). Mean copper content of the liver was 3230µg/g (normal range 20–50µg/g). Histology of the liver resembled that of ICC and Wilson disease. This case gives some indication of a level of chronic copper consumption in humans that may lead to toxicity, assuming that the individual concerned did not have a predisposition to copper toxicity.

Very little information is available on the reproductive and developmental toxicity of copper to humans. An epidemiological study of a population of Massachusetts's women found no association (after adjusting for confounding variables) between the occurrence of spontaneous abortion and exposure to copper in drinking water (>1 mg/litre) during 1976–1978. In a small trace element status study, a significant positive relationship between placental copper and birthweight and a negative correlation between the copper/zinc ratio and birthweight were found. This data is inadequate to assess the reproductive or developmental effects of copper in humans.

A number of epidemiological studies on cancer in the general population have been done. These studies have generally relied on serum copper concentrations as an indicator of an individuals copper status. It is questionable whether serum copper concentrations accurately reflect copper intake as it has been reported that in cases of chronic copper overload plasma copper concentrations are not elevated, and in one of the studies reported no significant correlation could be found between copper intake and copper blood level. These studies are therefore uninformative with respect to the possible aetiological role of copper in the disease.

Vulnerable groups

As copper is an essential metal, there are homeostatic mechanisms to maintain copper levels within defined limits. However, a number of disorders in homeostatic mechanisms can result in toxicity from exposure to copper at levels which are tolerated by the general population.

This form of copper toxicity is observed principally in patients with Wilson's disease and from the occurrence of infantile cirrhosis in areas of India (ICC, Indian Childhood Cirrhosis), and isolated clusters of cases in Germany, Austria and other countries (ICT, Idiopathic Copper Toxicosis) that have also been related to excess copper intake.

Wilson's disease is a condition with a well-defined genetic basis with patients exhibiting impaired biliary excretion of copper, which is believed to be the fundamental cause of copper overload. Wilson disease patients typically present with hepatic and/or neurologic dysfunction. The worldwide incidence of Wilson's disease is 1 in 30,000 and the corresponding prevalence of the heterozygous and asymptomatic carrier of a mutated ATPase gene is 1 in 90 (European Commission Health & Consumer Protection Directorate-General, 2003b).
ICC and ICT are conditions related to copper excess which may be associated with genetically–based copper sensitivity although this has not been demonstrated unequivocally.

Evaluation

Copper	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2001b)	10	Total	hepatotoxicity	human
UK (UK Expert Group on Vitamins and Minerals, 2003)	10	Total	forestomach, kidney and liver damage	animal
EU (European Commission Health & Consumer Protection Directorate-General, 2003b)	5	Total	hepatotoxicity	human
WHO/FAO (WHO, 1996)	10 (f), 12 (m)	Total	hepatotoxicity	human
ANZFA (ANZFA, 1999)*	13	Total	hepatoxicity	human

* Provisional maximum tolerable daily intake (PTDI)

Daily intakes of copper ranging from 2 to 32 mg in drinking water have been reported to cause symptoms of general gastric irritation. This low limit in water is of interest given that an intake of 2 mg/day is equivalent to average intakes in Australia and New Zealand. This discrepancy may result from the fact that in water (and in supplements) copper is present in the ionic form whereas in food, copper is present in the form of organic compounds (ANZFA, 1999). While there is little doubt that the uncontrolled ingestion of soluble inorganic copper salts in milligram quantities should be regarded with caution, levels of copper in food up to around 13 mg/day (assuming a body weight of 70 kg; 0.2 mg/kg bw/day) seem to have no detrimental effect on human health (WHO, 1996; ANZFA, 1999). This will take account of the quantity likely to be consumed from the usual diet (<10 mg/day) and will limit both the amount of copper that can be introduced by dietary fortification and the quantity of contaminating copper that can be regarded as tolerable.

US derived a NOAEL of 10 mg/day of copper on the basis of a double-blind study, where 10 mg copper as copper gluconate capsules was consumed daily for 12 weeks. Liver function tests were normal. From a case report, consumption of 30 mg/day as copper tablets for 2 years, followed by 60 mg/day for an additional period of time, resulted in acute liver failure. The NOAEL of 10 mg/day was considered protective of the general population. The UL does not apply to individuals with Wilson's disease, Indian childhood cirrhosis or idiopathic copper toxicosis.

The EU based the NOAEL on the same study as the US evaluation, however an uncertainty factor of 2 was considered appropriate on the NOAEL of 10 mg/day to allow for potential variability within the normal population.

High doses of copper can result in hepatoxicity. This effect is considered to be the most sensitive adverse effect induced by copper and is relevant for establishing an UL. Based on the data considered in the US and FAO/WHO evaluation, a level of 10 mg copper/day has been adopted as an UL. This UL does not apply to individuals with Wilson's disease, ICC, or ICT. In summary the ULs for **copper** in the various age groups are:

1-3 years	**1.8 mg/day**
4-8 years	**3.0 mg/day**
9-13 years	**5.0 mg/day**
14-18 years	**8.0 mg/day**
adults	**10 mg/day**

Permitted forms

The Applicant requested the following forms to be permitted for copper: copper gluconate, cupric sulphate, cupric citrate and cupric carbonate. Copper gluconate, cupric sulphate and cupric citrate are already permitted in Standard 2.9.1 – Infant Formula Products, and cupric carbonate is permitted in Standard 2.9.4 – Formulated Supplementary Sports Foods.

Within the assessment of copper toxicity by both the EU (European Commission Health & Consumer Protection Directorate-General, 2003b)and US (US Institute of Medicine, 2001b) copper toxicity was focussed on the copper II forms. All requested copper forms have a valency state of II. All four forms are easily dissociated and therefore, the toxicity would be similar.

In conclusion, the available evidence does not indicate that the different forms of copper have differences in toxicity. Therefore, the requested forms of copper are appropriate as permitted forms for copper.

Dietary intake

Intakes of copper were estimated at baseline and when formulated beverages are consumed. Estimated intakes were adjusted based on second day intake data from the NNSs.

The concentration of copper requested to be added to formulated beverages was 0.75 mg / 600 ml reference quantity.

Copper was not included in the 1995 Australian NNS. Therefore, in order to estimate intakes for the Australian population, the concentration data from the New Zealand NNS were matched to the most appropriate Australian food codes, then these values were used to estimate dietary intakes for the Australian population groups.

Estimated intakes increased from baseline by around 0.5 mg/per day when formulated beverages were consumed across the population groups assessed. The UL was not exceeded by any population groups assessed.

Table 15: Estimated dietary intakes of copper, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake MG/DAY (%UL)		95th percentile intake mg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	0.9 (45)	1.1 (65)	1.2 (65)	1.8 (100)
4-8 years, Aus	0.9 (30)	1.3 (45)	1.3 (45)	2.0 (65)
5-6 years, NZ	^1.1 (**)	NA	#1.5 (**)	NA
7-10 years, NZ	^1.3 (**)	NA	#1.7 (**)	NA
9-13 years, Aus	1.2 (25)	1.7 (35)	1.6 (30)	2.2 (45)
11-14 years, NZ	^1.3 (**)	NA	#1.9 (**)	NA
14-18 years, Aus	1.5 (20)	2.0 (25)	2.0 (25)	3.0 (40)
15-18 years, NZ	1.5 (20)	1.9 (25)	2.3 (30)	3.0 (35)
≥19 years, Aus	1.7 (15)	2.0 (20)	1.9 (20)	2.3 (25)
≥19 years, NZ	1.5 (15)	1.6 (15)	2.2 (20)	2.6 (25)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Dietary modelling indicates that intake for all population groups is predicted to be below the UL even for high consumers and applying a worst-case scenario where all specified products are replace with formulated beverages would be replaced by formulated beverages (Scenario 2).

All population groups, with the exception of 2-3 year olds, are estimated to have 95th percentile dietary intakes of copper below the UL. Estimated 95th percentile intakes for 2-3 year olds are at the UL (100%).

Copper intake from other sources includes drinking water. For the modelling, drinking water consumption is included in the dietary intake assessment using a copper concentration of 0.02 mg/L (from the 1997 New Zealand NNS). In Australia copper concentrations in drinking water range up to 0.8 mg/L with typical concentrations of about 0.05 mg/L (NHMRC and NRMMC, 2004). Therefore, intake of copper from drinking water has been included in the dietary intake assessment.

A number of conservative assumptions were used in the dietary modelling which may mean the 95th percentile intakes are still an overestimation to some extent. The conservative assumptions include that all drinks will be substituted for formulated beverages in the 2-3 year old.

The adverse effect on which the UL for copper was based is hepatoxicity. The UL was based on a 12-week study in healthy volunteers where copper supplementation at 10 mg/day did not result in effects on liver function.

There is one case report, where consumption of 30 mg of copper tablets per day for 2 years, followed by 60 mg/day for an additional period of time, resulted in acute liver failure.

The UL represents a quantitative level of total intake at which, or below no harm is expected to occur assuming nutrient adequacy is met. Therefore an estimated intake level at the UL generally does not raise any safety concerns, particularly as the dietary intake assessment includes the contribution from water. In this case, the predicted 95th percentile intake for 2-3 year olds is still well below a level at which adverse effects might be observed. The dietary modelling also predicts that the higher intakes estimated for 2-3 year olds will not be sustained in the older age groups (e.g. 4-8 year olds).

Overall, the potential to exceed the UL, even for 2-3 year olds, is considered to be low, given the conservative assumption in the Scenario 2 dietary modelling.

In conclusion, for the general population the addition of copper to formulated beverages at a level of 0.75 mg per 600 ml serve poses no appreciable public health and safety risk.

Comparison of estimated intakes with the UL is not appropriate when considering the health risk for individuals with Wilson's disease, Indian childhood cirrhosis or idiopathic copper toxicosis, as typically they respond adversely to levels of intake that might fall below the UL and, in some cases, at levels that approximate normal dietary intakes. Such individuals may therefore potentially be at risk even from natural fluctuations in the copper levels in foods. For individuals with Wilson's disease, Indian childhood cirrhosis or idiopathic copper toxicosis, consumption of formulated beverages with copper added, would be inappropriate.

Iodine

Hazard identification and characterisation

Chemistry

Iodine is a non-metallic group VII element (a halogen) existing in the valency states –1 (iodide) to +7 but not occurring free in nature. Iodides and iodates, its mineral forms, occur ubiquitously in igneous rocks and soils. The iodides in the sea accumulate in seaweeds, sea fish and shellfish. On land small amounts of iodide are taken up by plants, which have no essential nutritional requirement for this element, the plants being subsequently ingested by herbivores.

Function

Iodine is an important trace element that is required for the synthesis of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). These hormones have a key role in influencing cellular metabolism and metabolic rate.

Sources

Diet is the major source of iodine intake for humans. The major food categories contributing to dietary intake include seafood, milk and eggs, with meat and cereals being secondary sources. The iodine content of food is reflective of background levels in the environment as well as the use of iodine and its compounds in food production, processing and manufacturing.

In addition to dietary sources, various mineral supplements and medical preparations can further add to iodine intake.

Absorption, distribution, metabolism and excretion

Greater than 97% of ingested iodine is absorbed from the gastrointestinal tract, generally as iodide. Absorbed iodide enters the circulation where it is taken up primarily by the thyroid gland. The uptake of iodide by the thyroid gland is controlled by the thyroid-stimulating hormone (TSH) and is highly sensitive to dietary iodine intake. At low intakes representing iodine deficiency, uptake of iodide into the thyroid gland is increased and at very high intakes, iodine uptake into the thyroid gland decreases.

Once the physiological requirements for thyroid hormone synthesis have been met, the thyroid does not accumulate more iodine and any excess is excreted, primarily in the urine.

Iodine is largely excreted in the urine, mainly in the form of iodine. Very small amounts of iodine may be excreted in sweat, faeces and exhaled air.

Toxicity

A Final Assessment Report has been prepared for Application 493 – Iodine as a Processing Aid, which included a summary of available toxicity data of iodine. For a full review see this report (FSANZ, 2005).

A large number of human experimental, clinical, and epidemiological studies on the effects of excess iodine on human health have been reported and reviewed in detail by both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) ((WHO, 1989) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2004). These studies indicate that the principal direct effects of excessive iodine ingestion are on the thyroid gland and regulation of thyroid hormone production and secretion. Some individuals may experience a sensitivity type reaction to excess iodine, which is unrelated to thyroid gland function. Such reactions are typically associated with large doses of iodine (>300 mg/day), which would not be typical from dietary sources. There are also reports in the literature of iodine poisoning, but such cases are rare and typically associated with intakes of many grams. The focus of this evaluation is on the effects of excess iodine on thyroid function.

Excess iodine can produce an enlargement of the gland (goitre) and/or affect the production of the thyroid hormones. A diminished production of the thyroid hormones is referred to as hypothyroidism (and may be accompanied by goitre) and increased thyroid hormone synthesis and secretion by the thyroid gland is referred to as hyperthyroidism.

The effect on the thyroid depends on the current and previous iodine status of the individual and any current or previous thyroid dysfunction. For example, individuals with a history of iodine deficiency may be prone to the development of iodine-induced hyperthyroidism if iodine exposure increases later in life.

The literature indicates that the human response to excess iodine can be quite variable. Some individuals can tolerate quite large intakes without exhibiting any adverse effects on thyroid gland function, while others may respond adversely to levels close to recommended intakes.

