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

SURVEY OF NITRATES AND NITRITES IN FOOD AND BEVERAGES IN AUSTRALIA

September 2011

Summary

Background

Nitrate and nitrite ions are ubiquitous in the environment and occur naturally in plant foods as a part of the nitrogen cycle. Nitrate and nitrite, as the sodium or potassium salts, have also been used as food additives in cured meats for many years primarily to prevent growth and toxin production of *Clostridium botulinum*.

Human exposure to nitrate and nitrite occurs mainly through the ingestion of fruit and vegetables. The consumption of fruit and vegetables is widely recommended due to the strong evidence of beneficial effects for health. However, dietary nitrate and nitrite have also raised some concerns because of implications for adverse effects including methaemoglobinaemia (which results in reduced oxygen transport in the blood) and possible increased cancer risk.

In order to estimate the Australian dietary exposure to nitrate and nitrite, and to determine whether there are any risks to human health at current dietary exposure levels, FSANZ has funded and coordinated surveys for both nitrate and nitrite in Australian foods and beverages. Food regulatory agencies in State and Territory governments collected the food samples in their region.

Key findings

- The major sources of estimated nitrate dietary exposures across different population groups were vegetables (42-78%) and fruits (including juices) (11-30%). Highest concentrations of nitrate were generally found in leafy green vegetables, such as spinach, consistent with other international findings.

- Vegetables (44-57%) and fruits (including juices) (20-38%) were also the major contributors to estimated dietary nitrite exposure across the population groups. Nitrite exposure from processed meats accounts for only a relatively small amount of total dietary nitrite exposure (5-7%).

- Estimated Australian dietary nitrate and nitrite exposures are not considered to represent an appreciable health and safety risk.

- However, the health benefits of fruit and vegetables are widely accepted, including strong evidence of a protective effect of certain vegetables, legumes and fruit against the development of a number of non-communicable chronic diseases, among them cancer and cardiovascular disease.

Abbreviations

ADI	Acceptable Daily Intake
ATDS	Australian Total Diet Study
bw	Body weight
DIAMOND	Dietary Modelling of Nutritional Data – FSANZ's Dietary Modelling computer program
FAO	Food and Agriculture Organization
FSANZ	Food Standards Australia New Zealand
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KEKP	Kids Eat Kids Play (2007 Australian Children's Nutrition and Physical Activity Survey)
kg	Kilograms
LOR	Limit of Reporting
mg	Milligram (one thousandth of a gram)
NMI	The National Measurement Institute (NMI) (formerly the Australian Government Analytical Laboratory)
NNS	National Nutrition Survey
NOAEL	No observed adverse effect level
QHSS	Queensland Health Clinical and Statewide Services Division
the Code	The Australia New Zealand Food Standards Code
WHO	World Health Organization

Note: A glossary of terms can be found in Appendix 1

Introduction

Nitrate and nitrite ions are ubiquitous in the environment and occur naturally in plant foods as a part of the nitrogen cycle. Nitrate levels may vary significantly in fruit and vegetables dependent on a number of biotic and abiotic factors. Conversely, nitrite levels are generally relatively low in fresh undamaged vegetables but may increase in some nitrate rich vegetables after harvesting, particularly if stored at room temperature (reviewed in Maynard et al, 1976).

Nitrate and nitrite, as the sodium or potassium salts, have also been used as food additives in cured meats for many years primarily to prevent growth and toxin production of *Clostridium botulinum* which causes the illness botulism (Davidson et al., 2002, Sofos and Raharjo, 1995). The addition of nitrite or nitrate improves the microbiological safety of these foods and extends their safe shelf life. This offers significant benefits to consumers in terms of the availability of a variety of different foods that are safe, convenient and cost effective. An alternative to sodium nitrite for production of cured meats has not been identified despite significant research effort (EFSA, 2003).

The safety of nitrate and nitrite has been comprehensively reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Historically, there have been two main safety concerns around the presence of nitrate and nitrite in the diet. Those relate to the reaction of nitrite with haemoglobin to form methaemoglobin which can reduce oxygen transport in the blood, and a theoretical possibility of the potential for carcinogenicity through the formation of N-nitroso compounds in foods or in humans *in vivo*.

In order to estimate the Australian dietary exposure to nitrate and nitrite, FSANZ has funded and coordinated analytical surveys for both nitrate and nitrite. Food regulatory agencies in State and Territory governments collected the food samples in their region and provided these for analysis. These surveys have included:

- An assessment of nitrate and nitrite concentrations in processed foods conducted as part of the 21st Australian Total Diet Study (ATDS) which also examined sulphites, benzoates and sorbates.
- An assessment of nitrate and nitrite concentrations in fruit, vegetables and water as part of the 22nd ATDS which also estimated the dietary intake of the Australian population of the trace elements iodine, selenium, chromium, molybdenum and nickel.
- A supplementary survey of selected fruit and vegetables conducted in April 2010.

Background

The diet constitutes an important source of human exposure to nitrate and nitrite either as natural constituents of plant foods or as intentional additives. Drinking water can also be an important potential source of nitrate (Gangolli et al, 1994).

Nitrate and nitrite levels in fruit and vegetables

Significant concentrations of nitrate are found naturally in various fruits and vegetables. It has long been established that these levels are dependent upon a number of factors including; the use of fertilisers, location and soil type, carbon dioxide concentrations (in greenhouse vegetables), seasonal light intensity and duration of light exposure and water availability (reviewed in Maynard et al, 1976).

Nitrate concentrations in vegetables may also vary up to orders of magnitude dependent on the vegetable species and the part of the plant sampled. High concentrations of nitrate tend to accumulate in the leaves, roots, petioles or stems of certain plants meaning that leafy vegetables including lettuce or spinach, and root crops such as beetroot, may accumulate high concentrations of nitrate. In contrast, levels of nitrate in vegetables such as carrots or onions are likely to be lower (EFSA 2008; Maynard et al, 1976).

Nitrite concentrations generally tend to be low in fresh undamaged vegetables, however levels can increase rapidly in certain nitrate rich vegetables, particularly if pureed and stored at room temperature. In addition to temperature, this increase is dependent upon nitrate reductase activity in the plant and the level of bacterial contamination (Chung et al, 2004; Ezeagu, 1996; Lin and Yen, 1980; Phillips et al, 1968).

Nitrate and nitrite in drinking water

The World Health Organisation (WHO) and Australian drinking water guideline levels are 50 mg/L for nitrate (as NO_3^-) and 3 mg/L for nitrite (as NO_2^-). The guideline values are established to protect young infants from methaemoglobin formation, however the guideline advises that water with a nitrate concentration of up to 100 mg-nitrate/L can be used by adults and children over 3 months of age without risk of significant health effects (NHMRC, 2004).

The WHO has also set a provisional guideline level for nitrite in drinking water of 0.2 mg/L for long term exposure (WHO, 2008).

In Australia, nitrate concentrations in major public supplies of drinking water are typically below 0.15 mg/L, however elevated nitrate concentrations (200-300 mg-nitrate/L) have been recorded in groundwater sourced for drinking in some rural areas. Nitrite is rapidly oxidised to nitrate in water and is rarely detected in well-oxygenated or chlorinated water (NHMRC, 2004).

Food additive permissions in Australia and New Zealand

Standard 1.3.1 of the Australia New Zealand Food Standards Code (the Code) permits the addition of nitrite and nitrate, in the form of sodium or potassium nitrite and nitrate, to a range of food products.

Nitrate is permitted to be added to slow dried cured meats and fermented uncooked processed comminuted meat products to a maximum level of 500 mg/kg and to cheese and cheese products at a maximum level of 50 mg/kg.

Nitrite is permitted to be added to commercially sterile canned cured meats to a maximum level of 50 mg/kg and to cured meats, dried meats, slow dried cured meats and processed comminuted meat poultry and game products to a maximum level of 125 mg/kg. Permissions for nitrate and nitrite in the Code are shown in Table 1.

Table 1. Food additive permissions for nitrate and nitrite in Australia and New Zealand

Product	Additive	Permitted level
Cheese and cheese products	Nitrates (potassium and sodium salts)	50 mg/kg calculated as nitrate ion
Commercially sterile canned cured meat	Nitrites (potassium and sodium salts)	50 mg/kg total of nitrates and nitrites, calculated as sodium nitrite
Dried meat	Nitrites (potassium and sodium	125 mg/kg total of nitrates and

	salts)	nitrites, calculated as sodium nitrite	
Product	**Additive**	**Permitted level**	**Additive**
Slow dried cured meat	Nitrites (potassium and sodium salts) Nitrates (potassium and sodium salts)	125 mg/kg total of nitrates and nitrites, calculated as sodium nitrite 500 mg/kg total of nitrates and nitrites, calculated as sodium nitrite	
Processed comminuted meat, poultry and game products	Nitrites (potassium and sodium salts)	125 mg/kg total of nitrates and nitrites, calculated as sodium nitrite	
Fermented, uncooked processed comminuted meat products	Nitrates (potassium and sodium salts)	500 mg/kg total of nitrates and nitrites, calculated as sodium nitrite	

Objectives of the survey

The objectives of this survey were to analyse levels of nitrate and nitrite in Australian food and beverages, and to determine whether estimated dietary exposure to nitrate and nitrite poses a risk to human health and safety.

Survey design and analytical method

Sample selection

21st ATDS

Foods sampled as part of the 21st ATDS represented mainly processed foods for which there are permissions to contain preservatives in the Code. Foods sampled included those that may be expected to show regional variation (regional foods) and those available nationwide and not expected to show regional variation (national foods). For each food, three samples were combined to give a composite sample that was analysed to measure the levels of nitrate and nitrite. A detailed description of food sampling conducted as part of the 21st ATDS can be found at http://www.foodstandards.gov.au/_srcfiles/21st%20ATD%20Study%20report-Aug051.pdf

22nd ATDS

The 22nd ATDS analysed nitrate and nitrite concentrations in a selection of fresh produce including fruit, vegetables and other food products such as beverages and some snack foods. Two composite samples, of three purchases each, were collected in three capital cities, making six composite samples for each national food. For regional foods two composite samples, consisting of three purchases each, were collected in five capital cities, making ten composite samples for each regional food. The collection period varied slightly for each State or Territory in order to stagger the arrival of samples at the analytical laboratory, as soon as practicable after purchase. All perishable samples were frozen prior to forwarding to the laboratory. The analytical laboratory prepared foods in accordance with detailed instructions. Perishable foods were prepared within 48 hours of arrival at the laboratory. Full details of sample selection as part of the 22nd ATDS can be found at http://www.foodstandards.gov.au/_srcfiles/ATDS.pdf.