Individuals responding adversely to levels close to recommended intakes typically have an underlying thyroid disorder or have a long history of iodine deficiency.

For the majority of healthy individuals, the most sensitive endpoint for iodine toxicity is sub-clinical hypothyroidism, which is defined as an elevation in TSH concentration while serum thyroid hormone concentration is maintained within the normal range of values for healthy individuals. While not clinically adverse, such an effect, if persistent, could lead to clinical hypothyroidism. In healthy individuals, such effects are generally associated with intakes of 24 µg/kg body weight/day (1700 µg/day for a 70 kg person).

Vulnerable groups

Individuals with thyroid disorders or a long history of iodine deficiency may respond adversely at levels of intake below the UL.

Evaluation

Iodine	**UL in adults, µg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2001b)	1100	total	elevated TSH	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	500	Suppl	change in thyroid hormones	human
EU (European Commission Health & Consumer Protection Directorate-General, 2002a)	600	total	TSH levels	human
WHO/FAO (WHO, 1989)	1000	total	elevated TSH	human

* Guidance level

Intakes of approximately 1 mg iodine per day appear to be well tolerated by healthy adults. This level has been used by JECFA to establish a provisional maximum tolerable daily intake (PTDI) for iodine of 0.017 mg/kg bw. FSANZ has adopted this level as a safe intake level for the general healthy population. Individuals with thyroid disorders or a long history of iodine deficiency may respond adversely however at levels of intake below the UL. The adult UL of 1100 µg/day was adjusted on the basis of relative body weights.

In summary, the UL for **iodine** for the various groups are:

1-3 years **220 µg/day**
4-8 years **350 µg/day**
9-13 years **650 µg/day**
14-18 years **1000 µg/day**
Adults **1100 µg/day**

Dietary intake

Intakes of iodine were estimated at baseline and when formulated beverages are consumed.

The concentration of iodine requested to be added to formulated beverages was 37.5 µg/600 ml reference quantity.

Iodine was not assessed in the 1995 Australian or the 1997 New Zealand NNSs therefore, there were not concentration data available for the foods consumed in the NNSs. Iodine concentrations were available for a restricted range of foods or food groups from survey data or food composition data. A model was set up in DIAMOND assigning iodine concentrations to wider food groups. This type of model did not allow second day adjustments of intake to be made.

The concentrations of iodine in foods were only available from a limited number of sources. For Australia, the intake estimate was based primarily on unpublished 22nd Australian Total Diet Survey (TDS) data. For New Zealand, the intake estimate was based primarily on the data from the 2003/2004 New Zealand TDS first and then the 1997/1998 New Zealand TDS. However, where data gaps existed in the Australian data, New Zealand data were used, and visa versa. Where there were no recent TDS data, unpublished data from the Australian or New Zealand food composition programs were used for the respective countries. If data gaps still existed, international food composition data (German and UK) were used. For Australia, information from A493 – Iodine as a Processing Aid was also used.

Dietary iodine intakes were not assessed as a part of the 2002 New Zealand CNS, therefore, baseline estimates of intake were not available.

Estimated intakes increased from baseline by around 20 µg/per day when formulated beverages were consumed across the population groups assessed. The UL was not exceeded for any population group assessed, except for Australian children aged 2-3 years at the 95th percentile intake when formulated beverages are consumed. In reality, the UL is not likely to be exceeded, as unadjusted 95th percentile intakes are higher than those at the same level over a lifetime.

Table 16: Estimated dietary intakes of iodine, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake µG/DAY (%UL)		95th percentile intake µg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	106 (50)	124 (55)	206 (95)	232 (105)
4-8 years, Aus	109 (30)	131 (35)	217 (60)	243 (70)
5-6 years, NZ	N/A	NA	NA	NA
7-10 years, NZ	N/A	NA	NA	NA
9-13 years, Aus	130 (20)	156 (25)	276 (40)	314 (50)
11-14 years, NZ	N/A	NA	NA	NA
14-18 years, Aus	142 (15)	178 (20)	338 (35)	408 (40)
15-18 years, NZ	93 (9)	119 (10)	211 (20)	252 (25)
≥19 years, Aus	116 (10)	132 (10)	276 (25)	305 (30)
≥19 years, NZ	92 (8)	102 (9)	213 (20)	234 (20)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
NA = not assessed, because iodine was not included in the New Zealand 2002 CNS.

Risk characterisation

Healthy population

The data support the safety of iodine added to formulated beverages for the normal healthy population.

Dietary modelling indicates that intakes for all population groups is predicted to be below the UL even at the 95th percentile of intake and applying a worst-case scenario i.e. all products specified are replaced by formulated beverages.

At the 95th percentile of intake, with the exception of 2-3 year olds, the estimated intakes of iodine are below the UL. Estimated 95th percentile intakes for 2-3 year olds is estimated to only marginally exceed the UL (105%).

Due to the use of 24-hour dietary survey data, which tends to over-estimate habitual food consumption amounts for high consumers, it is likely that the 95th percentile dietary intake is an over-estimate. In addition, a number of conservative assumptions were used in the dietary modelling which may further add to the overestimation. For example, that all specified drinks would be substituted for formulated beverages for the 2-3 year old population group.

The UL represents a quantitative level of total intake at which, or below no harm is expected to occur assuming nutrient adequacy is met. Short-term excursions above the UL, particularly when these are of small magnitude (e.g. 105%), generally do not raise any safety concerns as the UL is not itself a threshold for toxicity. In this case, the predicted 95th percentile intake for 2-3 year olds is still well below a level at which adverse effects might be observed. The dietary modelling also predicts that the higher intakes estimated for 2-3 year olds will not be sustained in the older age groups (e.g. 4-8 year olds).

Overall, the potential to exceed the UL, even for 2-3 year olds, is considered to be low.

Vulnerable individuals

In relation to the vulnerable individuals identified in the hazard identification and characterisation, further consideration is necessary. Under certain circumstances these individuals may respond to excess iodine in the diet by developing thyrotoxicosis (also referred to as iodine-induce hyperthyroidism). Symptoms include rapid heartbeat, nervousness, weakness, heat intolerance, and weight loss. The most vulnerable are those over 40 years of age who have a long history of iodine deficiency, although individuals with underlying thyroid disorders may also be affected.

Comparison of estimated intakes with the UL is not appropriate when considering the health risk for these individuals, as typically they respond adversely to levels of intake that fall below the UL and, in some cases, at levels that approximate normal dietary intakes. Such individuals may therefore potentially be at risk even from natural fluctuations in the iodine levels in foods.

In the case of individuals with underlying thyroid disease, such as Graves' disease, consumption of formulated beverages with iodine would be inappropriate.

In the case of individuals with a long history of iodine deficiency, there may be cause for greater concern as such individuals may not be aware of their condition.

Conclusion

For the vast majority of the population, the addition of iodine to formulated beverages at a level of 37.5 μg per 600 ml serve poses no appreciable public health and safety risk. However, for individuals with underlying thyroid disease or have a long history of iodine deficiency may respond adversely to levels of intake that are safe for the general healthy population.

Iron

Hazard identification and characterisation

Chemistry

Iron is a transition metal and ubiquitous in biological systems. In aqueous solution, it exists in one of two oxidation states, Fe^{2+}, the ferrous form, and Fe^{3+}, the ferric form. Iron has a particularly high redox potential in solution. The interconversion of iron oxidation states is a mechanism whereby iron participates in electron transfer, as well as a mechanism whereby iron can reversibly bind ligands. The common biological ligands for iron are oxygen, nitrogen, and sulphur atoms.

Function

Four major classes of iron-containing proteins exist in the mammalian system: iron containing haem proteins (haemoglobin, myoglobin, cytochromes, others), iron sulphur enzymes (flavoproteins, haem-flavoproteins), proteins for iron storage and transport (transferring, lactoferrin, ferritin, haemosiderin), and other iron-containing or activated enzymes (sulphur, nonheam enzymes). In haem proteins, iron is bound to porphyrin ring structures with various side chains. In humans, the predominant form of haem is protoporphyrin-IX.

The movement of oxygen from the environment to the tissues is one of the key functions of iron. Oxygen is bound to an iron-containing porphyrin ring, either as part of the prosthetic group of haemoglobin within erythrocytes or as part of myoglobin as the facilitator of oxygen diffusion in tissues.

Myoglobin is located in the cytoplasm of muscle cells and increases the rate of diffusion of oxygen from capillary erythrocytes to the cytoplasm and mitochondria. The concentration of myoglobin in muscle is drastically reduced in tissue iron deficiency, thus limiting the rate of diffusion of oxygen from erythrocytes to mitochondria.

The cytochromes contain haem as the active site with the iron-containing porphyrin ring functioning to reduce ferric iron to ferrous iron. Cytochromes act as electron carriers.

Sources of iron

Dietary sources of iron include liver, meat, beans, nuts, dried fruits, poultry, fish, whole grains or enriched cereals, soybean flour and most dark green leafy vegetables. Iron in foods occurs in two main forms: haem and non-haem. The major sources of haem iron in the diet are haemoglobin and myoglobin from meat, poultry and fish. Non-haem iron present as foods is in the ferric form.

Absorption, distribution, metabolism and excretion

Modulation of absorption of iron from the gastrointestinal tract is the primary mechanism for regulation of body iron levels. The amount of iron absorbed from the diet can vary widely and depends on body iron stores and physiological requirements (generally, the rate of erythrocyte production). Absorption of haem and non-haem iron involves different mechanisms. In general haem iron uptake, which is via a specific haem receptor, occurs approximately 2- to 3-fold more extensively than that of non-haem iron and is largely independent of other dietary components. The mechanism by which non-haem iron enters intestinal mucosal cells is not clearly established, although there appear to be separate mechanisms for the uptake of ferrous and ferric iron. Uptake of non-haem iron depends initially on a low pH to effect solubilisation. Iron chelators, such as ascorbic acid, increase absorption by maintaining iron in solution. In the absence of chelators, ferric iron is generally less well absorbed than ferrous iron, due to its low solubility at higher pH. Dietary supplements are mostly inorganic salts. Iron supplements are also available in the form of the iron protein complex, ferritin, but poor absorption is reported.

Iron absorption from a diverse diet has been estimated to be approximately 15%. Women and children generally have lower iron stores than men, and thus absorb a greater percentage of the amount ingested. This is particularly pronounced during pregnancy with absorption of dietary iron increasing throughout gestation. Conversely, absorption is lower in postmenopausal women, in whom iron stores are generally high.

Iron is transported by the plasma transport protein, transferrin. In healthy adults approximately one-third of the total iron binding capacity is saturated. In conditions of iron overload or atransferrinaemia, non-protein-associated iron may also be detected in the plasma. Turnover of the total plasma iron pool (approximately 3 mg) is more than 10-fold every day. Approximately 80% of iron leaving the plasma is delivered to erythroid bone marrow. Iron in circulating erythrocytes is returned to plasma transferrin by means of reticuloendothelial cell phagocytosis.

Iron uptake by cells (other than during absorption from the intestinal lumen) occurs via binding of transferrin to the transferrin receptor, which is subsequently internalised within an endocytic vesicle.

Recent studies have identified a number of novel proteins which are also likely to be involved in iron transport into and within cells, although the function of these proteins in iron transport has yet to be determined.

Little of the absorbed iron is excreted. Very small losses occur in the faeces, by desquamation of gastrointestinal cells, in haemoglobin and bile, and via the urine. Substantial iron loss can occur through loss of blood.

Average, total daily iron losses for healthy adults are 1.0 mg for men and 1.3 mg for premenopausal women (assuming an average blood loss of 30 – 40 mL per menstrual cycle). Daily iron losses for children have not been measured directly but are estimated as 0.2 and 0.5 mg for infants and children aged 6 – 11 years, respectively.

Toxicity

Case reports of accidental poisoning with medicinal iron, especially in young children, indicate acute damage of gastrointestinal, hepatic, pancreatic and cardiovascular structures after ingestion of very high doses. An acute oral dose of 60 mg iron/kg body weight can be lethal but oral doses below about 10-20 mg iron/kg body weight do not cause acute systemic toxicity.

Adverse gastrointestinal effects (e.g. nausea, epigastric discomfort, constipation) have been reported following short-term oral dosage at 50-60 mg daily of supplemental non-haem iron preparations, particularly if taken without food.

Iron overload with clinical symptoms, including liver cirrhosis, has been reported in individuals receiving long-term, high-dose medical treatment with iron (160-1200 mg iron/day). Iron overload with clinical symptoms has also been found in subjects homozygous for hereditary haemochromatosis (a genetic disorder of iron storage), even at normal dietary iron intakes. Bantu siderosis, with liver cirrhosis and diabetes, has been attributed to chronic excess intake of highly available iron (50-100 mg iron/day) in beer; however, these adverse effects may be confounded by chronic alcohol intake and possibly by a genetic disorder.

Although a proportion of the population has serum ferritin levels indicative of elevated iron stores (above 200 μg/L for women and 300 μg/L for men), the point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects (such as liver fibrosis) is not known. The risk of adverse effects from iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low.

Epidemiological studies have reported associations between high iron intake and/or stores with increased risk of chronic diseases such as cardiovascular disease, type II diabetes and cancer of the gastrointestinal tract. However, these data are conflicting and do not provide convincing evidence of a causal relationship between iron intake or stores and such chronic diseases.

Vulnerable groups

A particularly sensitive subpopulation (up to 0.5% of the Caucasian population) are homozygous for hereditary haemochromatosis, who are susceptible to iron overload even at normal dietary iron intakes. Such individuals should avoid iron-supplements and highly iron-fortified foods. The majority of homozygotes are not diagnosed or identified, and they are not aware of their greater susceptibility until sufficient iron has accumulated to produce adverse effects.

Evaluation

Iron	UL, in adults mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2001b)	45	Total	gastrointestinal	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	17	Suppl.	gastrointestinal	human
EU (The Scientific Panel on Dietetic Products, 2005)	No UL		insufficient data	human

* Guidance level

The US identified a LOAEL of 60 mg/day of supplemental iron on the basis of a controlled, double blind study where gastrointestinal effects were examined in 97 Swedish male and female adults after intake of either a non-haem iron supplement (60 mg/day as iron fumarate), a supplement containing both haem iron and non-haem iron (18 mg/day, 2 mg from porcine blood and 16 mg as iron fumarate), or a placebo. The groups were similar with respect to gender, age, and basic iron status. The frequency of constipation and the total incidence of all side effects were significantly higher among those receiving non-haem iron than among those receiving either the combination of haem and non-haem iron or the placebo. Although most of the reported GI effects were minor, five individuals found them to be severe enough to stop taking the medication. Four of these withdrawals occurred during the non-haem containing iron treatment and one occurred just after changing from the non-haem-containing iron treatment to placebo.

The EU considered that the adverse gastrointestinal effects which have been reported after short-term oral dosage at 50-60 mg daily of supplemental non-haem iron preparations are not a suitable basis to establish an UL for iron from all sources. An UL could not be established for iron based on iron overload due to a poor correlation between iron intake and biochemical indicators of iron status, between biochemical indicators and actual body stores, or between body stores and adverse effects. Also the EU considered that an UL could not be established for iron (including haem iron) based on increased risk of chronic diseases such as cardiovascular disease, diabetes and cancer, due to the lack of convincing evidence of a causal relationship between iron intake or stores and chronic diseases.

The limited data indicate that supplemental intakes of non-haem iron at levels of 30 mg/day or more (in addition to iron intake from food) can be associated with indicators of high iron stores (e.g. elevated serum ferritin) in older adults. However, the point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects (such as liver fibrosis) is not known. Furthermore, epidemiological associations between high iron intake and/or stores and increased risk of chronic diseases such as cardiovascular disease, type II diabetes and cancer of the gastrointestinal tract are conflicting and do not provide convincing evidence of a causal relationship between iron intake or stores and such chronic diseases.

FSANZ considers the EU evaluation as more appropriate, since the gastrointestinal effects observed in short term studies are transient, reversible and occurred with non-haem iron without food. Therefore, the US level is not appropriate for the establishment for an UL. Data on effects on iron overload or risk on chronic diseases are insufficient to set an UL.

In conclusion, there is **insufficient data to set an UL for iron**. The limited data indicate that supplemental intakes of non-haem iron at levels of 30 mg/day or more (in addition to iron intake from food) can be associated with indicators of high iron stores (e.g. elevated serum ferritin) in older adults. However, the point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects (such as liver fibrosis) is not known.

Dietary intake

Intakes of iron were estimated at baseline and when formulated beverages are consumed. Estimated intakes were adjusted based on second day intake data from the NNSs.

The concentration of iron requested to be added to formulated beverages was 3 mg/600 ml reference quantity.

Estimated intakes increased from baseline by around 1-2 mg/per day when formulated beverages were consumed depending on the population groups assessed. There was no UL to compare the estimated intakes to.

Table 17: Estimated dietary intakes of iron, before and after formulated beverages are introduced into the diet

	Mean intake mg/day		95th percentile intake mg/day	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	8.2	9.4	12.2	13.4
4-8 years, Aus	9.3	10.8	13.7	15.5
5-6 years, NZ	^9.4	NA	#11.8	NA
7-10 years, NZ	^11.1	NA	#14.4	NA
9-13 years, Aus	12.3	14.3	22.1	23.2
11-14 years, NZ	^11.6	NA	#16.7	NA
14-18 years, Aus	13.9	16.4	24.5	29.4
15-18 years, NZ	12.8	16.3	19.4	25.2
≥19 years, Aus	12.7	13.8	20.9	22.9
≥19 years, NZ	12.2	13.5	18.5	22.0

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Dietary modelling indicated that when various drinks are substituted for formulated beverages, adolescents aged 14-18 years old would have the highest intake of iron (95th percentile intake 29.4 mg/day), which is still much lower than the concentrations found to result in adverse gastrointestinal effects. In older adults (>70 years), who represent a non-target group for formulated beverages, high consumers are estimated to have intakes of 17.5 mg of iron per day in Australia and 15.5 mg of iron per day in New Zealand. This level is below the level which is associated with indicate high iron store levels in older adults. Therefore, this would not be of concern.

A particularly sensitive subpopulation (up to 0.5% of the Caucasian population) are homozygous for hereditary haemochromatosis, who are susceptible to iron overload even at normal dietary iron intakes. Such individuals should avoid iron-supplements and highly iron-fortified foods. The majority of homozygotes are not diagnosed or identified, and are therefore not aware of their greater susceptibility until sufficient iron has accumulated to produce adverse effects.

In conclusion, for the general population the addition of iron to formulated beverages at a level of 3 mg per 600 ml serve poses no appreciable public health and safety risk. However, for individuals who are homozygous for hereditary haemochromatosis there is a potential increased risk of iron toxicity, especially for those individuals who are unaware of their increased susceptibility for iron overload.

Magnesium

Hazard identification and characterisation

Chemistry

Magnesium is a metallic element of group II of the periodic table and has an atomic weight of 24.3. Magnesium is the eighth most abundant element in the earth's crust. It does not occur as a pure metal in nature, but it is found in large deposits as magnetite, dolomite and other minerals.

Function

Magnesium is required as a cofactor for many enzyme systems. It is required for protein synthesis and for both anaerobic and aerobic energy generation and for glycolysis, either indirectly as a part of magnesium-ATP complex, or directly as an enzyme activator. Magnesium plays a multifunctional role in cell metabolism, (particularly at the level of key phosphorylations), and has a critical role in cell division. It has been suggested that magnesium is necessary for the maintenance of an adequate supply of nucleotides for the synthesis of RNA and DNA. Magnesium regulates the movement of potassium in myocardial cells and is also known to act as a calcium channel blocker. Magnesium is an important element in the metabolism and/or action of vitamin D, and is essential for the synthesis and secretion of parathyroid hormone.

Sources of magnesium

Magnesium is ubiquitous in foods, but the content varies substantially. Leafy vegetables, as well as grains and nuts, generally have a higher magnesium content (60-2700 mg/kg) than meats and dairy products (less than 280 mg/kg). A number of magnesium salts are used as food additives (see Standard 1.3.1 – Food Additives). Within Australia and New Zealand voluntary fortification with magnesium is allowed in various products up to 25% of the recommended daily intake (80 mg). In food derived from plant and animal sources, magnesium is mostly bound or chelated, e.g. to phytic acid, phosphates, chlorophylls or it is included in biological apatites (skeleton). In aqueous solutions, magnesium salts (e.g., sulphate, chloride, phosphate, citrate, and carbonate) are mostly dissociated depending on the concentration, pH and temperature. Most magnesium salts are hygroscopic and have a bitter taste.

Absorption, distribution, metabolism and excretion

The net absorption of magnesium from the diet is typically approximately 50 percent. High levels of dietary fibre from fruits, vegetables, and grains decrease magnesium absorption. Dietary protein is also known to influence intestinal magnesium absorption. Magnesium is absorbed along the entire intestinal tract, but the sites of maximal absorption appear to be the distal jejunum and ileum. It has been suggested that absorption occurs by both an unsaturable passive and saturable active transport system. Thus, in both adults and children, the fractional

absorption of magnesium is inversely proportional to the amount ingested. Magnesium is absorbed much more efficiently from the normal concentrations found in the diet than it is from the higher doses found in non-food sources. The presence of food likely counteracts the osmotic effect of the magnesium salts in the gut lumen.

Magnesium is abundant in the body with the largest amounts found in bone. It is also found in a variety of other tissues including muscle, liver, heart and kidneys. In plasma, half of magnesium present is in the ionised form. About 20% is bound to proteins, the remaining 80% is unbound. Most intracellular magnesium is found bound to the endoplasmic reticulum.

In normal individuals, the kidney seems to maintain magnesium homeostasis over a rather wide range of magnesium intakes. Thus, hypomagnesaemia has not been documented following the intake of high levels of dietary magnesium in the absence of either intestinal or renal disease.

Magnesium is excreted primarily in the urine. The extent of urinary excretion, and thereby the homeostasis of magnesium, is influenced by a wide variety of hormones, including calcitonin, thyroxine, glucocorticoids, glucagons and angiotensin. Under normal conditions, the kidney tubule reabsorbs 95% of the filtered load of magnesium and about 5% is excreted in urine.

Toxicity

Magnesium, when ingested as a naturally occurring substance in foods, has not been shown to exert any adverse effects. However, adverse effects of excess magnesium intake have been observed with intakes from non-food sources such as various magnesium salts used for pharmacologic purposes. Magnesium derived from plant or animal sources has not been demonstrated to induce diarrhoea or other adverse effects in healthy persons, probably as magnesium is bound to matrices and hence is mostly not easily dissociable. On the other hand easily dissociable magnesium salts (e.g. chloride or sulphate; included are compounds like MgO becoming readily dissociable after the reaction with gastric hydrochloric acid) that are present in water many supplements and drugs exert dose-dependent laxative effects.

Easily dissociable magnesium salts, especially the sulphate are used as ‘osmotic’ and ‘saline’ laxatives, respectively. Nevertheless mild diarrhoea can be taken as the most sensitive non-desirable effect if magnesium supplements are taken for nutritional purposes.

However, it must be kept in mind that adaptation of the bowel to higher oral magnesium intake is known, that a mild laxative effect may be desirable ('four patients reported mild diarrhoea in the magnesium group, and a similar number felt that their bowel function improved with less constipation'), that mild laxative effects have been frequently observed also in the placebo groups (perhaps caused by taste adjusters, vehicles a.o.), that a given daily dose of magnesium is better tolerated when it is divided into several portions, and finally that the galenic form (aqueous solution, capsules, tablets, etc.) may play a role. Data from the literature included children, pregnant women, tetanic, hypertensive and cardiac patients as well as volunteers. Papers were only considered when the presence or absence of 'mild diarrhoea' was stated. Studies where magnesium was derived from plant or animal sources were not considered, since these forms are poorly dissociable (e.g. phytates).

As discussed, mild diarrhoea is the most sensitive non-desirable effect of orally administered easily dissociable magnesium salts. From the available data it can be concluded that mild diarrhoea occurs in a small percentage of adult subjects at oral doses of about 360/365 mg magnesium per day, this level being regarded as the LOAEL.

Larger pharmacological doses (e.g. doses > 2500 mg/day) of magnesium can clearly result in more serious adverse effects, such as metabolic alkalosis, hypokalaemia, paralytic ileus and cardio respiratory arrest.

Vulnerable groups

Individuals with impaired renal function are at greater risk of magnesium toxicity. However, magnesium levels obtained from food are insufficient to cause adverse reactions even in these individuals. Patients with certain clinical conditions (e.g. neonatal tetany, hyperuricaemia, hyperlipidemia, lithium toxicity, hyperthyroidism, pancreatitis, hepatitis, phlebitis, coronary artery disease, arrhythmia, and digitalis intoxication) may benefit from the prescribed use of magnesium in quantities exceeding the upper limit in the clinical setting.

Evaluation

Magnesium	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2000a)	350	non-food	Osmotic diarrhoea	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	400	Suppl	Osmotic diarrhoea	human
EU (European Commission Health & Consumer Protection Directorate-General, 2001b)	250	not normally present in foods and beverages	Osmotic diarrhoea	human

* Guidance level only

Diarrhoea was chosen by the US, UK and EU as the most sensitive toxic manifestation of excess magnesium intake from non-food sources. Therefore, it is considered appropriate to set an UL for magnesium for readily dissociable magnesium salts (e.g., chloride, sulphate, aspartate, lactate) and compounds like MgO in nutritional supplements, water, or added to food and beverages.

The US established an UL of 350 mg/day for adolescents and adults aged 9 and over. Although a few studies have noted mild diarrhoea and other mild gastrointestinal complaints in a small percentage of patients at levels of 360 to 380 mg/day, it is noteworthy that many other individuals have not encountered such effects even when receiving substantially more than this level of supplementary magnesium. It was assumed that children are as susceptible to the osmotic effects of non-food sources of magnesium as are adults. Thus, by adjusting the value for adults on a body-weight basis an UL for children at a magnesium intake of 5 mg/kg bw /day can be established. For children, aged 1-3 years, the UL would be 65 mg/day, and aged 4-8 years 110 mg/day.