2010 Survey of selected fruit and vegetables

Food items were collated into a sampling plan which included food preparation techniques consistent with how the food was prepared for analysis for the 21st and 22nd ATDS. Samples for testing were collected by food regulatory agencies in the Australian Capital Territory, Western Australia and Queensland from a variety of retailers during May 2010. Jurisdictions sampled three purchases of each food type. For each food type, the products available on retail shelves were reviewed and purchased. Where possible, two samples of the same product, each with different batch numbers/date markings were purchased to account for variation between batches. In this instance, the same products with different batch dates were composited together for analysis.

Sample preparation

The 52 foods selected according to the above sampling plans that were analysed for nitrate and nitrite are set out in Appendix 2, Table A1. Foods were collected and forwarded to the analytical laboratory as soon as practicable. All perishable samples were refrigerated or frozen prior to forwarding to the laboratory. All the foods examined in the study were prepared to a 'table ready' state before analysis (refer to Appendix 2, Table A2 for details on food preparation instructions). For example, potatoes were boiled and bacon was dry fried until cooked through. A number of the foods surveyed in this study, such as ham and cheese, were available in a table ready form and required no further preparation.

Sample analysis

Analysis was conducted by Queensland Health and Scientific Services (QHSS) for food samples as part of the 21st ATDS, and by the National Measurement Institute (NMI) for foods sampled during the 22nd ATDS. Inter-laboratory checks were also conducted for certain fruit and vegetables as part of the 22nd ATDS. Some inconsistencies were identified between laboratories. Therefore, some fruit and vegetables were resampled in 2010. Symbio Alliance conducted the nitrate and nitrite measurements in additional samples. All analyses were carried out in the food samples in accordance with accredited quality assurance procedures and the results were provided to FSANZ. The Limit of Reporting (LOR), which is the lowest concentration level at which the laboratory is confident in the quantitative results reported, ranged from 0.6 (liquid matrix) to 10 mg/kg for sodium nitrate (solid matrix) and 0.6 (liquid matrix) to 7.5 mg/kg (solid matrix) for sodium nitrite dependent upon laboratory method. Analytical methods are summarised in Table 2.

Table 2: Methods of Analysis for nitrate and nitrite

Laboratory	Method	Reference
QHSS	FIA/ Spectrophotometry	QIS 12641 based on the method of Kirk and Sawyer in Pearson's Composition and Analysis of foods
NMI	Ion chromatography	Based on method 4110B from APHA Standard method for the examination of waters. 20th Edition
SymBio Alliance	Spectrophotometry	NATA accredited method based on AOAC 973.31

The concentration of nitrate and nitrite can be expressed as a number of different units including mg/L, mg/kg, mg/L nitrate-nitrogen, mg/L nitrite-nitrogen, or also in terms of number of moles, and as the sodium salt. In this report, units are reported as the sodium salt (mg/kg) unless otherwise specified. Conversion factors between nitrate and nitrite and the sodium salts of nitrate and nitrite were based on the figures shown in Table 3. To convert

NO_3 to $NaNO_3$ data were divided by 0.73 and to convert NO_2 to $NaNO_2$ data were divided by 0.67.

Table 3: Conversion of nitrate and nitrite ions to the sodium salt.

mM	mg/L NO_3	mg/L NO_2	mg/L $NaNO_3$	mg/L $NaNO_2$
1	62	46	85	69

Estimating dietary exposures to sodium nitrate

A dietary exposure assessment (dietary modelling) is a tool used to estimate the exposure to (or intake of) agricultural and veterinary residues, contaminants, nutrients, food additives and other substances from the diet. To estimate dietary exposure to food chemicals, food consumption data is combined with food chemical concentration data. Food regulators have used dietary modelling techniques internationally for many years to determine if dietary exposures to specific food chemicals present an unacceptable risk to public health and safety.

To estimate the dietary exposures to sodium nitrite and sodium nitrate for each individual, the concentration of these chemicals in each analysed food was multiplied by the amount of food consumed and summed over all foods to determine the exposure to sodium nitrate and sodium nitrite from the whole diet (Equation 1).

Equation 1: Dietary exposure calculation

Dietary Exposure = sodium nitrite or sodium nitrate concentration x food consumption

In addition, approximately 5% of ingested nitrate is converted into nitrite in the saliva of humans (Appendix 6.2). This additional (endogenous) nitrite exposure also needs to be taken into account in the total dietary exposure assessment of nitrite. Therefore, 5% of the sodium nitrate concentration was added to the concentration of sodium nitrite for each food (Equation 2) before applying (Equation 1). This accounts for total nitrite exposure obtained through endogenous conversion of nitrate in the saliva and exogenous nitrite exposure in the diet to be calculated.

Equation 2: Total sodium nitrite concentration calculation

Total Sodium Nitrite Concentration (including endogenous formation) = sodium nitrite concentration in the food + (5% of sodium nitrate concentration in the food)

The dietary exposure assessments for all food chemicals [sodium nitrate, sodium nitrite and total sodium nitrite (endogenous and exogenous sodium nitrite)], were conducted using a computer program known as DIAMOND (Dietary Modelling of Nutritional Data), which was designed to automate dietary exposure calculations. DIAMOND multiplied the allocated sodium nitrate, sodium nitrite and total sodium nitrite concentrations for each food consumed in the national nutrition surveys (NNS) with the amount of that food that each survey respondent consumed. This gave an estimation of each individual's exposure to sodium nitrate, sodium nitrite and total sodium nitrite from each food. Once this had been completed for all of the foods, the total amount of sodium nitrate, sodium nitrite and total sodium nitrite consumed from all foods was summed for each individual. Population statistics (e.g. mean and 90th percentile exposures) for each age group were then derived from the individuals' ranked intakes.

DIAMOND enables the dietary exposure assessments to be conducted using actual diets for males and females aged 2 years and above, as derived from national nutrition surveys. The dietary exposures to each chemical were calculated for each individual in the survey before mean dietary exposure results were derived for the specified age categories. Use of specific food consumption data greatly improves the reliability and accuracy of the dietary exposure estimates, and takes into account the different eating patterns of consumers.

Population groups assessed

Dietary exposures were estimated for:

- infants aged 9 months
- children aged 2-5 years
- children aged 6-12 years
- children aged 13-16 years
- 17 years and above
- females aged 16-44 years

Dietary exposure assessments were conducted for children because children generally have higher exposures due to their smaller body weight and they consume more food per kilogram of body weight compared to adults. A detailed description of food consumption data, model diet construction, food mapping and assumptions and limitations in dietary exposure assessment are at Appendices 4 to 4B.

Treatment of analytical values below the LOR

Nitrate and nitrite can be distributed in foods at very low concentrations and occur naturally in the environment. These amounts may make a substantial contribution to dietary exposures and should be accounted for. Therefore, it was not reasonable to assume they were not present in the food when the analytical results were less than the LOR. To allow for this uncertainty, the results for dietary exposures to sodium nitrate, sodium nitrite and total sodium nitrite were presented as a range, using the mean analytical concentration. The lower end of the range was calculated based on the assumption that results below the LOR were equal to zero (lower bound mean). A more conservative approach assumes the concentrations were present at half of the LOR and is indicated by an inner point in the range (middle bound mean). The upper end of the range, representing a very conservative 'worst-case' estimate, was calculated on the assumption that results below the LOR were equal to the LOR (upper bound mean). Where sodium nitrite or sodium nitrate concentrations were expected to be from intentionally added sources only, analytical results that were less than the LOR were assumed to be zero in all cases (e.g. sodium nitrite in cottage cheese).

Analytical results

Nitrate

The mean analytical concentrations for lower and upper bound concentrations for nitrate are shown in Appendix 3, Table A3. Upper bound mean nitrate concentrations (expressed as sodium nitrate) were highest in raw and fresh cooked spinach (2741-2963 mg/kg), canned beetroot (2009 mg/kg), fresh parsley (1957 mg/kg), raw celery (1527 mg/kg) and raw lettuce (1144 mg/kg). For banana, broccoli, cabbage, cucumber, potato crisps, pumpkin, salami, and strawberries concentrations were between 100 and 450 mg/kg. All other surveyed foods had nitrate concentrations of less than 100 mg/kg.

These results are relatively consistent with a comprehensive survey of nitrate concentrations in vegetables in Europe which examined 41,969 analytical results from 20 member states and Norway (EFSA, 2008). A large variation in median nitrate concentrations was observed ranging from around 1 mg/kg in peas to a high of 4,800 mg/kg for rucola (expressed as the nitrate ion). Median concentrations for cabbage, cauliflower and onions were 223 mg/kg, 122 mg/kg and 60 mg/kg, respectively. For cucumber and tomatoes, median reported concentrations were 156 and 26 mg/kg, respectively. High concentrations were typically reported in leafy vegetables including spinach (785 mg/kg), silverbeet (1,510 mg/kg) and mixed lettuce (1,878 mg/kg).

The results for nitrate are also consistent with those observed in a 2007 New Zealand survey. Mean concentrations of nitrate (expressed as sodium nitrate) were: cabbage (331 mg/kg), lettuce (1590 mg/kg), celery (1610 mg/kg), broccoli (133 mg/kg), spinach (990 mg/kg), beetroot, canned (763 mg/kg), potato (129 mg/kg), carrot (58 mg/kg) and pumpkin (67 mg/kg). Mean nitrate levels in bacon (36.5 mg/kg), ham (16.6 mg/kg) and luncheon sausage (30.9 mg/kg) were typically lower (Thomson et al, 2007). Some variation in results between surveys is expected because it is known that nitrate concentrations are influenced in particular by the season, methods of production and sunlight available. In addition, differences in survey methodology and reporting are likely to contribute to variations in reported nitrate concentrations for the same commodity between surveys and countries.

Nitrite

Upper bound mean nitrite concentrations (expressed as sodium nitrite) were generally highest in processed meats including bacon (27 mg/kg), frankfurts (30 mg/kg), ham (28 mg/kg), luncheon sausage (35 mg/kg), and strassbourg (35 mg/kg). The upper bound mean concentration of sodium nitrite was 38 mg/kg in spinach and 29 mg/kg in pumpkin. Other foods or beverages that reported upper bound mean concentrations of more than 10 mg/kg included beans, broccoli, cabbage, cucumber, grapes, parsley, peaches, peaches, pineapple and strawberry. White wine also contained nitrite at a concentration of above 10 mg/kg. All other foods and beverages contained nitrite at concentrations close to, or below the LOR. Individual results are shown in Appendix 3, Table A4.