The EU established an UL of 250 mg/day for children, adolescents and adults aged 4 and over, while for younger children no upper limit was established. Based on a NOAEL of 250 mg magnesium per day and an uncertainty factor of 1.0 an UL of 250 mg magnesium per day can be established for readily dissociable magnesium salts (e.g., chloride, sulphate, aspartate, lactate) and compounds like MgO in nutritional supplements, water, or added to food and beverages. This UL does not include magnesium normally present in foods and beverages. An uncertainty factor of 1.0 was justified in view of the fact that data are available from many human studies involving a large number of subjects from a spectrum of lifestage groups, including adults, pregnant and lactating women, and children. In addition, the NOAEL was based on a mild, transient laxative effect, without pathological sequelae, which is readily reversible and for which considerable adaptation can develop within days. This UL holds for adults, including pregnant and lactating women, and children from 4 years on. As no data were available for children from 1 to 3 years, and since it was considered that extrapolation of the UL for older children and adults on the basis of body weight was inappropriate, no UL could be established for this age group.

FSANZ considered the UL established by the US as the most comprehensive and therefore the age-derived ULs for **magnesium, not naturally occurring in food** are:

1-3 years	**65 mg/day**
4-8 years	**110 mg/day**
9 and over	**350 mg/day**

Dietary intake

Intakes of magnesium were only assessed from added sources from food. Intakes were estimated when formulated beverages are consumed. Baseline intakes were not calculated because it was assumed that no food products are fortified with magnesium.

The concentration of magnesium requested to be added to formulated beverages was 80 mg/600 ml reference quantity.

Estimated intakes were adjusted based on second day intake data from the NNSs.

Dietary magnesium intakes were not assessed as a part of the 2002 New Zealand CNS, therefore, baseline estimates of intake were not available.

Estimated mean intakes were between 20 and 65 mg/per day and between 75 and 170 mg/day when formulated beverages were consumed depending on the population groups assessed. The UL was not exceeded for any population groups assessed, except for Australian children aged 2-3 years at the 95th percentile intake when formulated beverages are consumed.

Table 18: Estimated dietary intakes of magnesium from formulated beverages only, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake mg/day (%UL)	95th percentile intake mg/day (%UL)
Age group	**Scenario 2***	**Scenario 2***
2-3 years, Aus	33 (50)	95 (150)
4-8 years, Aus	44 (40)	105 (95)
5-6 years, NZ	NA	NA
9-13 years, Aus	54 (15)	123 (35)
7-10 years, NZ	NA	NA
11-14 years, NZ	NA	NA
14-18 years, Aus	63 (20)	169 (50)
15-18 years, NZ	45 (15)	120 (35)
≥19 years, Aus	32 (9)	106 (30)
≥19 years, NZ	22 (6)	76 (20)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
NA = not assessed, because magnesium was not included in the New Zealand 2002 CNS.

Risk characterisation

Dietary modelling was undertaken on the assumption that there is no baseline exposure to magnesium, since the adverse effects associated with magnesium are only observed to occur when magnesium is not in a food matrix. It could be assumed that magnesium would not be in a food matrix when added to formulated beverages, therefore the UL for magnesium, not naturally occurring in food, is applicable when assessing the risk of adding magnesium to formulated beverages. Formulated beverages could be consumed on an empty stomach, therefore, osmotic diarrhoea could occur at a high consumption level. Children aged 2-3 years at the 95th percentile intake of magnesium are predicted to exceed the UL (50% of UL at mean intake and 150% at 95th percentile of intake).

The UL is based on a mild reversible adverse effect, osmotic diarrhoea. The age-specific UL was derived from the adult UL on a body weight basis, which might not be appropriate. For an adverse effect such as osmotic diarrhoea, the intake level at which the diarrhoea occurs might be more related to the concentration of magnesium in the intestines than to a daily dose.

It is unlikely that children aged 2 to 3 years would consume the daily reference quantity (600 ml) of formulated beverages in one serve on an empty stomach. Because of these assumptions in setting age-specific ULs for magnesium, not naturally occurring in food, the mildness and reversibility of the adverse effect, and the assumption that the intake would be in one serving on an empty stomach, the risk of adverse effects in 2-3 years old is considered to be relatively low.

For all other population groups, there was no appreciable risk of adverse effects related to high intake of magnesium.

In conclusion, the addition of magnesium to formulated beverages at a level of 80 mg per 600 ml serve poses no appreciable public health and safety risk.

Manganese

Hazard identification and characterisation

Chemistry

Manganese (Mn) is an abundant metallic element that can exist in a variety of oxidation states. Mn^{2+} and Mn^{3+} are the most biologically important. Within this assessment, the word manganese refers to ionic manganese, except when specific manganese compounds are mentioned.

Function

Manganese is a component of a number of enzymes and activates a range of others. Glycosyl transferases are specifically activated by manganese.

Sources of manganese

Manganese is present both naturally and as a result of contamination in soils, sediments and water. Manganese is present in foods, particularly tea, green vegetables, nuts, bread and other cereals. The level of manganese in drinking water in Australia in reticulated supplies can range up to 0.25 mg/L, with typical concentrations of manganese usually less than 0.01 mg/L (NHMRC and NRMMC, 2004).

Absorption, distribution, metabolism and excretion

Absorption takes place in the small intestine via a carrier-mediated mechanism; passive diffusion may also occur. Absorption is generally low but appears to be higher in infants and young animals. Bioavailability of manganese from different food types is variable, but appears to be generally low, due to poor solubility.

In the portal blood manganese may bind to albumin and α_2 macroglobulin. A small proportion of manganese is oxidised to Mn^{3+}, and enters the systemic circulation, possibly by binding to transferrin. Manganese accumulates in mitochondria-rich tissues such as liver and pancreas. Manganese also accumulates in the brain, particularly in the globus pallidus, striatum and substantia nigra.

Manganese is excreted largely in the faeces, mostly as a result of biliary excretion, although some direct secretion also occurs. A small amount of manganese is excreted in the urine.

Toxicity

Manganese has low acute toxicity. Occupational exposure, for example in manganese mines and smelters, to high levels of inhaled manganese has been associated with manganism, a neurotoxic condition similar to Parkinson's disease. This condition occurs as a result of inhalation exposure to high levels of manganese and is not relevant to the assessment of lower levels of manganese in food. Drinking water contaminated with manganese has also been associated with neurological and behavioural effects.

There is an association between manganese accumulation and liver disease but this may be due to impaired biliary excretion caused by the liver disease rather than manganese toxicity. Effects on the immune system have been reported.

Manganese is a known neurotoxin at high occupational levels of inhalation exposure. However, it has also been suggested that exposure from lower levels in drinking water may result in more subtle neurological effects in human populations. The reported symptoms include muscle pain, fatigue, tremor, memory problems and impaired reflexes. Neurological effects have been reported at estimated intakes of 3.6-4.6 mg manganese from water, through comparable intakes have been negative in other studies. Other more limited data suggest that adverse effects may occur at even lower intake levels in children.

Animal data are also available and indicate similar neurotoxic effects to those reported in humans. However, the neurotoxic effects are inevitably of a less subtle nature than the symptoms assessed in human studies and so these have not been considered further. Animal studies have also reported adverse effects on haematology and reproductive parameters. The lowest dose affecting the central nervous system was found in a study with growing male rats, in which 50 μg $MnCl_2.4H_2O$/rat, initially equivalent to about 0.28 mg Mn/kg bw for 15 or 60 days and reported to increase significantly the monoamine oxidase in the brain and to cause neuronal degeneration in the cerebral and cerebella cortex (Chandra and Shukla, 1978).

There are human studies reporting effects of manganese contained in drinking water. Assuming a consumption of 2 litres of drinking water/day, the cohorts showing the reported neurological effects were exposed to at least 28 mg Mn/day (Kawamura et al, 1941), 0.16-0.5 and 3.6-4.4 Mn/day (Kondakis *et al*., 1989) and 0.48-0.69 mg Mn/day (He *et al*., 1994), plus the contribution from food. In another study, 0.6-4.3 mg Mn/day from drinking water plus contribution from food showed no effects (Vieregge *et al*., 1995). However, the limitations of these studies including the uncertainty of the contribution from food make firm conclusions difficult.

The margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to manganese beyond that normally present in food and beverages could represent an adverse health risk without evidence of any health benefit.

Vulnerable groups

Anaemic individuals may be vulnerable to the toxic effects of manganese due to the increased absorption that occurs in states of iron deficiency. Groups with impaired biliary clearance, such as patients with liver disease or older people, may also be susceptible to manganese accumulation and toxicity. It has also been reported that ethanol and long-term use of anti-psychotic drugs increases the susceptibility of humans to manganese toxicity.

Evaluation

Manganese	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2001b)	11	Total	neurotoxicity	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	4	Suppl	neurotoxicity	human
EU (European Commission Health & Consumer Protection Directorate-General, 2000i)**	No UL		neurotoxicity, insufficient data	

* Guidance level

** EU considered the available data not suitable for establishing an upper limit, however characterised the risk such that oral exposure above levels normally present in food could represent a risk of adverse health effects..

The US established a NOAEL of 11 mg/day of manganese from food based on the data presented by Greger (Greger, 1999). Greger reviewed information indicating that people eating Western-type and vegetarian diets may have intakes as high as 10.9 mg/day of manganese. Because no adverse effects due to manganese intake have been noted, at least in people consuming Western diets, 11 mg/day is a reasonable NOAEL for manganese from food. A LOAEL of 15 mg/day can be identified on the basis of an earlier study by Davis and Greger (Davis and Greger, 1992). At this dose, there were significant increases in serum manganese concentrations after 25 days of supplementation and in lymphocyte manganese-dependent superoxide dismutase activity after 90 days of supplementation. Because of the lack of evidence of human toxicity from doses less than 11 mg/day of manganese from food, an uncertainty factor of 1.0 was selected. The adult UL of 11 mg/day was adjusted for other age groups on the basis of relative body weights.

The EU decided there were limitations with the human data and the non-availability of NOAELs for critical endpoints from animal studies produced a considerable degree of uncertainty. Therefore, a UL could not be set. The margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit.

FSANZ considers the US evaluation as less appropriate, since adjustment on a body weight basis for age groups is inappropriate, when the UL is based on the level currently in a Western diet. If this basis is taken, it would be more appropriate to base the age-specific UL on their intake levels. Therefore, the EU approach is more appropriate. There are limitations with the human data and the margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very small. Oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit.

In conclusion, oral exposure to **manganese beyond the normally present in food and beverages could represent a risk of adverse health effects** without evidence of any health benefit.

Dietary intake

Intakes of manganese were only estimated at baseline. Estimated intakes were adjusted using second day intake data from the NNSs.

The concentration of manganese requested to be added to formulated beverages was 1.25 mg/600 ml reference quantity.

Manganese was not included in the 1995 Australian NNS. Therefore, in order to estimate intakes for the Australian population, the concentration data from the New Zealand NNS were matched to the most appropriate Australian food codes, then these values were used to estimate dietary intakes for the Australian population groups.

Estimated intakes for manganese were between two and five milligrams per day at the mean level of intake, and between four and eight milligrams per day at the 95th percentile of intake, depending on the population group.

Table 19: Estimated dietary intakes of manganese, before and after formulated beverages are introduced into the diet

	Mean intake MG/DAY	**95th percentile intake mg/day**
Age group	**Baseline**	**Baseline**
2-3 years, Aus	2.7	4.9
4-8 years, Aus	3.0	5.5
5-6 years, NZ	^2.7	#3.5
7-10 years, NZ	^3.1	#4.0
9-13 years, Aus	3.5	6.5
11-14 years, NZ	^3.5	#4.5
14-18 years, Aus	3.9	7.3
15-18 years, NZ	3.8	6.4
≥19 years, Aus	4.6	7.9
≥19 years, NZ	4.6	7.6

^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.

Risk characterisation

The NHMRC has proposed an adequate intake for manganese in 2-3 years old to be at the same level as the UL (NHMRC, 2004). The adequate intake was based on current mean intake of manganese in Australia and New Zealand. This indicates that addition of manganese to formulated beverages is inappropriate, since there is a risk of adverse health effects without evidence of any health benefit.

For all other age groups, the margin between oral effect levels and the estimated intake from food is very small. Therefore, the addition of manganese to a formulated beverage could pose a public health and safety risk.

In conclusion, there are potential safety concerns with the addition of manganese to formulated beverages at a level of 1.25 mg in a 600 ml serve.

Molybdenum

Hazard identification and characterisation

Chemistry

Molybdenum (Mo) does not exist naturally in the metallic state, but occurs in association with other elements. Molybdenum exists in several valency states, e.g. $Mo^{II}O$, $Mo^{IV}S_2$, $Mo^{VI}O_3$, and as the stable salts $(NH_4)_2Mo^{VI}O_4$ (ammonium molybdate), $(NH_4)_6Mo^{VI}_7O_{24}.4H_2O$ (ammonium molybdate tetrahydrate) and $Na_2Mo^{VI}O_4.2H_2O$ (sodium molybdate dihydrate).

Function

Molybdenum is ubiquitous in food and water as soluble molybdates. Molybdenum-containing enzymes are found in many plants and animal organisms. In plants and lower organisms these enzymes are involved in the bacterial fixation of N_2, in the conversion of NO_3 to NH_3, in protein synthesis and in some redox reactions. In human and animal tissues the enzymes xanthine dehydrogenase (XD)/oxidase (XO), aldehyde oxidase (AO) and sulphite oxidase (SO) require molybdopterin as cofactor and part of the enzyme molecule. In molybdopterin, molybdenum is bound by two S atoms to the pterin. The redox potential of Mo^V/Mo^{VI} is appropriate for the electron exchange with flavinmononucleotides. Molybdenum is therefore an essential component of flavin- and Fe-containing enzymes.