Comparatively fewer data are available for nitrite concentration in surveys of foods and beverages internationally, however nitrite concentrations were generally comparable with concentrations in processed meats in surveys conducted in New Zealand and France (Thomson et al, 2007; Menard et al, 2008). Concentrations of nitrite were generally higher in fruit and vegetables in Australia than in the New Zealand survey (Thomson et al, 2007). Upper bound concentrations were more comparable with those reported in France (Menard et al, 2008). The storage temperature and length of storage of samples of some fruit and vegetables in the current survey prior to purchase was not known. Unfavourable conditions such as high storage temperature and long storage periods have previously been shown to increase nitrite levels in vegetables (Aworh *et al.* 1978, 1980 and Chung *et al.* 2004) and may have contributed to results in this study.

A 1996-1997 survey commissioned by Queensland Health found that of the 107 vegetable samples analysed, 18 samples had nitrite (expressed as sodium nitrite) levels of greater than 5 mg/kg. Nitrite concentrations of up to 10 mg/kg were seen in cabbage and celery; concentrations in lettuce ranged up 20 mg/kg and levels in silverbeet were up to 50 mg/kg (unpublished data). In a separate survey of nitrate and nitrite in Australian leafy vegetables, Parks et al., (2008) reported that fresh leafy vegetables available during a 6-month period on the Australian market can range in nitrate-N from 12 to 1400 mg/kg fresh weight and nitrite-nitrogen from 0 to 37.5 mg/kg. Meah et al., (1994) also reported a wide variation in nitrite (expressed as the ion) levels for lettuce (not detected (nd)-15 mg/kg), celery (nd-19 mg/kg), potatoes (nd-60 mg/kg) and beetroot (nd-71 mg/kg) in the United Kingdom. Nitrite

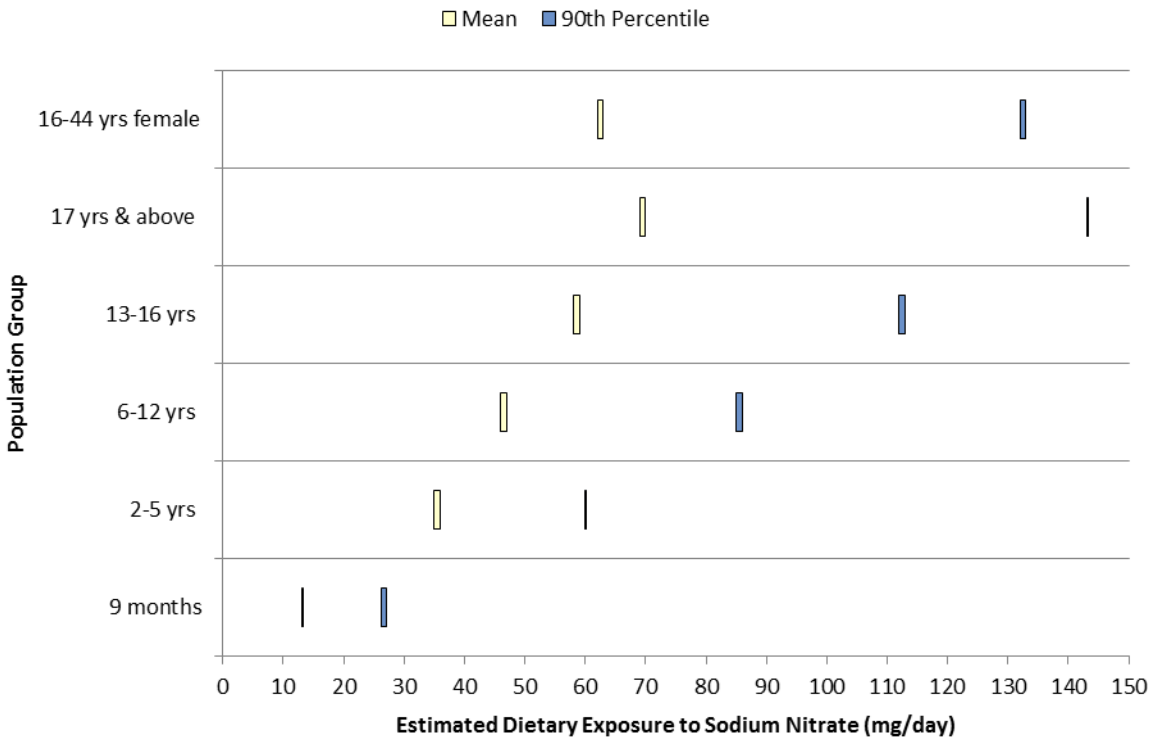
concentrations in spinach, cabbages and tomatoes were below the limit of detection. A recent survey of nitrite in vegetables available in Hong Kong found that nitrite concentrations were generally low (around 1 mg/kg) with higher levels reported in some cabbage (*ca* 3 mg/kg) and beetroot (*ca* 8 mg/kg).

Estimated dietary exposure to sodium nitrate and sodium nitrate

Sodium nitrate

The estimated mean and 90th percentile dietary exposures to sodium nitrate, in milligrams per day, for each age category are given in Table A9 in Appendix 5 and in Figure 1 below. For dietary exposures expressed in mg/kg bw/day, see Table A10 in Appendix 5. Across the three scenarios there was a minimal to no estimated range of mean and 90th percentile dietary sodium nitrate exposures, reflecting the low number of analytical samples at below the LOR (Figure 1). The estimated dietary exposure to sodium nitrate for the mean and 90th percentile increased with increasing age. Infants aged 9 months had the lowest estimated dietary exposure and people aged 17 years and above had the highest.

Figure 1: Range of mean and 90th percentile estimated dietary exposure to sodium nitrate, in milligrams per dayɤ



ɤ lower end of the range represents where all <LOR analytical results have a concentration of zero; the upper end of the range represents where all <LOR analytical results have a concentration equal to the LOR.

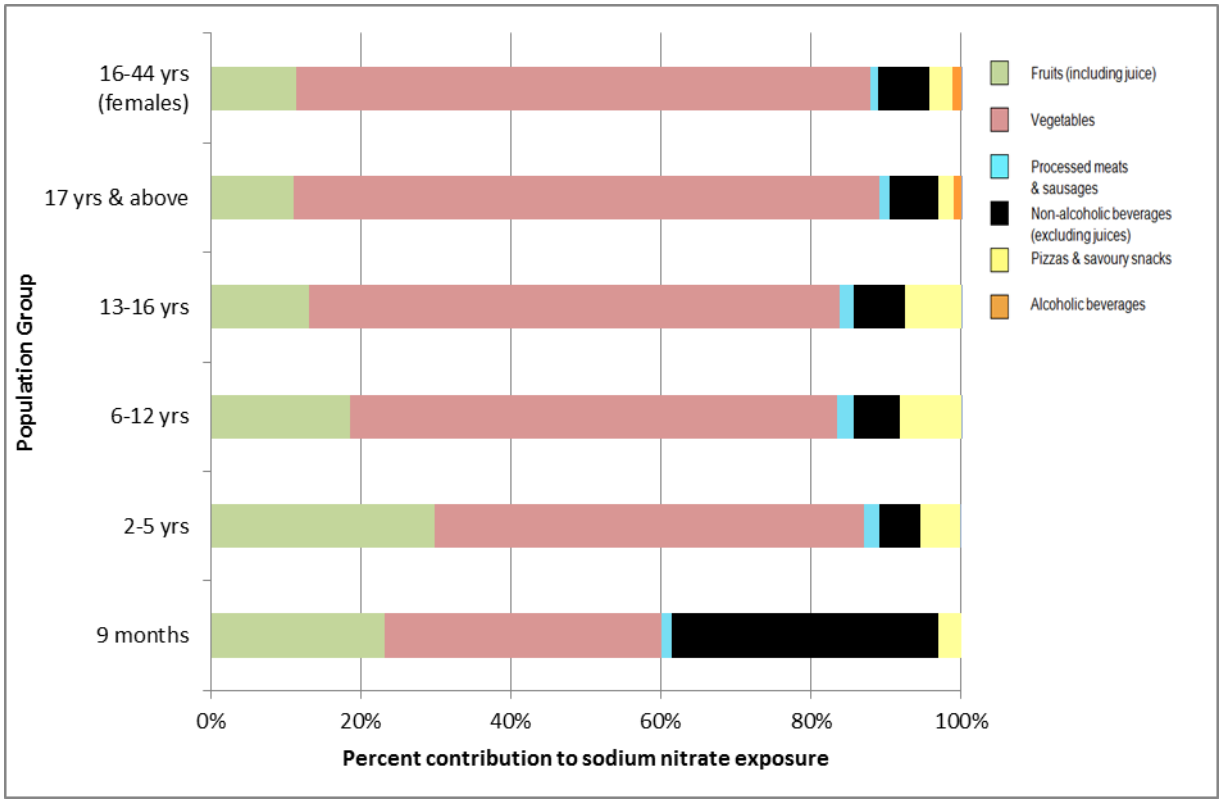
Major contributing foods

As shown in Figure 2, the major sources of sodium nitrate dietary exposures across the different population groups were vegetables (42-78%) and fruits (including juices) (11-30%). Non-alcoholic beverages (excluding juices) were also a major contributing food group for infants aged 9 months.

More specific details regarding the major food group contributors to sodium nitrate are presented in Table A11 and A12 of Appendix 5. Lettuce (5-18%), stalk and stem vegetables (7-13%), starchy root vegetables (7-12%) and green leafy vegetables (cooked) (7-10%) were major contributors to dietary exposure to sodium nitrate for all age categories 2 years and above. Leafy vegetables and herbs (14%) and starchy root vegetables (8%) were the major contributing vegetables to sodium nitrate dietary exposures for infants aged 9 months. For children aged 2-12 years, bananas, tropical fruits and figs (7-13%) were the major contributing foods to sodium nitrate exposure, followed by root vegetables (starchy) (11-12%). Bananas and plantains were the major contributing fruit to estimated dietary exposures for infants.

Based on the theoretical infant diet, bottled water (including plain mineral water and soda water) (17%) was the major contributor to sodium nitrate exposure for infants aged 9 months. Other non-alcoholic beverage food groups that were major contributors to sodium nitrate dietary exposures were infant formula (8%) and non-bottled water (7%).

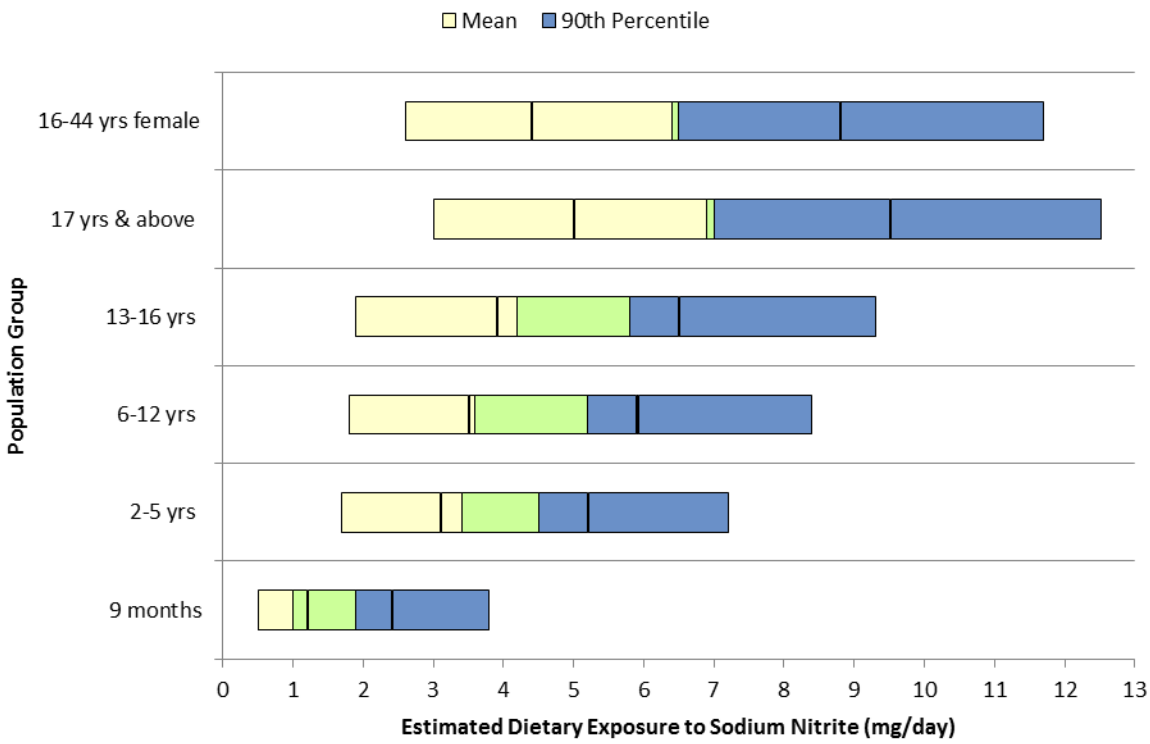
Figure 2: Contributors to dietary exposure to sodium nitrate



Sodium Nitrite

The estimated mean and 90th percentile sodium nitrite dietary exposures for all age categories included in this survey , are presented in Table A13 (in milligrams per day (mg/day) and in Table A14 (in milligrams per kilogram body weight per day (mg/kg bw/day) of Appendix 5A. Generally, as age increased, the estimated mean and 90th percentile dietary exposures (in mg/day) increased. Infants aged 9 months had the lowest estimated dietary exposures and people aged 17 years and above had the highest (Figure 3).

Figure 3: Range of mean and 90th percentile estimated dietary exposure to sodium nitrite, in milligrams per day☿



☿ lower end of the range represents where all < LOR analytical results have a concentration of zero; the upper end of the range represents where all < LOR analytical results have a concentration equal to the LOR. The upper end of the mean range and the lower end of the 90th percentile range for some population groups overlap. This is represented by the green on the figure. Note: the black line on each mean range and 90th percentile range represents where all < LOR analytical results have a concentration equal to half the LOR.

Major contributing foods

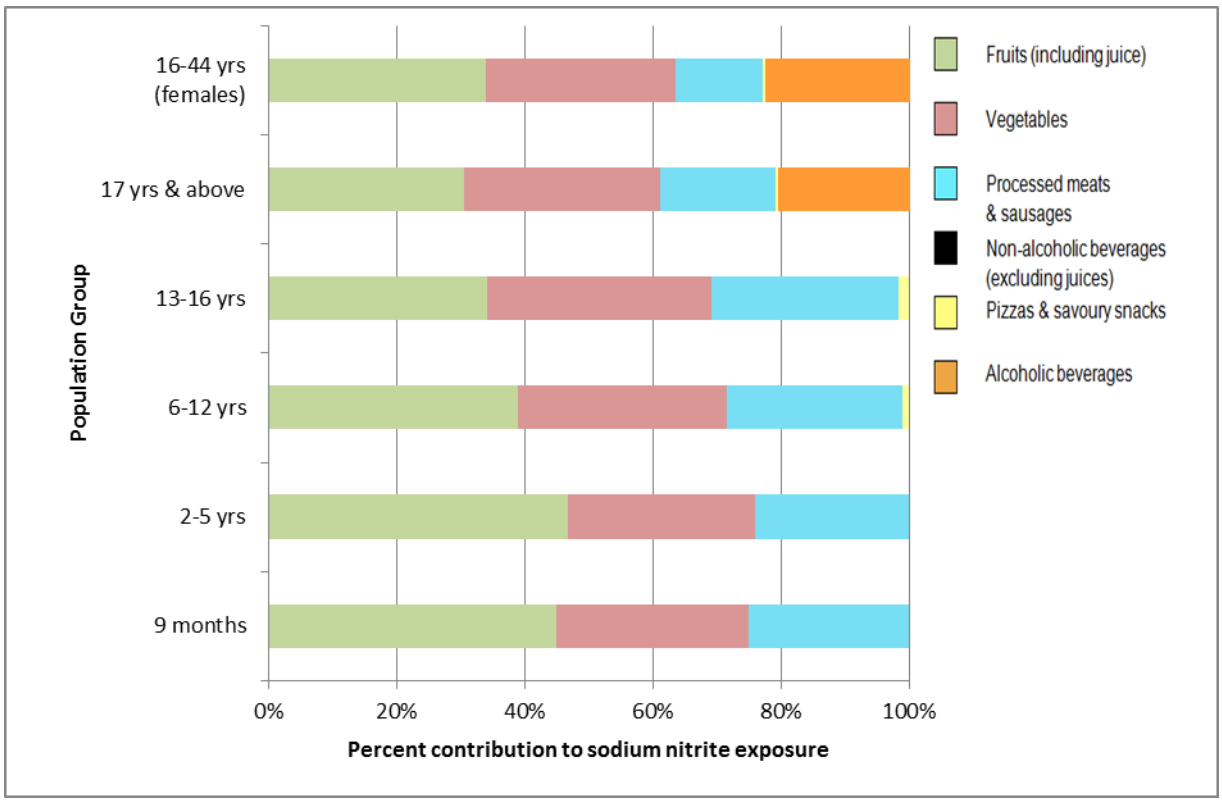
Fruits (including processed and juices), vegetables (including cooked) and processed meats and sausages were the major dietary sources of sodium nitrite exposure across all age categories examined (Figure 4), contributing 31-47%, 29-35%, 13-31% respectively. Alcoholic beverages were also a major source of sodium nitrite intake for the population group aged 17 years and above (20%) and females aged 16-44 years (22%). More specific details regarding the major food group contributors to sodium nitrite intake are presented in Table A15 and A16 of Appendix 5A.

The fruits that had the highest contributions to estimated dietary sodium nitrite exposures for children aged 2-16 years were pineapple (9-11%), berries (6-12%) and bananas, tropical fruits and figs (<5-9%). For infants aged 9 months, the fruits that had the highest contribution were bananas and plantains (8%), berries and jams (8%), grapes (6%) and dried apricots, peel, cherries, ginger and fruit leathers (6%). For population groups aged 17 years and above, the major contributing fruits were fresh stone fruits and persimmon (10%) and pineapple (6-8%).

'Pumpkin, squash and zucchini' (12-13%) and 'cucumbers, capsicums, chokos and chillies' (6-8%) were the major contributing vegetables to sodium nitrite dietary exposures for children aged 2-16 years, the population aged 17 years and above and for females aged 16-44 years. Similarly, 'pumpkin, squash, marrows and zucchini' was the major vegetable contributor for infants aged 9 months (12% of estimated exposure).

Deli meats in whole pieces or cuts (except bacon) were a major source of sodium nitrite exposure for all age groups (8-15%). These results may be explained by the use of sodium nitrite as a preservative in cured meats. Bacon and pancetta was a major contributing food group for children aged 13-16 years only. No other deli meats (e.g. salami, frankfurts, strassburg, luncheon) were major contributing foods to estimated sodium nitrite dietary exposures for population groups aged 2 years and above. 'Pork (except bacon) and deli meats (except frankfurts and poultry-based)' (18%) and sausages and frankfurts (11%) were the major contributing processed meat foods for infants aged 9 months.