Sources of molybdenum

Good food sources of molybdenum are sorghum, leafy vegetables (levels depending on soil content, those grown on neutral or alkaline soil are rich in molybdenum, those grown on leached acid soil are molybdenum deficient, legumes (beans), grains (cereals, wheat germ), organ meats, milk and eggs. Some 40% of molybdenum in cereals is lost on milling. Fruits, root vegetables, and muscle meat are pour sources. High concentrations have been found in shellfish.

Absorption, distribution, metabolism and excretion

Animals

The rate of gastrointestinal absorption of molybdenum depends on its chemical nature and the animal species. Ingested Mo^{VI} but not Mo^{IV} is readily absorbed from the duodenum and proximal jejunum. Water-soluble molybdates, thiomolybdates and oxothiomolybdates and molybdenum in herbage and green vegetables are absorbed to 75-97% by laboratory animals and ruminants. Insoluble MoS_2 is not absorbed; Mo^{IV} compounds are not readily absorbed. Intestinal absorption is inhibited by high intraluminal sulphate concentrations, probably because of competition for the common carrier. Silicates also inhibit the absorption of dietary molybdates.

Absorbed molybdenum rapidly appears in the blood loosely attached to the erythrocytes, specifically bound to α2-macroglobulins. In rodents it is distributed mainly to the liver, converted to molybdate and 36-90% of the total dose is excreted in the urine, less than 1% in the bile and only some in the faeces.

In rabbits and guinea pigs molybdenum is deposited in the tissues within 4 hours after initial high blood and bile levels and eliminated within 72 hours by the kidneys. In horses, cattle and sheep faecal elimination is about half the urinary elimination because of limited absorption. Some bone storage was noted. Molybdenum crosses the placenta. Sulphate reduces the utilisation of molybdenum by some tissues and increases the urinary molybdenum excretion. Molybdenum is reabsorbed by the renal tubules but this reabsorption is reduced by S-containing and by acid proteins. The reabsorbed molybdenum deposits in liver, lung, bone and skin. It is responsible for fluoride storage and aids retention of fluoride in the bone of old rats as well as decreasing caries in rats. Small amounts of molybdenum increase antibody formation, e.g. agglutinins

^{99}Mo injected into dogs was concentrated in liver, kidney, pancreas, pituitary, thyroid and adrenals but none appeared in brain, white marrow or fat. The biological half-life varies from a few hours to several days in small laboratory animals and is related to the Cu and S metabolism.

Humans

Water-soluble molybdenum compounds and molybdenum in herbage and green vegetables are absorbed by humans at 40-50%. The absorption rate from drinking water may be the same as from food. Twenty five percent of absorbed molybdenum appears rapidly in the blood loosely attached to the erythrocytes, specifically bound to α2-macroglobulins, normal whole blood levels are 2-6 µg/L and serum levels are 0.55 µg/L. In man, the highest levels appear in kidney, liver and bone, raised levels appear also in adrenals, fat and omentum. There is no bioaccumulation, with tissue levels rapidly returning to normal once exposure stops. Increased exposure at the work place or through drinking water is balanced by increased urinary excretion.

16-27% of intravenously administered ^{99}Mo to human subjects was excreted in 5 days in the urine. Faecal excretion over 10 days was 1-7%. Molybdenum was rapidly cleared from the blood within 24 hours.

Data on the molybdenum status of normal tissues are unreliable. Quoted blood and serum levels vary by 4 orders of magnitude. Serum levels of molybdenum rise in liver functional defects, hepatitis, hepatic tumours and after certain drugs. Raised blood levels are seen in uraemia, rheumatic disorders and cardiovascular disease. Human liver contains 1.3-2.9 mg molybdenum/kg dry matter, kidney 1.6 mg/kg dry matter, lung 0.15 mg/kg dry matter, brain and muscle 0.14 mg/kg dry matter, hair 0.07-0.16 mg/kg.

Toxicity

Molybdenum compounds appear to have low toxicity in humans. More soluble forms of molybdenum have greater toxicity than insoluble or less soluble forms. The UL in this report applies to all forms of molybdenum. There are limited toxicity data for molybdenum in humans; most of the toxicity data are for animals, especially ruminants. Ruminants are more sensitive to molybdenum than monogastric animals, but the basis for the toxicity of molybdenum in ruminants is not relevant for humans, because in ruminants this toxicity is always associated with 'conditioned' copper-deficiency. In monogastric laboratory animals, molybdenum has been associated with reduce growth or weight loss, renal failure, skeletal abnormalities, infertility, anaemia, diarrhoea, and thyroid injury.

Since none of these effects have been observed in humans, it is impossible to determine which ones might be considered most relevant to humans.

Molybdenum toxicity in animals varies according to age, species, sex, and duration of exposure. In ruminants, the relative amounts of copper and sulphur in the diet are also important determinants of toxicity, but the effect of molybdenum on copper metabolism in humans is probably not significant.

There are no adequate human data for establishing a UL. Growth depression occurs in rats at 2-8 mg molybdenum/kg bw/day and skeletal changes at 7.5 mg molybdenum/kg bw/day. Reproductive and developmental changes were found in rats at 1.6-2 mg molybdenum/kg bw/day. In mice infertility and early pup deaths were noted at 1.5 mg molybdenum/kg bw/day. In rabbits skeletal changes and nephrotoxicity were found at 5 mg molybdenum/kg bw/day, while skeletal changes, bodyweight loss and anaemia were seen at 25-46 mg molybdenum/kg bw/day. Reduced growth occurred in guinea pigs at 75 mg molybdenum/kg bw/day. Adverse spermatogenic effects were seen in calves at 4 mg molybdenum/kg bw/day. Thiomolybdate intoxication can occur in experimental animals at intakes of 5 mg molybdenum/kg bw.

From these studies the critical effects of molybdenum in the rat and mouse appear to be effects on reproduction, particularly foetal development.

In a 9 weeks study in SD rats on the effects of molybdenum supplementation on oestrus activity, fertility and foetal development, 5 groups, each of 21 female weaning rats, were given for 6 weeks a basic diet containing 0.025 mg molybdenum/kg diet as well as 6.3 mg Cu/kg diet, and additionally in their drinking water doses of 0, 5, 10, 50 and 100 mg molybdenum/L as sodium molybdate ($Na_2MoO_4.2H_2O$) for 3 weeks until the 21st day of gestation. Six animals in each group were sacrificed after 6 weeks to determine the oestrus cycle length. The remaining 15 animals in each group were mated with untreated males and allowed to continue gestation for 21 days. The average mean weekly supplementary molybdenum intakes were 0.0, 0.64, 1.12, 5.81 and 11.56 mg molybdenum/rat (equivalent to 0, 0.91, 1.6, 8.3 and 16.7 mg molybdenum/kg bw/day assuming an average rat weight of 100 g). There was no effect on fertility, food and water consumption. Oestrus cycle was prolonged from 1.6 mg/kg bw/day and higher supplementation. Gestational weight, litter size and foetal weights were less than controls for the groups fed 1.6 mg/kg bw/day and higher doses. Histopathology showed delayed histological development of foetal structures, delayed oesophageal development, delayed transfer of foetal haematopoeisis from liver to bone marrow, and delayed myelination of the spinal cord at doses of ≥1.6 mg/kg bw/day. Foetal resorption increased at doses of 1.6 mg/kg bw/day and higher. Molybdenum supplementation at dose levels of 1.6 mg/kg bw/day and higher increased SO and XDH/XO activity, however this effect was less apparent in pregnant animals. The NOAEL was 0.9 mg molybdenum/kg bw/day ((Fungwe *et al*., 1989), reviewed by the EU).

This study in rats is pivotal because of its satisfactory design (according to EU), the use of an adequate number of test animals, demonstration of a clear dose-response relationship and clear toxicological endpoints.

Few data are available on human toxicity following ingestion. Food or water must contain more than 100 mg/kg to produce signs of toxicity, which include diarrhoea, anaemia and high levels of uric acid in the blood.

Elevated uric acid levels, which are associated with the onset of gout, are hypothesised to be caused by stimulation of xanthine oxidase by high molybdenum intake.

Evaluation

Molybdenum	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2001b)	2	Total	reproductive effects	rat
UK (UK Expert Group on Vitamins and Minerals, 2003)*	0.23	Total	insufficient data	
EU (European Commission Health & Consumer Protection Directorate-General, 2000d)	0.6	Total	reproductive effects	rat

* Guidance level, the level is the current estimated maximum intake from the UK diet.

Because of deficiencies in human studies, inadequate data exist to identify a causal association between excess molybdenum intake in normal, apparently healthy individuals and any adverse health outcomes. In addition, studies have identified levels of dietary molybdenum intake that appear to be associated with no harm. Thus, the US and EU selected reproductive effects in rats as the most definitive toxicological indices, while the UK found the data inadequate to establish an UL.

Based on studies in rats and mice, the EU and US established a NOAEL of 0.9 mg/kg/day. The US used an uncertainty factor of 30 (10 for interspecies and 3 for intraspecies), while the EU used an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies).

The US used a UF of 3 for intraspecies variation that was based on the expected similarity in pharmokinetics of molybdenum among humans. The reason for this difference was explained by the US that the main concern for an intraspecies factor of 10 was based on concerns on possible interactions with copper and concerns about copper-deficient humans. Recent information suggests that molybdenum does not have any effect on copper metabolism in humans (Turnland and Keyes, 2000). The US used the NOAEL of 0.9 mg/kg bw/day and divided this by the overall uncertainty factor of 30 to obtain an UL of 30 μg/kg bw/day for humans. This value of 30 μg/kg bw/day was multiplied by the average of the reference bodyweight and the resulted UL for adults was rounded to 2000 μg/day. Since no specific data for other age groups are available the adult UL was adjusted on the basis of relative weight. The UL is also applicable for pregnant and lactating women, since the adverse effect was based on reproductive effects.

The EU used an uncertainty factor of 100. This comprised a factor of 10 for protecting sensitive human sub-populations with inadequate copper intake or with deficient copper metabolism in view of the species differences in antagonism between molybdenum and copper, and another factor of 10 to cover the lack of knowledge about reproductive effects of molybdenum in humans and incomplete data on the toxicokinetics in man. Because the exposure in this 9-week rat study is sufficient to cover the relevant period of foetal development, a further uncertainty factor is unnecessary. This provides an UL of approximately 0.01 mg/kg bw/day, equivalent to 0.6 mg/person/day for adults, which is also applicable to pregnant and lactating women. The UL for children was derived by extrapolating from the adult UL on a body weight basis. .

FSANZ evaluation

The US evaluation based the decreased uncertainty factor for intraspecies variation on recent information on the possible interaction between molybdenum and copper. However, this publication was not published in a peer-reviewed scientific paper, but in a book chapter. Since 2001 (US assessment), there haven't been any scientific publications on the interaction between molybdenum and copper. A reduced uncertainty factor may therefore be premature and so at this point in time the EU evaluation is considered the most appropriate. The adult UL was adjusted on a body weight basis for the various age groups.

In summary, the UL for **molybdenum** for the various groups are:

1-3 years	**100 µg/day**
4-8 years	**200 µg/day**
9-13 years	**350 µg/day**
14-18 years	**500 µg/day**
Adults	**600 µg/day**

Dietary intake

There were no food composition data available to enable a comprehensive intake assessment to be conducted for molybdenum. Whilst there are small amounts of data available, these data were either not from Australian or New Zealand sources, were not extensive enough across the whole diet, were not in the correct format or had not been assessed for accuracy.

Risk characterisation
For molybdenum a UL of 600 µg/day for adults has been established based on reproductive effects in rats. Some food composition data are available for molybdenum although these are not sufficient to undertake a complete dietary intake assessment at the present time.

In the absence of a complete dietary intake assessment, it is not currently possible to evaluate the safety of the addition of molybdenum to formulated beverages.

Phosphorus

Hazard identification and characterisation

Chemistry

Phosphorus is a group 5 element of the periodic table and has an atomic weight of 30.97. Phosphorus is most commonly found in nature in its pentavalent form in combination with oxygen, as phosphate (PO_4^{3-}).

Function

Phosphorus is a constituent of all major classes of biochemical compounds. Structurally, phosphorus occurs as phospholipids, which are a major constituent of most biological membranes, and as nucleotides and nucleic acids. Phosphorus plays an important role in carbohydrate, fat and protein metabolism and is essential for optimum bone health. The energy that is required for most metabolic processes is derived from the phosphate bonds of adenosine triphosphate and other high energy phosphate compounds.

Clinical studies employing chronic phosphorus supplementation were the first to show that high phosphorus intakes influence the parathyroid-vitamin D axis, which maintains calcium balance in the body. The phosphorus loading in humans operates through mechanisms of nutritional or secondary hyperparathyroidism similar to those observed in animals fed excess phosphorus.

Sources of phosphorus

Dietary sources that are rich in phosphorus include red meats, dairy products, fish, poultry and bread and other cereal products. A number of phosphate salts are used in foods and soft drinks as additives.