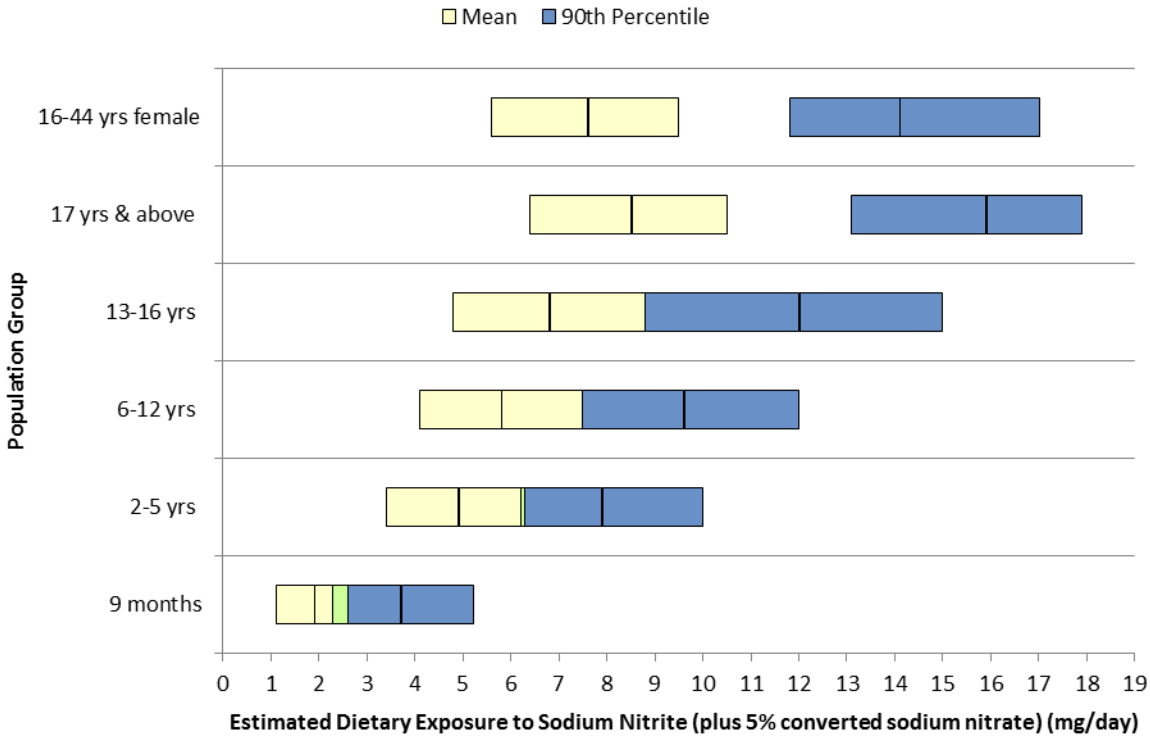
Figure 4: Contributors to dietary exposure to sodium nitrite



Total Sodium Nitrite

A proportion (approximately 5%) of ingested nitrate is converted into nitrite in the saliva of human beings. Therefore it is necessary to consider this endogenous contribution to total nitrite exposure in addition to nitrite exposure in the diet. The estimated mean and 90th percentile dietary exposures to total sodium nitrite, in milligrams per day, for each age category are given in Table A17 in Appendix 5B and in Figure 5 below. For dietary exposures expressed in mg/kg bw/day, see Table A18 in Appendix 5B. The estimated mean and 90th percentile for total sodium nitrite increased with age across the three scenarios.

Figure 5: Range of mean and 90th percentile estimated dietary exposure to total sodium nitrite, in milligrams per day☿



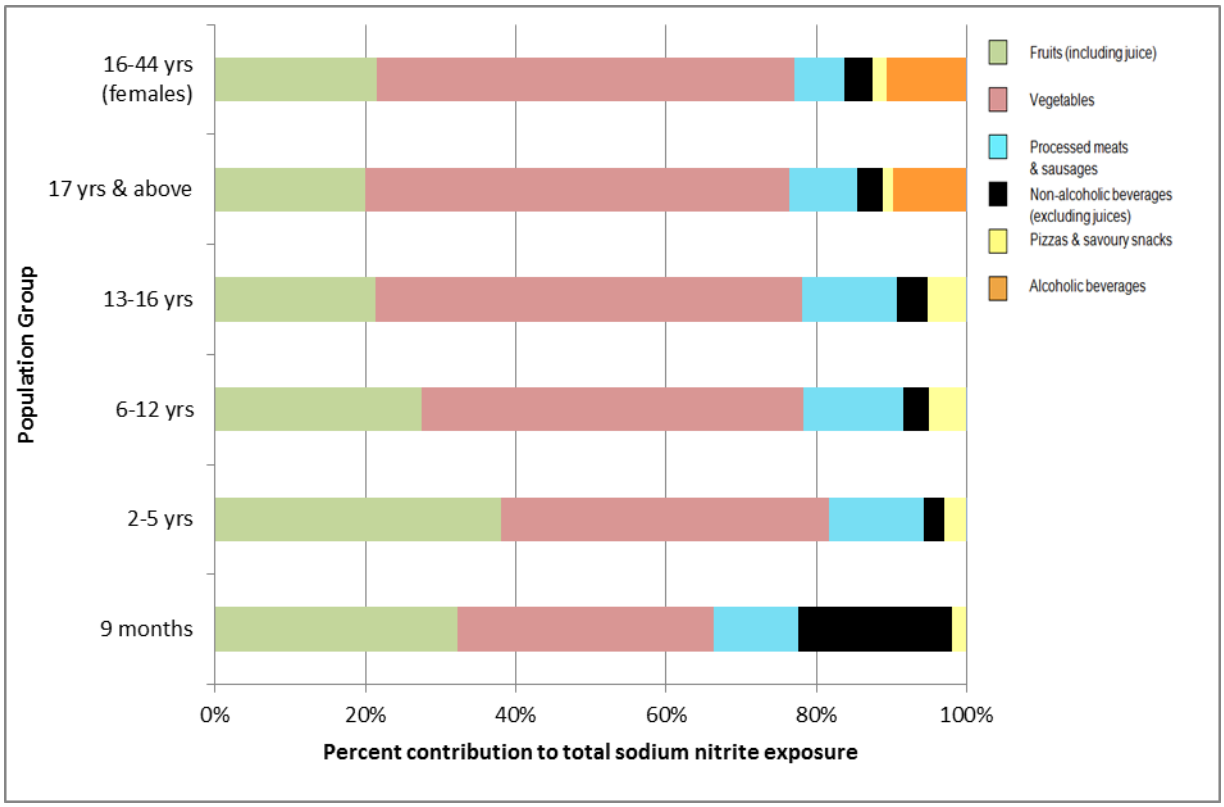
☿ lower end of the range represents where all < LOR analytical results have a concentration of zero; the upper end of the range represents where all < LOR analytical results have a concentration equal to the LOR. The upper end of the mean range and the lower end of the 90th percentile range for some population groups overlap. This is represented by the green on the figure.
Note: the black line on each mean range and 90th percentile range represents where all < LOR analytical results have a concentration equal to half the LOR.

Major contributing foods

As shown in Figure 6, the major contributors to total sodium nitrite dietary exposures across the different population groups were vegetables (44-57%) and fruits (including juices) (20-38%). Alcoholic beverages were also a major contributing food group for people aged 17 years and above and females aged between 16 and 44 years.

More specific details regarding the major food group contributors to total sodium nitrite are presented in Table A19 and A20 of Appendix 5B. Deli meats in whole pieces or cuts (except bacon) (5-7%), pumpkin, squash and zucchini (6-8%), starchy root vegetables (5-7%) and cucumbers, capsicums, chokos and chillies (5-6%) were major contributors to dietary exposure to total sodium nitrite for all age categories 2 years and above. In addition, bananas, tropical fruits and figs (6-11%) and berries (5-8%) were also major contributors for children aged 2-12 years.

Figure 6: Contributors to dietary exposure to total sodium nitrite



Health significance of survey results

Background

There is an extensive toxicological database for nitrate and nitrite. These studies were last reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002. JECFA established an Acceptable Daily Intake for nitrate and nitrite on the basis of studies in rats (Appendix 6.10). EFSA and the International Agency for Research on Cancer (IARC) have also evaluated potential health effects associated with nitrate and nitrite ingestion in food (EFSA 2008; 2010; Grosse, 2006). These evaluations include an analysis of toxicological and epidemiological studies for nitrate and nitrite that have been published since the JECFA evaluation. In this report, the pharmacokinetics and toxicology of nitrate and nitrite have been summarised to provide context to the consideration of possible health risks that may be associated with current estimated dietary exposure levels in the Australian population (Appendix 6). The assessment has considered the JECFA, IARC and EFSA reviews, together with other relevant data in humans.

Summary of nitrate and nitrite toxicity

Nitrate is generally regarded to be of relatively low toxicity. The toxicological sequelae of nitrate exposure are considered to be virtually entirely attributable to its conversion to nitrite. Nitrite reacts with haemoglobin (Hb) to form methaemoglobin (MetHb) in the blood which is the critical toxicological endpoint following nitrate and nitrite exposure. MetHb levels of up to 10% are typically not associated with clinical signs in humans. At higher levels, MetHb is associated with clinical signs including cyanosis, impaired aerobic respiration, metabolic acidosis, and in severe cases, death (Table 4). Levels of up to around 5% MetHb were not

considered adverse by JECFA, or in a review of MetHb following acute exposure to MetHb inducing chemicals (Solecki, 2005; Speijers and Brandt, 2002).

Table 4: Clinical signs associated with elevated MetHb levels in blood (Wright et al, (1999)).

% MetHb	Clinical signs
<10	None
10-20	Cyanotic skin discolouration
20-30	Anxiety, headache, tachycardia
30-50	Fatigue, confusion, dizziness, tachypnea, increased tachycardia
50-70	Coma, seizures, arrhythmias, acidosis

Other toxicological endpoints are summarised in detail in Appendix 6. In addition to methaemoglobinaemia, the other main concern related to nitrate and nitrite exposure has been the potential for increased cancer risk. The available evidence does not support a conclusion that nitrate intake from the diet is associated with increased cancer risk. There was equivocal evidence of an increased incidence of squamous cell papilloma and carcinoma in the forestomach in female mice given sodium nitrite at 165 mg/kg bw/day. Nitrite was not carcinogenic in male mice or male and female rats. Epidemiological evidence that nitrite in the diet is associated with increased cancer risk in human beings is equivocal. It is known that nitrite and dietary amines can react to form N-nitroso compounds, but whether endogenous nitrosation takes place under actual food intake conditions in sufficient amounts to pose a risk to human health is uncertain.

Nitrate exposures associated with elevated blood MetHb levels in infants, children and adults

MetHb levels in blood of human adults were not increased at exposures of up to 15 mg/kg bw/day for 28 days (Table 5). In infants, aged 3-8 months, 16.5 – 21 mg/kg bw/day nitrate (ions) administered as nitrate-rich vegetables, did not induce elevated MetHb blood levels. Oral administration of bolus doses of 50-100 mg/kg bw/day nitrate were associated with MetHb levels of around 5-8% in the blood of infants. Detailed summaries of these studies are at Appendix 6.4.1.

Table 5: MetHb levels in adults and infants exposed to sodium nitrate or nitrate in food

Nitrate exposure	Dose (mg/kg bw)	Study duration	Age	Number of subjects	Percentage MetHb	Reference
Sodium nitrate	10	Single dose	Adult	8	No change	Colbers et al, (1996)
Sodium nitrate	15	28 d	Adult	10	0.1-0.6%	Lambers et al, (2000)
Nitrate (ions)	50	2-18 d	11 d to 11 m	4	Maximum of 5.3%	Cornblath and Hartmann, (1948)
Nitrate (ions)	100	6-9 d	2 d to 6 m	4	Maximum of 7.5%	Cornblath and Hartmann, (1948)
Nitrate (ions)	16.5 -21	7 d	3.5 – 8 m	7	0.8 (0.2-3.4)	Kubler, (1958)

Blood MetHb levels following exposure to nitrate in drinking water have been investigated in infants and children (Table 6 and Appendix 6.4.2). The weight of evidence supports that exposure of infants and children to drinking water containing concentrations of up to 100 mg/L nitrate is not associated with increased MetHb levels. Assuming a high water intake of 150 mL/kg bw/day, this is equivalent to about 15 mg/kg bw/day nitrate, consistent with observations from controlled exposure studies.

Table 6: MetHb levels in infants and children exposed to nitrate in drinking water

Nitrate (ion) Concentration (mg/L)	Dose* (mg/kg bw/day)	Age	Number of subjects	Percentage MetHb	Reference
0	0	90-180 d	89	0.8	Simon et al., (1964)
50-100	11	90-180 d	38	0.8	
>100	15	90-180 d	25	0.7	
5	<1	>91 d	556	0.97	Shuval et al., (1972)
50-90	10	>91 d	1426	0.99	
<43	3	1-8	37	0.98	Craun et al., (1981)
95-482	18	1-8	62	1.13	
<44	7	≤ 9 y	234	1.4-1.8	Diskalenko et al., (1968)†
792	119	≤ 9 y	126	2.1-3.3	
898	135	≤ 9 y	208	3.1-7.1	
8.8	1	12-14 y	10	0.8	Subbotin et al., (1961)†
101	15	12-14 y	11	5.3	

- †Cited in Craun et al., (1981) * Unless water intake was detailed in the study, an intake of 150 mL/kg bw/day was assumed

Nitrite exposure associated with elevated blood MetHb levels in infants and adults

Approximately 6.3% of ingested nitrate on a molar basis, or 5% by mass, of ingested nitrate is converted to nitrite in the saliva of humans (reviewed in Appendix 6.2). In many cases,

endogenous nitrate reduction by saliva is the primary source of dietary nitrite exposure in humans, because dietary nitrate exposure in the diet far exceeds that of nitrite. Therefore, nitrate exposure studies can be used to support an estimated nitrite exposure that is not associated with elevated MetHb levels in blood. The equivalent sodium nitrite exposure at 15 mg/kg bw/day sodium nitrate is approximately 0.75 mg/kg bw/day sodium nitrite. This exposure to nitrite did not cause an increase in MetHb levels in infants, children or adults following administration of nitrate in controlled experimental studies, or in drinking water. Studies in infants support that MetHb levels are not elevated below exposures of up to 0.75 mg/kg sodium nitrite. Oral doses of 0.3 and 1.2 mg/kg bw/day nitrite administered to healthy infants aged 1-3 months for 10 days did not increase MetHb levels. At higher doses of 3.7 and 5.2 mg/kg bw/day MetHb concentrations increased to about 3-4%, and were maintained at that level for the 10 day experimental period (Toussaint and Selenka, 1970). In adult volunteers, bolus oral doses of sodium nitrite of approximately 2.4 mg/kg bw caused maximum MetHb levels of around 3-4% (Hunault et al, 2009). However, MetHb levels following exposure in the diet over the course of the day would be expected to be lower due to the short blood half-life of nitrite and MetHb. Overall, the available data support that exposure of up to 0.75 mg/kg bw/day sodium nitrite is not associated with elevated MetHb levels in humans.

Options for establishing an Acceptable Daily Intake or Acute Reference Dose

Available human data were not sufficient to support the establishment of an Acceptable Daily Intake (ADI), or an Acute Reference Dose due to confounding factors in nitrate and nitrite exposure studies. These included bacterial contamination, the concurrent presence of nitrate and nitrite in water, and limited exposure data in drinking water studies. Laboratory animal studies were not considered entirely suitable for establishment of a Reference Health Standard because quantitative differences exist in the conversion of nitrate to nitrite in the oral cavity (Speijers and van den Brandt, 2002; Walker, 1990; 1996) and species differences are also evident in MetHb formation and reduction rates in blood (See Appendix 6.3).

Risk Characterisation

Estimated mean dietary exposure to sodium nitrate ranged from 1.0 to 2.1 mg/kg bw/day for all population groups. At the 90th percentile, estimated dietary exposure ranged from 2.1 to 3.5 mg/kg bw/day. For nitrite, estimated mean dietary exposure ranged from 0.15 to 0.36 mg/kg bw/day, and exposure at the 90th percentile ranged from 0.3 to 0.6 mg/kg bw/day for all population groups. Exposures for nitrate and nitrite at the 90th percentile are below levels that were not associated with elevated MetHb in blood of adults, children or infants in experimental and drinking water studies. Therefore, estimated dietary exposure to sodium nitrate and sodium nitrite in Australian food is not considered to represent an appreciable human health and safety risk.

Vegetables (42-78%) and fruits (including juices) (11-30%) were the major sources of sodium nitrate dietary exposures across the different population groups. For sodium nitrite, the major contributors to total dietary exposures across the different population groups were also vegetables (44-57%) and fruits (including juices) (20-38%). Deli meats (except bacon) represented only around 5-7% of total dietary exposure to sodium nitrite. As such, the risks associated with nitrate and nitrite exposure also need to be considered in terms of the benefits of consumption of fruit and vegetables (See below). Overall, it is considered that because the estimated exposures to nitrate and nitrite are unlikely to result in any appreciable health risks, the strong evidence of health benefits from fruit and vegetable consumption outweigh the risks associated with nitrate and nitrite exposure.

Beneficial Effects of Fruit and Vegetables.

The Dietary Guidelines for Australian Adults[1] recommend eating plenty of vegetables, legumes and fruits. Regularly including a variety of vegetables, legumes and fruits in the diet will provide a wide range of vitamins, minerals, dietary fibres and beneficial, non-nutrient phytochemicals found in plant foods for very few kilojoules. Vegetables include green leafy varieties, red and yellow and starchy vegetables. Fruits include those high in vitamin C and those high in vitamin A (and its analogues).

The Dietary Guidelines concluded that 'there is strong evidence of a protective effect of certain vegetables, legumes and fruit against the development of a number of non-communicable chronic diseases, among them cancer, cardiovascular disease, type 2 diabetes, hypertension, and cataract and macular degeneration of the eye. This may, in part, be mediated through phytochemicals. Adults are encouraged to consume on average at least two helpings of fruit and five of vegetables each day, selected from a wide variety of types and colours and served cooked or raw, as appropriate.'
The Dietary Guidelines for Children and Adolescents in Australia[2] reference the *Australian Guide to Healthy Eating* which recognises the importance of fruits and vegetables in a healthy diet for all sections of the population. It recommends "consumption of between one and two servings of fruit and two to four of vegetables each day for children aged 4–7 years; one to two servings of fruit and three to five of vegetables each day for children aged 8–11 years; and three to four servings of fruit and four to nine of vegetables each day for adolescents (12–18 years)."

Risk Management Considerations

Data from this survey indicated that estimated levels of nitrite dietary exposure levels are below the threshold for elevated MetHb levels in blood of infants, children and adults. As the current estimated dietary exposures to sodium nitrate and sodium nitrite are not considered to represent an appreciable human health and safety risk neither a regulatory nor a non-regulatory approach to risk management is considered necessary.

Conclusion

The large majority of estimated dietary nitrate and nitrite exposure occurred through the ingestion of fruit and vegetables. Exposure to nitrate and nitrite through uses as a food additive represented only a relatively small proportion of dietary exposure. Current estimated Australian dietary nitrate and nitrite exposures are not considered to represent an appreciable health and safety risk. Any health risks that may be associated with ingestion of nitrate and nitrite in the diet, are outweighed by the strong evidence of health benefits of consumption of fresh fruit and vegetables as part of a balanced diet.

[1] National Health and Medical Research Council. Dietary Guidelines for Australian Adults. Canberra 2003.

[2] National Health and Medical Research Council. Dietary Guidelines for Children and adolescents in Australia incorporating the infant feeding guidelines for health workers. Canberra 2003.

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Appendix 1: Glossary of Terms

Acceptable Daily Intake (or ADI): The amount of a specific substance (for instance a food additive, or a residue of pesticide) in food or drinking water that can be ingested daily over a lifetime without an appreciable health risk.

Acute reference dose (ARfD): An estimate of the amount of a substance in food and/or drinking-water, normally expressed on a body-weight basis, that can be ingested in a period of 24 h or less, without appreciable health risk to the consumer.

Exposure: The amount of a chemical contaminant that is ingested by a consumer.

Lower bound mean: An estimate of the mean concentration of a chemical in a food or dietary intake assuming analytical results reported as being below the LOR equal zero.

Middle bound mean: An estimate of the mean concentration of a chemical in a food or dietary intake assuming analytical results reported as below the LOR are equal to half the value of the LOR.
Respondent

Upper bound mean: An estimate of the mean concentration of a chemical in a food or dietary intake assuming analytical results reported as below the LOR are equal to the value of the LOR.