Absorption, distribution, metabolism and excretion

Food phosphorus is a mixture of inorganic and organic forms. Intestinal phosphatase hydrolyze the organic forms contained in ingested protoplasm and thus most phosphorus absorption occurs as inorganic phosphate. On a mixed diet, net absorption of total phosphorus in various reports ranges from 55 to 70 percent in adults and from 65 to 90 percent in infants and children. There is no evidence that this absorption efficiency varies with dietary intake. There is no apparent adaptive mechanism that reduces phosphorus absorption at high intakes. A portion of phosphorus absorption is by way of a saturable active transport facilitated by 1,25-dihydroxyvitamin D. However, the fact that fractional phosphorus absorption is virtually constant across a broad range of intakes suggests that the bulk of phosphorus absorption occurs by passive, concentration-dependent processes. Phosphorus absorption is reduced by ingestion of aluminium-containing antacids and by pharmacologic doses of calcium carbonate. There is no significant interference with phosphorus absorption by calcium at intakes within the typical adult range.

Approximately 80% of the body phosphorus is present in the skeleton and the remainder is distributed in soft tissues and extracellular fluid. About 70% of the phosphorus in blood is as a constituent of phospholipids; the remainder is present as inorganic phosphate, about 85% free and 15% protein-bound.

Excretion of endogenous phosphorus is mainly through the kidneys. Inorganic serum phosphate (P_i) is filtered at the glomerulus and reabsorbed in the proximal tubule. The transport capacity of the proximal tubule for phosphorus is limited; it cannot exceed a certain number of mmol per unit time. This limit varies inversely with parathyroid hormone (PTH) concentration; PTH thereby adjusts renal clearance of P_i. In the healthy adult, urine phosphorus is essentially equal to absorbed diet phosphorus, less small amounts of phosphorus lost in shed cells of skin and intestinal mucosa. This regulation of phosphorus excretion is apparent from early infancy. In infants, as in adults, the major site of regulation of phosphorus retention is at the kidney.

Toxicity

P_i rises as total phosphorus intake increases. Excess phosphorus intake from any source is expressed as hyperphosphatemia, and essentially all the adverse effects of phosphorus excess are due to the elevated P_i in the extracellular fluid.

The principal effects that have been attributed to hyperphosphatemia are: 1) adjustments in the hormonal control system regulating the calcium economy, 2) ectopic (metastatic) calcification, particularly in the kidney, 3) in some animal models, increased porosity of the skeleton, and 4) a suggestion that high phosphorus intakes could reduce calcium absorption by complexing calcium in the chyme.

It has been reported that high intakes of polyphosphates, such as are found in food additives, can interfere with absorption of iron, copper, and zinc; however, described effects are small, and have not been consistent across studies. For this reason it was not considered feasible to use trace mineral status as an indicator of excess phosphorus intake.

Most of the studies that describe harmful effects of phosphorus intake used animal models. In extrapolating these data to humans, it is important to note that the phosphorus density of human diets represent the extreme low end of the continuum of standard diets for pets and laboratory animals.

The US stated that a UL can be defined as an intake associated with the upper boundary of adult normal values of serum P_i. No reports exist of untoward effects following high dietary phosphorus intakes in humans. Essentially all instances of dysfunction (and, hence, all instances of hyperphosphatemia) in humans occur for non-dietary reasons (for example, end-stage renal disease, vitamin D intoxication). Therefore, data on the normal adult range for serum P_i are used as the basis for deriving a UL for adults.

The higher values for serum P_i in infancy are manifestly tissue-safe levels, and if they are taken as an approximation of the upper normal human value (on the ground that there is no basis for assuming major differences in tissue susceptibility to metastatic mineralization at different ages), the corresponding ingested intake in an adult would be over 10.2 g (330 mmol)/day.

If the normal adult range is used, the upper boundary of adult normal values of serum P_i is reached at a daily phosphorus intake of 3.5 g. There is no evidence that individuals consuming this intake may experience any untoward effects. No benefit is evident from serum P_i values above the usual normal range in adults.

Vulnerable groups

Hyperphosphatemia from dietary causes becomes a problem mainly in patients with end-stage renal disease or in such conditions as vitamin D intoxication. When functioning kidney tissue mass is reduced to less than ~20 percent of normal, the glomerular filtration rate becomes too low to clear typical absorbed loads of dietary phosphorus, and then even sharply reduced phosphorus diets may still be excessive as they lead to hyperphosphatemia.

Evaluation

Phosphorus	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2000a)	4,000	Total	serum inorganic phosphorus levels	human
UK (UK Expert Group on Vitamins and Minerals, 2003)*	250	supplemental	gastrointestinal	human
EU	no assessment available			

* Guidance level only

Only the US set a UL for total phosphorus intake and is therefore more relevant than the UK evaluation.

The US stated that no benefit is evident from serum P_i values above the usual normal range in adults. Moreover, information is lacking concerning adverse effects in the zone between normal P_i and levels associated with ectopic mineralization. Therefore, the US kept with the pharmacokinetic practice where the relationship between intake and blood level is known, an uncertainty factor of 2.5 is chosen. An UL of 4.0 g/day for adults is calculated by dividing a NOAEL of 10.2 g/day by an uncertainty factor of 2.5.

The US calculated a UL for children up to 8 years of 3 g/day by dividing the NOAEL for adults (10.2 g/day) by an uncertainty factor of 3.3 to account for potentially increased susceptibility due to smaller body size. There is no evidence to suggest increased susceptibility to adverse effects during adolescence. Therefore, the same UL specified for adults was selected. Because of an increasing prevalence of impaired renal function after age 70, a larger uncertainty factor of 3.3 seems prudent, and the UL for adults of this age is set at 3.0 g/day. During pregnancy, absorption efficiency for phosphorus rises by about 15 percent, and thus, the UL associated with the upper end of the normal range will be about 15 percent lower, that is, about 3.5 g/day.

During lactation, the phosphorus economy of a woman does not differ detectably from the non-lactating state. Hence the UL for this physiologic state is not different from the non-lactating state, 4.0 g/day.

In summary, the UL for **phosphorus** for the various groups are:

1-3 years	**3.0 g/day**
4-8 years	**3.0 g/day**
9-13 years	**4.0 g/day**
14-18 years	**4.0 g/day**
19-70 years	**4.0 g/day**
71 years and over	**3.0 g/day**
Pregnancy	**3.5 g/day**
Lactation	**4.0 g/day**

Dietary intake

Intakes of phosphorus were estimated at baseline and when formulated beverages are consumed. Estimated intakes were adjusted based on second day intake data from the NNSs.

The concentration of phosphorus requested to be added to formulated beverages was 250 mg/600 ml reference quantity.

Estimated intakes were calculated for two more specific population groups for phosphorus compared to other nutrients. This was because these groups had specific ULs. The additional groups assessed were older people aged 71 years and over, and women of child bearing age (16-44 years) as a proxy to represent pregnant and lactating women. Where respondents aged 71 years or over were included in the collated results for the age group of 19 years and above, they were assigned their own respective UL. Where the estimates were calculated for the general population that may have included females 16-44 (e.g. 14-18 years), the UL for the general population was used. Only when females aged 16-44 years were assessed in isolation was the UL for pregnancy used. (A separate comparison of intakes against the UL of 4 g/day for lactation was not calculated, as the pregnancy UL of 3.5 g/day was a worst case scenario).

Estimated intakes increased from baseline by around 100 mg/per day when formulated beverages were consumed across the population groups assessed. The UL was not exceeded for any population group assessed.

Table 20: Estimated dietary intakes of phosphorus, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake mg/day (%UL)		95th percentile intake mg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	1052 (35)	1150 (40)	1504 (50)	1655 (55)
4-8 years, Aus	1143 (40)	1270 (45)	1737 (60)	1860 (60)
5-6 years, NZ	^1020 (**)	NA	#1301 (**)	NA
7-10 years, NZ	^1164 (**)	NA	#1546 (**)	NA
9-13 years, Aus	1402 (35)	1549 (40)	2259 (55)	2375 (60)
11-14 years, NZ	^1339 (**)	NA	#1792 (**)	NA
14-18 years, Aus	1589 (40)	1758 (45)	2735 (70)	2980 (75)
15-18 years, NZ	1568 (40)	1685 (40)	2462 (60)	2585 (65)
16-44 years females, Aus	1374 (40)	1470 (40)	2145 (65)	2245 (65)
16-44 years females, NZ	1421 (40)	1494 (45)	2141 (60)	2231 (65)
≥19 years, Aus	1490 (40)	1574 (40)	2459 (65)	2608 (65)
≥19 years, NZ	1484 (40)	1541 (40)	2335 (60)	2427 (60)
71+ years, Aus	1247 (40)	1281 (45)	1941 (65)	1982 (65)
71+ years, NZ	1235 (40)	1254 (40)	1733 (60)	1765 (60)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.

^ mean adjusted intake, from MOH 2003, averaged for males and females.

90th percentile adjusted intake, from MOH 2003, averaged for males and females.

** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.

NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

The main route of exposure to phosphorus is through the diet. Toxicological evaluation and dietary intake data indicate that both children and adult Australian and New Zealand consumers are unlikely to approach the UL set for phosphorus, at the 95th percentile of intake assuming use in formulated beverages (55-75% UL for children of the various age-groups and 65% UL for adult Australians 19 years and above and 60% UL for adult New Zealanders aged 19 years and above).

For pregnant women an UL of 3.5 g/day was established. The intake in females of childbearing age (16-44 years) at the 95th percentile intake was 2.2 g/day when formulated beverages are consumed (65% UL). For older adults (71 years and over) a lower UL was established of 3.0 g/day. This age group did not exceed the UL at the 95th percentile intake (2.0 g/day for Australians or 65% UL and 1.8 g/day or 60% UL for New Zealanders, aged 71 and above). Therefore, dietary intake of phosphorus for consumers from all population groups is considered to be within the safe range of intake for both mean and high consumers.

It is concluded that addition of phosphorus to formulated beverages at a level of 250 mg in a 600 ml serve poses no appreciable public health and safety risk, assuming baseline levels of use in other foods.

Selenium

Hazard identification and characterisation

Chemistry

Selenium is a metallic group VI element that is abundant and which can exist in 4 oxidation states (-2, +1, +2 and +6).

Function

The biologically active form of selenium is selenocysteine. Selenocysteine is incorporated into selenoproteins, of which over thirty have been identified to date. The selenoproteins include the glutathione peroxidases, which protect against oxidative damage, the iodothyronine deiodinases (involved in the production of the hormone triiodothyronine from thyroxine), selenoprotein P (which is involved in antioxidant and transport functions) and the thioredoxin reductases (maintenance of the intracellular redox state). Selenium is essential to humans at low levels but potentially toxic at high levels of exposure. Selenium is widely distributed in rocks and soils; however, its distribution is uneven. Selenium was known as a toxicant before being recognised as a nutrient.

Sources of selenium

The selenium content of food varies depending on the selenium content of the soil where the animal was raised or the plant was grown: organ meats and seafood, 0.4-1.5 μg/g; muscle meats, 0.1 to 0.4 μg/g; cereals and grains, less than 0.1 to greater than 0.8 μg/g; dairy products, less than 0.1 to 0.3 μg/g; and fruits and vegetables, less than 0.1 μg/g ((WHO, 1987).

Thus, the same foodstuffs may have more than a ten-fold difference in selenium content. Plants do not appear to require selenium and most selenium metabolism by plants occurs through sulphur pathways in which selenium substitutes for sulphur. Thus, plant content of selenium depends on the availability of the element in the soil where the plant was grown. Unlike plants, animals require selenium. Meat and seafood are therefore more reliable dietary sources of selenium.

Absorption, distribution, metabolism and excretion

Absorption of selenium is efficient and is not regulated. More than 90 percent of selenomethionine, the major dietary form of the element, is absorbed by the same mechanism as methionine itself. Although little is known about selenocysteine absorption, it appears to be absorbed very well also. An inorganic form of selenium, selenate (SeO_4^{2-}), is absorbed almost completely, but a significant fraction of it is lost in the urine before it can be incorporated into tissues. Another inorganic form of selenium, selenite (SeO_3^{2-}), has a more variable absorption, probably related to interactions with substances in the gut lumen, but is better retained, once absorbed, than is selenate. Absorption of selenite is generally greater than 50 percent. Although selenate and selenite are not major dietary constituents, they are commonly used to fortify foods and as selenium supplements.

Two pools of reserve selenium are present in humans and animals. One of them, the selenium present as selenomethionine, depends on dietary intake of selenium as selenomethionine. The amount of selenium made available to the organism from this pool is a function of turnover of the methionine pool and not the organism's need for selenium. The second reserve pool of selenium in the selenium present in liver glutathione peroxidase (GSHPx-1). In rats, 25 percent of total body selenium is present in this pool. As dietary selenium becomes limiting for selenoprotein synthesis, this pool is downregulated by a reduction of GSHPx-1 messenger RNA concentration. This makes selenium available for synthesis of other selenoproteins.

Selenomethionine, derived mainly from plants, enters the methionine pool in the body and shares the fate of methionine until catabolised by the transsulfuration pathway. The resulting free selenocysteine is further broken down with liberation of a reduced form of the element, which is designated selenide. Ingested selenite, selenate, and selenocysteine are all apparently metabolised directly to selenide. This selenide may be associated with a protein that serves as a chaperone. The selenide can be metabolised to selenophosphate, the precursor of selenocysteine in selenoproteins and of selenium in transfer RNA, or it can be converted to excretory metabolites, some of which have been characterised as methylated forms.

The mechanism that regulates production of excretory metabolites has not been elucidated, but excretion has been shown to be responsible for maintaining selenium homeostasis in the animal. The excretory metabolites appear in the urine, primarily, but when large amounts of selenium are being excreted, the breath also contains volatile metabolites (e.g. dimethylselenide, garlic breath).

Toxicity

FSANZ[5] reviewed selenium toxicity in 1999 as part of Proposal P157 – Metal Contaminants (ANZFA, 1999). Since then new evaluations have become available and therefore the safety of selenium has been revisited.

Selenium has a variety of toxic endpoints in both animals and humans. In humans, the first signs of chronic toxicity appear to be pathological changes to the hair and nails, followed by adverse effects on the nervous system. Common clinical features are hair loss and structural changes in the keratin of hair and of nails, the development of icteroid skin, and gastrointestinal disturbances. A positive association between dental caries and urinary selenium have been reported. Changes in biochemical parameters have also been reported. The available studies indicate the development of selenosis (chronic selenium poisoning) is associated with selenium intakes in excess of 0.85 mg/day (0.014 mg/kg bw for a 60 kg adult). Selenium toxicity is cumulative.

Supplementation studies in humans indicate that up to 0.3 mg/day additional selenium is not associated with overt adverse effects over a short period of time, although specific symptoms have not always been investigated. However, one study, which specifically considered symptoms of selenosis, indicated that 0.2 mg/day additional selenium for up to 10 years did not result in symptoms of selenosis. In addition to reduced growth rates, similar symptoms to those in humans are found in animals treated with selenium.

Selenium sulphide, which is not a permitted form in the Code, is carcinogenic but other selenium compounds are not. Selenium compounds are not mutagenic *in vivo*. Adverse effects have been reported on the reproductive system of various animals, though not primates. Reproductive toxicity is not an issue that has been examined in detail in the available human epidemiological studies.

The most sensitive indicators of selenium toxicity are changes in the nails and hair. In a study by Yang ((Yang *et al*., 1989a; Yang *et al*., 1989b) conducted in an area of China where dietary selenium exposure is high, selenium intakes were correlated with blood levels to determine the intakes at which marginal selenium toxicity occur. This was at a total intake of 0.91 mg/day selenium.

Children

Studies have shown that a human milk selenium concentration of 60 μg/L was not associated with known adverse effects. Therefore, this will give a conservative estimate to derive upper limits for children and adolescents.

[5] as the Australia New Zealand Food Standards Authority (ANZFA)

Evaluation

Selenium	UL in adults, mg/day	Total diet / suppl	Critical effect	human /animal data
US (US Institute of Medicine, 2000c)	0.40	Total	hair loss and changes in nail pathology	human
UK (UK Expert Group on Vitamins and Minerals, 2003)	0.45	Total	hair loss and changes in nail pathology	human
EU (European Commission Health & Consumer Protection Directorate-General, 2000e)	0.30	Total	hair loss and changes in nail pathology	human
WHO/FAO (WHO, 1987)#	0.40	Total	hair loss and changes in nail pathology	human
ANZFA (ANZFA, 1999)#	0.75	Total	hair loss and changes in nail pathology	human

Provisional Tolerable Daily Intake

The EU considered the study of Yang the most relevant study for selenium toxicity. A NOAEL of 850 µg/day was derived. The NOAEL used was derived from a study on a large number of subjects and is expected to include sensitive individuals. It was decided to use an uncertainty factor of 3 to account for the remaining uncertainties of the studies used in deriving an UL. An upper limit of 0.30 mg/day was derived for adults. This value covers selenium intake from all sources of food, including supplements.

The US established a NOAEL of 800 µg/day, based on the Chinese studies, which is protective for the population in the United States and Canada. An uncertainty factor of 2 was selected to protect sensitive individuals. The toxic effect is not severe, but may not be readily reversible, so a UF greater than 1 is needed. An UL of 0.40 mg/day was derived for adults.

The UK concluded that the intake of 0.91 mg selenium/day produced slight effects and was close to a NOAEL. Because of this an uncertainty factor of 2 was applied for LOAEL to NOAEL extrapolation. Because this is based on a population study, an uncertainty factor for inter-individual variation is not required. An UL for total selenium intake of 0.45 mg/day can therefore be derived.

The evaluation of FAO/WHO (FAO/WHO, 2002) for the upper limit of selenium was based on a risk assessment report from the International Programme on Chemical Safety (WHO, 1987). A comprehensive account of the clinically significant biochemical manifestations of chronic and acute intoxication from selenium arising from high concentrations in food, drinking water, and the environment were published jointly by WHO and the United Nations Environment Programme and the International Labour Organisation (WHO, 1987). This report stresses that the signs and symptoms of human overexposure to selenium are not well defined. Common clinical features are hair loss and structural changes in the keratin of hair and of nails, the development of icteroid skin, and gastrointestinal disturbances. An increased incidence of nail dystrophy has been associated with consumption of high-selenium foods supplying more than 900 µg/day. These foods were grown in selenium-rich (seleniferous) soil from specific areas in China. A positive association between dental caries and urinary selenium output under similar circumstances was reported. Sensitive biochemical markers of impending selenium intoxication have yet to be developed.

In their absence it is suggested that the upper tolerable nutrient intake level (UL) for selenium should be set, provisionally, at 400 μg/day for adults. It is noteworthy that a maximum tolerable dietary concentration of 2 mg/kg dry diet was suggested for all classes of domesticated livestock and has proved satisfactory in use (National Research Council, 1980). This suggests that the proposed UL of 400 μg/day for human subjects provides a fully adequate margin of safety.

The previous evaluation by FSANZ (as ANZFA) had established the level of 750 μg/day for a toxicological endpoint, which is not life-threatening. Homeostatic mechanisms present in adults act to compensate for excessive intakes of selenium and the toxicity at this level, and at higher levels (850-959 μg/day) associated with clinical signs of toxicity, were considered to be reversible. Chronic selenium intake of 750 μg/day was therefore proposed as the provisional tolerable daily intake for selenium. Insufficient data were available from which to estimate the safe upper limit to population mean intakes of selenium for most other age groups and for pregnant and lactating women.

The most sensitive indicators for selenium toxicity are changes in nails and hair. More severe adverse effects on the nervous system are difficult to analyse and therefore less easily detected. The effects of selenium toxicity, i.e. adverse effects on the nervous system, are serious and cumulative, and necessitate the setting of an upper limit. The most sensitive indicators of selenium toxicity are changes in nails and hair; therefore these endpoints are used for establishing an upper limit.

The more recent evaluations by the US and the FAO/WHO are considered the most comprehensive and have been used to derive the following upper limits for **selenium**:

1-3 years	**90 μg/day**
4-8 years	**150 μg/day**
9-13 years	**280 μg/day**
14-18 years	**400 μg/day**
adults	**400 μg/day**

Permitted forms

The Applicant requested the following forms to be permitted for selenium: seleno methionine, sodium selenate, and sodium selenite. Seleno methionine and sodium selenite are already permitted in Standard 2.9.1 – Infant Formula Products and sodium selenate is permitted in Standard 2.9.4 – Formulated Supplementary Sports Foods.

Within the assessment of selenium toxicity by both the EU (European Commission Health & Consumer Protection Directorate-General, 2000e) and US (US Institute of Medicine, 2000c) inorganic selenites and selenates as well as selenomethionine were included. The US stated that the limited data available in humans suggest that chronic toxicities from inorganic and organic forms have similar clinical features but differ in rapidity of onset and relationship to tissue selenium concentrations.

In conclusion, the available evidence does not indicate that the different forms of selenium have differences in toxicity. Therefore, the requested forms of selenium are appropriate as permitted forms for selenium.

Dietary intake

Intakes of selenium were estimated at baseline and when formulated beverages are consumed.

The concentration of selenium requested to be added to formulated beverages was 17.5 µg/600 ml reference quantity.

Selenium was not assessed in the 1995 Australian NNS. Therefore, a model was set up in DIAMOND assigning selenium concentrations to food groups in order to estimate selenium intakes for Australian population groups. The concentration data used in the dietary modelling were derived from Australian analytical surveys that were collected for the purposes of conducting dietary exposure assessments for P157 – Metal contaminants in foods, a previous proposal raised during the Review of the Code. Only Australian survey data were used for the assessment for Australia.

Selenium was assessed in the 1997 New Zealand NNS, therefore the concentration data were specific to New Zealand foods.

Estimated intakes for selenium were not adjusted for Australia, but were adjusted for New Zealand based on second day intake data from the 1997 NNS. Baseline intakes for New Zealanders aged 5-14 years from the 2002 Children's Nutrition Survey were also adjusted using second day intakes (Ministry of Health, 2003). The unadjusted estimated intakes for selenium for the Australian population groups will be higher at the 95th percentile than those for similar age groups that have adjusted intakes.

Estimated intakes increased from baseline around 10 to 20 µg/per day when formulated beverages were consumed depending on the population group assessed. The UL was not exceeded for any population groups assessed.

Table 21: Estimated dietary intakes of selenium, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake µG/DAY (%UL)		**95th percentile intake µg/day (%UL)**	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	32 (35)	42 (45)	70 (80)	92 (100)
4-8 years, Aus	41 (30)	53 (35)	93 (60)	111 (75)
5-6 years, NZ	^28.3 (**)	NA	#37.7 (**)	NA
7-10 years, NZ	^35.3 (**)	NA	#52.6 (**)	NA
9-13 years, Aus	54 (20)	68 (25)	117 (40)	138 (50)
11-14 years, NZ	^42.6 (**)	NA	#63.4 (**)	NA
14-18 years, Aus	69 (15)	86 (20)	160 (40)	192 (50)
15-18 years, NZ	48 (10)	59 (15)	70 (20)	85 (20)
≥19 years, Aus	70 (15)	77 (20)	165 (40)	178 (45)
≥19 years, NZ	51 (15)	56 (15)	78 (20)	87 (20)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.

** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Dietary modelling indicates that selenium intakes for all population groups are predicted to be below the UL even for high consumers and applying a worst-case scenario i.e. all products specified are replaced by formulated beverages (Scenario 2).

All population groups at the 95th percentile intake, with the exception of 2-3 year olds, are estimated to have intakes of selenium below the UL. Estimated 95th percentile intakes for 2-3 year olds is estimated to be at the UL (100%).

Due to the use of 24-hour dietary survey data, which tends to over-estimate habitual food consumption amounts for high consumers, it is likely that the 95th percentile dietary intake is an over-estimate. In addition, a number of conservative assumptions were used in the dietary modelling which may further add to the overestimation. For example, that all specified drinks would be substituted for formulated beverages in the 2-3 year old population group.

The UL represents a quantitative level of total intake at which, or below no harm is expected to occur assuming nutrient adequacy is met. Therefore estimated intake levels at the UL, generally do not raise any safety concerns as the UL is not itself a threshold for toxicity. In this case, the predicted 95th percentile intake for 2-3 year olds is still well below a level at which adverse effects might be observed. The dietary modelling also predicts that the higher intakes estimated for 2-3 year olds will not be sustained in the older age groups (e.g. 4-8 year olds).

Overall, the potential to exceed the UL, even for 2-3 year olds, is considered to be low.

It is concluded that addition of selenium to formulated beverages at a level of 17.5 μg in a 600 ml serve poses no appreciable public health and safety risk.

Zinc

Hazard identification and characterisation

Chemistry

Zinc is an abundant group IIB post-transition metallic element. It occurs in nature in various forms. Zinc is present in the earth's crust and in seawater. Zinc is found in all plant and animal tissues, particularly inside the nuclei.

Function

Zinc is essential for growth and development, testicular maturation, neurological function, wound healing and immunocompetence. Over 300 zinc enzymes have been discovered covering all six classes of enzymes and in different species of all phyla. Zinc has structural, regulatory or catalytic roles in many enzymes.

Additionally, it maintains the configuration of a number of non-enzymatic proteins such as pre-secretory granules of insulin, some mammalian gene transcription proteins and thymulin. Well known zinc containing enzymes include superoxide dismutase, alkaline phosphatase and alcohol dehydrogenase.

Sources of zinc

Zinc is found in all plant and animal tissue, particularly inside the nuclei. Good food sources of zinc include red meat, whole wheat, raisins, unrefined cereals (high content, low bioavailability) and fortified cereals.

Tap water can contain high concentrations of zinc as a result of corrosion of zinc-coated pipes and fittings (NHMRC and NRMMC, 2004). Zinc concentrations in galvanised iron rainwater tanks are typically 2 mg/L to 4 mg/L but have been reported as high as 11 mg/L. In major Australian reticulated supplies, the concentration of zinc ranges up to 0.26 mg/L, with a typical concentration of 0.05 mg/L. Drinking water guidelines in Australia and New Zealand (Ministry of Health, 2000) recommend concentrations should not exceed 3 mg zinc/L, based on aesthetic considerations (taste).

Other sources of zinc, excluding dietary intakes, include zinc supplements, inhalation of zinc metal or oxide fumes in industrial settings and storage of food and drink in galvanised containers.