Appendix 2: Foods sampled

Table A1: Foods sampled and analysed for nitrates and nitrites

Vegetables	Fruit
Baked beans in tomato sauce, canned	Apples, unpeeled
Beans, green, cooked	Avocadoes
Beetroot, canned	Banana
Broccoli, cooked	Grapes, both red and green
Cabbage, cooked	Mango
Carrots, cooked	Orange, fresh
Celery, raw	Peach, canned in natural juice
Cucumber, raw	Peach, fresh
Lettuce, raw	Pineapple, fresh
Mushroom, cooked	Strawberries
Olives, preserved	Sultana
Onions, cooked	Tomato, raw and canned
Parsley, fresh	Watermelon
Peas, frozen, cooked	
Potatoes, cooked	**Dairy**
Pumpkin, cooked	Cheese, cheddar, full fat
Spinach, fresh, cooked	Cheese, cottage
Spinach, fresh, raw	Cheese, processed, cheddar type
Sweetcorn, kernels, frozen	Dip, cream cheesed based
Beverages, alcoholic	**Meat and meat products**
Wine, white	Bacon
	Frankfurts
Beverages, non alcoholic	Ham
Juice, orange	Luncheon Sausage uniform texture (Fritz)
Soft drink	Sausages, beef
Tea	Salami
Water, bottled, still	Strassburg
Water, tap	
	Snack foods
Infant food and beverages	Potato crisps
Infant dessert, fruit	Pizza, meat & vegetable containing

Table A2: Individual food preparation instructions

Food	Preparation
Apples, unpeeled	▪ Core and chop apples into small cubes ▪ Mix the cubes together thoroughly.
Avocadoes	▪ Slice avocadoes in half, remove stone, and scoop out flesh with a stainless steel spoon. ▪ Chop avocado flesh. ▪ Mix the cubes together thoroughly.
Bacon	▪ Remove bacon rind. ▪ Chop and mix together thoroughly. ▪ Fry the bacon until tender.
Baked beans in tomato sauce, canned	▪ Include sauce. Mix together thoroughly.
Banana	▪ Remove skin. ▪ Chop banana flesh. ▪ Mix together.
Beans, green	▪ Top and tail beans and remove 'string' if necessary. ▪ Microwave until cooked. ▪ Chop beans and mix together.
Beetroot, canned	▪ Drain the contents of each of the three cans. ▪ Chop and mix together.
Broccoli	▪ Remove stalks and cut into flowerets. ▪ Wash in water. ▪ Mix thoroughly. ▪ Microwave until cooked. ▪ Chop and mix together.
Cabbage	▪ Remove outer leaves and discard. ▪ Slice cabbage thinly. ▪ Mix thoroughly. ▪ Boil cabbage in unsalted water until cooked.
Carrots	▪ Top and tail the carrots. ▪ If the carrots are unblemished, rinse only, if not, peel and remove blemishes. ▪ Slice carrots thinly. ▪ Boil carrot slices in unsalted water.
Celery, raw	▪ Separate celery stalks, remove leaves and base of stalk and discard. ▪ Wash stalks in water. ▪ Chop and mix together.
Cucumber, raw	▪ Top and tail cucumber and discard ends. ▪ Chop and mix together.
Cheese, cheddar, full fat	▪ Chop into small cubes. ▪ Mix together.
Cheese, cottage	▪ Chop and mix together.
Cheese, processed, cheddar type	▪ Chop into small cubes. ▪ Mix together.
Dip, cream cheese based	▪ Mix together.
Frankfurts	▪ Separate into individual links. ▪ Prepare as per label. ▪ Chop. ▪ Mix together.
Grapes	▪ Remove stalks from grapes and discard. ▪ Chop grapes. ▪ Remove seeds. ▪ Mix together.
Ham	▪ Chop and mix together thoroughly.
Infant dessert	▪ Combine 300 grams from each purchase into a large glass or

	stainless steel bowl. ▪ Mix.
Juice, orange	▪ Shake and invert containers to ensure thorough mixing.
Lettuce, raw	▪ Remove shrivelled outer leaves or roots, if any. ▪ Weigh 100 g from each purchase of washed lettuce, including some outer and some inner leaves (i.e. 300 g in total). ▪ Some purchases may consist of more than one lettuce. Ensure that for such a purchase the 100 g is made up of approximately equal proportions of the lettuces in the purchase. ▪ Chop and mix together.
Luncheon Sausage, uniform texture (Fritz)	▪ Chop. ▪ Mix together.
Mango	▪ For each purchase, slice mangoes in half, remove stone, and scoop out flesh with a stainless steel spoon. ▪ For each purchase, chop mango flesh and mix the cubes together thoroughly. ▪ Mix the cubes together thoroughly.
Mushroom	▪ Wash the mushrooms and wipe dry with paper towel. ▪ Chop and mix.
Olives, preserved	▪ Drain the olives. ▪ Discard the liquid. ▪ Chop and mix thoroughly in a large bowl.
Onions	▪ Peel off skin, discard. ▪ Chop and cook onion flesh. ▪ Mix thoroughly in a large bowl.
Oranges	▪ Peel and discard peel. ▪ Using gloved hands, break the oranges into segments into a large bowl (glass or stainless steel). ▪ Take care to include all juice. ▪ Mix the segments thoroughly in the bowl using gloved hands.
Parsley, fresh	▪ Chop and mix together.
Peach, canned in natural juice	▪ Include a representative proportion of juice. ▪ Chop and mix together.
Peach, fresh	▪ Do not peel peaches. ▪ Split peaches, remove stone and chop peaches. ▪ Mix the cubes together thoroughly.
Peas, frozen	▪ Microwave until cooked.
Pineapple, fresh	▪ Remove leaves and peel. ▪ Chop flesh.
Pizza, meat and vegetable-containing	▪ Chop. ▪ Mix together.
Potatoes	▪ Wash thoroughly. ▪ Peel and halve potato. ▪ Cook in boiling water until soft/cooked. ▪ When cooked, drain potatoes. ▪ Chop finely and mix together.
Potato crisps	▪ Mix the crushed potato chips/crisps thoroughly in a large bowl.
Pumpkin	▪ Wash thoroughly. ▪ Chop coarsely, leave unpeeled. ▪ Cook in boiling water until soft/cooked. ▪ When cooked, remove skin. ▪ Mix together.
Salami	▪ Remove skin from salami. ▪ Chop. ▪ Mix together.
Sausage, beef	▪ Dry fry and cool. ▪ Chop. ▪ Mix together thoroughly.
Soft drink	▪ Mix in a large stainless steel or glass bowl.

Spinach, fresh, cooked	▪ Remove stalks below the leaves and mix leaves. ▪ Microwave until cooked. ▪ Chop and mix together.
Spinach, fresh, raw	▪
Strassburg	▪ Chop. ▪ Mix together.
Strawberries	▪ Wash and remove leaves and stalks. ▪ Chop and mix together.
Sultanas	▪ Chop ▪ Mix together.
Sweetcorn, frozen	▪ Microwave until cooked.
Tea	▪ Brew one cup (250 ml) of tea using a teabag from each of the individual purchases. ▪ Wait 5 minutes for the tea to infuse. ▪ Mix the three cups of tea together thoroughly. ▪ Let cool.
Tomatoes, raw	▪ Chop and mix together.
Tomatoes, canned	▪ Mix entire contents of can until homogenised.
Water, bottled still	▪ Mix in a large stainless steel or glass bowl.
Water, tap	▪ Mix in a large stainless steel or glass bowl.
Watermelon	▪ Cut a cross section of watermelon from the melon. ▪ Remove the skin and seeds and chop into cubes. ▪ Perform this procedure until at least 500 grams of skinless and seedless watermelon pieces have been chopped. ▪ Mix thoroughly.
Wine	▪ Shake and invert the bottles several times to ensure thorough mixing of the contents. ▪ Mix in a large stainless steel or glass bowl.

Appendix 3: Analytical results

Table A3. Minimum and maximum concentration of sodium nitrate in foods and beverages

Food	No. of analyses	No. of samples <LOR	Minimum	Maximum	Lower Bound Mean	Upper Bound Mean
			mg/kg	mg/kg	mg/kg	mg/kg
Apples, unpeeled	9	1	<6.8	56.2	19.9	20.5
Avocadoes	10	3	<6.8	50.7	17.9	19.9
Bacon	15	0	22.0	90.0	54.0	54.0
Baked beans in tomato sauce, canned	6	1	<6.8	23.3	16.7	17.8
Banana	9	0	63.3	200.4	107.4	107.4
Beans, green, cooked	10	1	<6.8	808.2	400.0	400.7
Beetroot, canned	6	0	1643.8	2328.8	2009.1	2009.1
Broccoli, cooked	13	1	<6.8	424.7	224.7	225.2
Cabbage, cooked	10	0	93.2	616.4	346.4	346.4
Carrots, cooked	10	9	<6.8	8.9	0.9	7.0
Celery, raw	22	0	274.0	3013.7	1527.0	1527.0
Cucumber, raw	13	0	86.3	479.5	247.5	247.5
Cheese, cheddar, full fat	15	0	0.0	0.0	0.0	0.0
Cheese, cottage	9	1	0.0	10.0	0.0	1.1
Cheese, processed, cheddar type	9	3	<10.0	18.0	3.3	6.7
Dip, cream cheese based	15	3	<10.0	25.0	2.7	14.7
Frankfurts	15	0	25.0	78.0	53.5	53.5
Grapes	13	2	<6.8	52.1	18.5	19.5
Ham	15	0	20.0	90.0	47.0	47.0
Infant dessert, fruit	6	0	13.7	26.0	19.0	19.0
Juice, orange	10	2	<6.8	15.1	9.9	11.3
Lettuce, raw	22	0	520.6	2898.6	1143.6	1143.6
Luncheon sausage, uniform texture (Fritz)	15	0	35.0	60.0	51.7	51.7
Mango	6	2	<6.8	12.9	8.3	10.5
Mushroom, cooked	10	8	<6.8	10.7	1.8	7.3
Olives, preserved	2	0	20.6	23.3	21.9	21.9
Onions, cooked	10	4	<6.8	15.1	7.2	9.9
Oranges, fresh	10	3	<6.8	20.6	8.7	10.8
Parsley, fresh	22	0	205.5	4467.1	1957.1	1957.1
Peach, canned in natural juice	6	0	7.4	10.6	8.9	8.9
Peach, fresh	10	0	7.3	17.8	10.8	10.8
Peas, frozen, cooked	10	5	<6.8	9.6	4.3	7.7
Pineapple, fresh	10	3	<6.8	12.33	6.7	8.8

Pizza, meat and vegetable-containing	15	0	10.0	40.0	19.1	19.1
Potatoes, cooked	10	0	63.0	191.8	98.7	98.7
Potato crisps	2	0	397.3	465.8	431.5	431.5
Pumpkin, cooked	13	0	10.3	452.1	165.8	165.8
Salami	15	0	16.0	335.0	129.4	129.4
Sausages, beef	15	9	<10.0	10.0	0.7	6.7
Soft drink	6	2	<0.6	3.0	0.9	1.1
Spinach, fresh, cooked	22	0	671.2	3663.0	2741.4	2741.4
Spinach, fresh, raw	6	0	1990.0	3940.0	2962.5	2962.5
Strassburg	15	2	<10.0	70.0	44.3	45.6
Strawberries	10	0	95.9	232.9	172.2	172.2
Sultanas	3	0	21.0	21.9	21.6	21.6
Sweetcorn, kernels, frozen	6	2	<6.8	17.8	8.7	11.0
Tea	6	0	2.3	3.0	2.6	2.6
Tomatoes, raw and canned	14	0	6.9	41.6	16.4	16.4
Water, bottled still	6	0	9.3	43.8	21.5	21.5
Water, tap	16	3	<0.6	16.4	2.0	2.1
Watermelon	10	5	<6.8	24.7	8.0	11.4
Wine, white	11	1	<6.8	17.8	12.8	13.5

Notes to table:

1. Results are derived from composite samples.
2. Two means are given in the Table; the ‘Lower Bound Mean’ is derived assuming values below the LOR are equal to ‘0’, and the ‘Upper Bound Mean’ is derived assuming that values below the LOR are equal to the LOR.