Absorption, distribution, metabolism, and excretion

Absorption of zinc takes place in the small intestine and appears to be a carrier-mediated transport process which is not saturated under normal physiological conditions. At high intakes, zinc is also absorbed through a non-saturable process or passive diffusion. Absorption of dietary zinc ranges from 15 to 60%. Mechanisms for the transport of zinc across the intestinal wall, its export into plasma and its uptake into other tissues are uncertain. Once in plasma, zinc is carried by a number of proteins that include albumin, transferrin and caeruloplasmin. Most of the absorbed zinc is excreted in the bile and eventually lost in the faeces. There appears to be no specific zinc ‘store’ in the body.

Tissue content and activity of zinc-dependent processes are maintained over a wide range of dietary zinc intakes. When zinc intake is increased, the fractional absorption decreases and intestinal excretion increases while urinary losses remain fairly constant. Endogenous faecal zinc losses may increase several fold to maintain zinc homeostasis with high intakes. At very low zinc intakes, absorption can increase to between 59-84% and faecal and urinary losses decrease accordingly. When these primary homeostatic mechanisms are not sufficient to handle large dietary excesses of zinc, the excess zinc is lost via the hair. The kinetics of zinc absorption and elimination follow a two-component model. The initial rapid phase has a half-life in humans of 12.5 days and the slower pool turns over with a half-life of approximately 300 days.

Interactions with a number of dietary factors influence zinc uptake. Ligands, such as phytate, form insoluble complexes with zinc and prevent absorption. Calcium increases binding of zinc by phytate. Larger doses of calcium can decrease net zinc absorption. High iron content in the diet decreases zinc absorption.

Earlier reports indicated that folic acid can also inhibit zinc retention and metabolism, but more recent evidence indicates that folic acid does not adversely affect zinc status. Copper and zinc compete for absorption but it appears unlikely that modestly increased intakes of copper interfere with zinc absorption. Histidine, methionine and cysteine are thought to facilitate zinc absorption (these amino acids remove zinc from the zinc-calciumphytate complexes).

Toxicity

FSANZ[6] reviewed zinc toxicity in 1999 as part of Proposal P157 – Metal Contaminants (ANZFA, 1999). Since then new evaluations have become available and therefore the safety of zinc has been revisited.

Animals

Very high doses of zinc in animal studies can cause neural degeneration, acinar cell necrosis and metaplasia in the pancreas, decreased haematocrit and decreased white blood cell count. Very high doses have also been shown to cause reproductive toxicity in rats. Lower doses have resulted in reduced ceruloplasmin activity and decreased haemoglobin levels.

Zinc has been found to give positive results in some in vitro and in vivo genotoxicity tests. The weight of evidence from the in vitro and in vivo genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels at elevated doses, has no biologically relevant genotoxicity activity. No data have been identified on the carcinogenicity of zinc.

Humans

Acute toxicity is infrequent in humans. Several cases of food poisoning are described resulting from storage of food or drink in galvanised containers. Symptoms of acute zinc toxicity include nausea, vomiting, epigastric pain, abdominal cramps and diarrhoea. One study reported symptoms of lethargy and light-headedness. This change in presenting symptoms could be a result of the type of zinc (in this case zinc sulphate) ingested. Zinc acetate (25-50 mg, three times per day), given to Wilson's disease patients to prevent copper accumulation was reported to cause less dyspepsia than equivalent doses of zinc sulphate. Emetic doses of zinc have been estimated to correspond to 225-450 mg. An industrial hazard associated with inhalation of zinc oxide fumes is 'metal fume fever'. Subjects present with malaise, fever, headache, nausea and dryness of mouth and throat.

Studies of chronic and sub-chronic toxicity of zinc are well documented. Prolonged intakes of zinc supplements ranging from 50 mg/day up to 300 mg/day have been associated with a range of biochemical and physiological changes. These changes include hypocupraemia, leucopaenia, neutropaenia, sideroblastic anaemia, decreased concentrations of plasma copper and decreased activity of the copper containing enzymes, superoxide dismutase and caeruloplasmin, altered lipoprotein metabolism and impaired immune function. Many of these biochemical and physiological changes are similar to those observed during copper deficiency. Nevertheless, there are problems with hazard identification in that these changes are not specific to copper deficiency and the clinical relevance of some are unknown.

[6] as the Australia New Zealand Food Standards Authority (ANZFA)

Vulnerable groups

Sensitive sub-populations may include subjects with haemochromatosis and/or insulin dependent diabetes. A small study suggests that zinc supplementation increases the levels of glycosylated haemoglobin in diabetics.

Zinc excess in water may decrease iron absorption. Hepatic zinc concentration is increased in haemochromatosis and there is some evidence that zinc absorption may be increased

Evaluation

Zinc	**UL in adults, mg/day**	**Total diet / suppl**	**Critical effect**	**human /animal data**
US (US Institute of Medicine, 2001b)	40	Total	reduced copper status	human
UK (UK Expert Group on Vitamins and Minerals, 2003)	25	Suppl	reduction in copper absorption	human
EU (European Commission Health & Consumer Protection Directorate-General, 2003e)	25	Total	reduced copper status	human
FAO/WHO (FAO/WHO, 2002)	45	Total	reduced copper status	human
ANZFA (ANZFA, 1999)*	60	Total	reduced copper status	human

* Provisional Tolerable Daily Intake.

The selection of reduced copper status was chosen as the critical effect based on 1) the consistency of findings from studies measuring the interaction of zinc and copper, 2) the sensitivity of endothelial superoxide dismutase (ESOD) activity as a marker for this effect, and 3) the quality and completeness of the database for this endpoint. The data on the effects of zinc on HDL cholesterol concentration were not consistent from study to study and therefore were not used to derive a UL.

Systemic evidence of copper deficiency in humans may be observed at doses of 150 mg/day in humans, but doses as low as 50 mg/day may indicate a threshold effects, as observed by changes in biochemical markers of copper deficiency (ANZFA, 1999).

The US set a LOAEL of 60 mg/day based on a study of Yadrick and coworkers (Yadrick *et al*., 1989) who evaluated copper status after supplemental intake of 50 mg/day as zinc gluconate in 18 healthy female subjects (aged 25 to 40 years) for 10 weeks. ESOD activity was significantly lower than pretreatment values. Although no dietary zinc or copper intakes were reported, a level of dietary zinc can be estimated at approximately 10 mg/day for females. A LOAEL of 60 mg/day was calculated by adding the supplemental intake of 50 mg/day with the rounded estimate of dietary intake, 10 mg/day. Support for a LOAEL of 60 mg/day is provided by other studies showing altered copper balance after zinc supplementation.

The US selected an uncertainty factor of 1.5 to account for inter-individual variability in sensitivity and for extrapolation from a LOAEL to a NOAEL. Because reduced copper status is rare in humans, a higher UF was not justified.

For children a study in infants fed 5.8 mg/L of zinc for six months did not reveal effects of zinc on serum copper or cholesterol concentrations or other adverse effects. This would result in an intake of 4.5 mg/day for infants 0 through 6 months of age. This NOAEL was divided by a UF of 1.0 to obtain an upper limit of 4 mg/day (rounded down) for infants 0 through 6 months. No adverse effects of zinc in children and adolescents could be found. Due to a dearth of information, the UL for young infants was adjusted for older infants, children and adolescents on the basis of relative body weight. Values have been rounded down.

The EU set a NOAEL of 50 mg/day, based on the absence of any adverse effects on a wide range of relevant indicators of copper status (as the critical endpoint) in the various. Subjects were 25 and 21 healthy post-menopausal women and 19 healthy young men. Duration of supplementation was for 90 days and for 14 weeks. Total zinc and copper intakes were tightly controlled in the metabolic studies in which the zinc intake was 53 mg/day. Total zinc intake was 40 mg/day in the second study. An uncertainty factor of 2 is applied owing to the small number of subjects included in relatively short-term studies but acknowledging the rigidly controlled metabolic experimental conditions employed. EU recommended an UL of 25 mg/day.

FAO/WHO made a distinction between zinc absorption in different diets was made. According to the report, certain diets have high zinc bioavailability (50%), moderate bioavailability (35%) or low bioavailability (15%). The Australian and New Zealand diet can be considered to have high bioavailability (refined diets low in cereal fibre, low in phytic acid content and with phytate/zinc (molar) ratio <5; adequate protein content principally from non-vegetable sources, such as meats, fish). In the WHO/FAO report, consideration was given to countries where diets have low bioavailability, which often are developing countries with a high level of zinc deficiency. Since the emphasis in this report was on developing countries, the UL is considered less relevant for Australia and New Zealand.

Based on the data considered in the US evaluation the UL for **zinc** for the various age groups are:

1-3 years	**7 mg/day**
4-8 years	**12 mg/day**
9-13 years	**23 mg/day**
14-18 years	**34 mg/day**
adults	**40 mg/day**

Dietary intake

Intakes of zinc were estimated at baseline and when formulated beverages are consumed.

The concentration of zinc requested to be added to formulated beverages was 3 mg/600 ml reference quantity.

Estimated intakes were adjusted based on second day intake data from the NNSs. Dietary modelling has been conducted only for food intake. Intake through other sources (i.e. supplements and drinking water) was not included in the modelling.

Estimated intakes increased from baseline by between 1 and 2 mg/per day when formulated beverages were consumed depending on the population groups assessed.

The UL was not exceeded for the majority of population groups assessed, apart from Australian children aged 2-3 years at the mean level of intake, at baseline and when consuming formulated beverages, and for Australian children aged 2 to 8 years at the 95th percentile level of intake, at baseline and when consuming formulated beverages.

Table 22: Estimated dietary intakes of zinc, before and after formulated beverages are introduced into the diet, and percent of UL

	Mean intake mg/day (%UL)		95th percentile intake mg/day (%UL)	
Age group	**Baseline**	**Scenario 2***	**Baseline**	**Scenario 2***
2-3 years, Aus	7.5 (110)	8.8 (130)	10.4 (150)	11.9 (170)
4-8 years, Aus	8.2 (70)	9.7 (80)	11.7 (100)	13.7 (115)
5-6 years, NZ	^8.1 (**)	NA	#10.3 (**)	NA
7-10 years, NZ	^9.7 (**)	NA	#13.4 (**)	NA
9-13 years, Aus	10.9 (45)	12.9 (55)	16.5 (70)	18.2 (80)
11-14 years, NZ	^10.2 (**)	NA	#15.5 (**)	NA
14-18 years, Aus	12.7 (35)	15.3 (45)	21.3 (65)	25.6 (75)
15-18 years, NZ	13.0 (40)	16.6 (50)	22.3 (65)	26.4 (80)
≥19 years, Aus	11.9 (30)	13.0 (35)	18.4(45)	20.5 (50)
≥19 years, NZ	12.3 (30)	13.7 (35)	19.6 (50)	22.7 (60)

* Scenario 2 = when people substitute all water based flavoured drinks, bottled water and fruit juices and drinks they consumed with formulated beverages.
^ mean adjusted intake, from MOH 2003, averaged for males and females.
# 90th percentile adjusted intake, from MOH 2003, averaged for males and females.
** not calculated, because the age groups in the summary report did not allow comparison of the mean or high percentile intake with the UL, and the raw data from the survey were not available to allow the age groups to be disaggregated to allow this calculation.
NA = not assessed, because the raw data from the New Zealand 2002 CNS were not in DIAMOND to allow scenario 2 estimates to be calculated.

Risk characterisation

Toxicological evaluation and dietary intake data indicate that children aged 2-3 years as well as children aged 4-8 years in Australia may be exceeding the UL for zinc, both at the mean and at the 95th percentile dietary intake at baseline and for Scenario 2, when added to formulated beverages at 3 mg in a 600 ml serving. For these calculations, intake from other sources, e.g. galvanised containers and supplements, have not been included.

For adults in both Australia and New Zealand estimated zinc intakes, both at baseline and when added to formulated beverages, are below the UL.

In conclusion, children up to 8 years of age are predicted to exceed the UL at the 95th percentile of intake of dietary zinc for baseline and Scenario 2. At the 95th percentile intake for adolescents up to the age of 18 years the intake is 80% of the UL of zinc for Scenario 2.

Chronic zinc toxicity is associated with symptoms of copper deficiency. These overt adverse effects (e.g. anaemia, neutropaenia, impaired immune responses) are evident only after feeding zinc in the form of dietary supplements in excess of 150 mg/day for long periods. It is much more difficult to identify the critical effect of zinc excess at intakes below 100-150 mg per day.

The UL for zinc is based on reduced copper status. The LOAEL was set at 60 mg/day based on a 10-week study in 18 healthy female subjects. At this level the endothelial superoxide dismutase activity (the most sensitive indicator of copper status) was significantly lower than pre-treatment values. Other studies support this LOAEL.

The UL for children was based on levels in infants that did not reveal effects of zinc on serum copper concentrations or other adverse effects. Due to a dearth of information, the UL for young infants was adjusted for older infants, children and adolescents on the basis of relative body weight.

Chronic zinc toxicity is associated with symptoms of copper deficiency. These adverse effects include anaemia, neutropaenia and impaired immune response. Furthermore, the potential contribution from other sources (e.g. dietary supplements) has not been taken into consideration in the dietary intake assessment.

In conclusion, there are potential safety concerns for children and adolescents up to the age of 18 years were the addition of zinc to formulated beverages to be permitted. For adults, addition of zinc to formulated beverages at a level of 3 mg per 600 ml serve poses no appreciable public health and safety risk.

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