Table A4: Mean, minimum and maximum levels of nitrites in foods, expressed as sodium nitrite

Food	No. of analyses	No. of samples <LOR	Minimum	Maximum value	Lower Bound Mean	Upper Bound Mean
			mg/kg	mg/kg	mg/kg	mg/kg
Apples, unpeeled	9	9	<7.5	<7.5	0.0	7.5
Avocadoes	10	10	<7.5	<7.5	0.0	7.5
Bacon	15	0	12.0	45.0	26.5	26.5
Baked beans in tomato sauce, canned	6	6	<7.5	<7.5	0.0	7.5
Banana	9	3	<7.5	11.2	3.6	6.3
Beans, green, cooked	10	8	<7.5	56.7	6.5	12.5
Beetroot, canned	6	6	<7.5	<7.5	0.0	7.5
Broccoli, cooked	13	8	<7.5	109.0	9.2	14.0
Cabbage, cooked	10	5	<7.5	26.0	8.9	12.6
Carrots, cooked	10	10	<7.5	<7.5	0.0	7.5
Celery, raw	22	22	<1.5	<7.5	0.0	6.0
Cucumber	13	5	<1.5	116.4	15.4	18.7
Cheese, cheddar, full fat	15	15	<5.0	-	0.0	0.0
Cheese, cottage	9	9	<5.0	-	0.0	0.0
Cheese, processed, cheddar type	9	9	<5.0	-	0.0	0.0
Dip, cream cheese based	15	9	<5.0	-	0.0	0.0
Frankfurts	15	1	<5.0	70.0	29.6	29.9
Grapes	13	2	<1.5	19.4	10.0	10.8
Ham	15	0	8.0	50.0	27.9	27.9
Infant dessert, fruit	6	6	<7.5	<7.5	0.0	7.5
Juice, orange	10	10	<7.5	<7.5	0.0	7.5
Lettuce, raw	22	19	<1.5	2.8	0.3	6.1
Luncheon sausage, uniform texture (Fritz)	15	0	18.0	70.0	34.9	34.9
Mango	6	3	<7.5	14.9	5.5	9.2
Mushroom, cooked	10	10	<7.5	-	0.0	7.5
Olives, preserved	2	2	<7.5	-	0.0	7.5
Onions, cooked	10	10	<7.5	-	0.0	7.5
Oranges, fresh	10	10	<7.5	-	0.0	7.5
Parsley, fresh	22	13	<7.5	24.5	6.2	10.6
Peach, canned in natural juice	6	6	<7.5	<7.5	0.0	7.5
Peach, fresh	10	0	11.6	22.4	16.9	16.9
Peas, frozen, cooked	10	8	<7.5	10.3	1.8	7.8
Pineapple, fresh	10	0	9.6	22.4	16.7	16.7
Pizza, meat and vegetable-containing	15	8	<5.0	8.0	1.2	3.9
Potatoes, cooked	10	9	<7.5	9.9	1.2	6.4
Potato crisps	2	2	<7.5	<7.5	0.0	7.5
Pumpkin, cooked	13	6	<1.5	194.0	26.7	30.3
Salami	15	2	<5.0	18.0	8.5	9.2
Sausage, beef	15	0	<5.0	-	0.0	0.0

Soft drink	6	6	<0.6	-	0.0	0.6
Spinach, fresh, cooked	22	18	<1.5	5.0	0.4	6.1
Spinach, fresh, raw	6	0	20.0	55.0	37.5	37.5
Strassburg	15	0	8.0	80.0	34.7	34.7
Strawberries	10	0	12.7	23.9	17.5	17.5
Sultanas	3	3	<1.5	-	0.0	5.5
Sweetcorn, kernels, frozen	6	6	<7.5	-	0.0	7.5
Tea	6	6	<0.6	-	0.0	0.3
Tomatoes, raw and canned	14	8	<1.5	13.1	1.9	6.5
Water, bottled still	6	6	<0.6	-	0.0	0.6
Water, tap	16	16	<0.6	-	0.0	0.6
Watermelon	10	8	<7.5	16.4	2.6	8.6
Wine, white	11	1	<1.5	20.9	10.8	11.6

Notes to table:

1. Results are derived from composite samples.
2. Two means are given in the Table; the ‘Lower Bound Mean’ is derived assuming values below the LOR are assigned a value of ‘0’, and the ‘Upper Bound Mean’ is derived assuming that values below the LOR are equal to the LOR.

Appendix 4: Dietary Exposure Assessment

Food consumption data

In 2007, the Australian Children's Nutrition and Physical Activity Survey, also known as Kids Eat Kids Play (KEKP), was conducted involving 4,487 children aged 2-16 years. This survey employed a 24 hour recall, with a second 24 hour recall for all KEKP participants, conducted on a non-consecutive day. The availability of two days of food consumption data provides a more realistic estimate of long term consumption of infrequently consumed foods, because it takes into account those who may eat a food on one day of the survey but not on the other. Using one 24-hour recall may capture an unusual eating occasion for an individual that does not describe how they normally eat. The survey was conducted over a seven month time period, from February to August 2007 and the results were released in 2008. There were approximately 3,000 individual foods reported as consumed in KEKP. These data have been used in the dietary exposure assessments for children aged 2-16 years. The results of the 2007 KEKP were weighted to represent the overall population of Australian children because stratified sampling with non-proportional samples was used in the KEKP survey. DIAMOND, the FSANZ computer program used for dietary exposure estimates, first calculates each individual's total exposure of food chemicals using their actual food consumption data (Equation 1). The individual's unweighted exposure is then multiplied by their sampling weight and divided by the sum of sampling weights for all respondents in the survey sample, as shown in Equation 3. From these individual weighted exposures, the weighted mean population exposure is generated. Weighting is applied after all other calculations are undertaken. The derivation of the appropriate weights for non-proportionate sampling is described in the user guide to the 2007 KEKP (CSIRO, 2008).

Equation 3: Weighting calculation

$$\textit{Individual weighted exposures} = \frac{(y_i \times sample\,weight \times n)}{sum\,of\ consumer\,weights}$$

Where y_i = consumption or intake for each consumer i, and n= number of consumers.

In 1995, the Australian National Nutrition Survey (NNS) surveyed 13,858 Australians aged two years and above using a 24 hour recall. A second (non-consecutive) day of food consumption data was collected from approximately 10% of NNS participants. Results were released in 1998. These food consumption data were used in this dietary exposure assessment for all population groups aged 17 years and above.

Both the 1995 NNS and 2007 KEKP did not survey children aged below two years of age. Therefore a model diet was constructed to allow the dietary exposure assessments for sodium nitrate and sodium nitrite to be conducted for infants aged nine months. This is discussed in more detail below.

Construction of the model diet for infants aged 9 months

To enable sodium nitrate, sodium nitrite and total sodium nitrite dietary exposures for nine month old infants to be estimated, a model diet was constructed. The model diet was based on information on recommended energy intakes, mean body weight and the proportion of milk and solid foods in the diet for a nine month old infant, and 2007 KEKP data on foods consumed by a two year old child. The recommended energy intake for a nine month old boy

(FAO, 2004) at the 50th percentile weight (WHO, 2007) was used as the basis for the theoretical diet. Boys' weights were used because boys tend to be heavier than girls at the same age and therefore have higher energy and food requirements. The body weight of a 50th percentile nine month old boy was 8.9 kg.

It was assumed that 50% of energy intake was derived from infant formula and 50% from solids and other fluids (Hitchcock *et al*., 1986). The patterns of consumption of a two year old child from the 2007 KEKP survey were scaled down and used to determine the 50% solid and other fluids portion of the 9 month old's diet. Certain foods such as nuts, tea, coffee and alcohol were removed from the diet since nuts can be a choking risk (National Health and Medical Research Council, 2001) and coffee and alcohol are unsuitable foods for infants (ACT Community Care, 2000). Following this, the foods analysed for sodium nitrite, total sodium nitrite and sodium nitrate were matched, where possible, to the foods they most accurately represented in the infant diet. In some cases, an exact match could not be made therefore assumptions based on similarities were used. These assumptions are listed in the "Assumptions and limitations" section below. For cases where more than one food in the infant diet could be matched to an analysed food, the analysed food with the highest concentration was used. Foods that could not be assigned a concentration were left with a zero concentration and excluded from the exposure assessment for infants aged 9 months. This information is presented in Table A7 of Appendix 4B.

The amount of water consumed in the model infant diet was not directly derived from the foods consumed by 2 year old children. Water consumption, from bottled and non-bottled sources, was estimated using the estimated fluid requirement for 9 month old infants of 135 ml/kg bw/day (Mahan and Arlin, 1992). In the 2007 KEKP survey, approximately 9% of 2 year old children consumed bottled water. To ensure conservative exposure estimates for sodium nitrate, sodium nitrite and total sodium nitrite this percentage was rounded to 10% and applied to the total quantity of water + infant formula in the model infant diet (see Equation 4). It was assumed that infant formula was made up using tap water only.

Equation 4: Water consumption calculations

Total fluid requirements (ml)	=	135 ml/kg bw/day
	=	1,201.5 ml
	=	Infant formula (ml) + bottle water (ml) + non-bottled water (ml) + soft drink (ml) + fruit juice (ml)
Bottled water consumption (g)	=	{Total fluid requirements (ml) – soft drink (ml) – fruit juice (ml)} * 10%
	=	117 g
Non-bottled water consumption (g)	=	Total fluid requirements (ml) – soft drink (ml) – fruit juice (ml) – bottled water (ml) – infant formula (ml)
	=	506 g

Table A6 in Appendix 4B lists the mean consumption amounts for each food in the model infant diet. Since the model diet was based on mean food consumption amounts only, a distribution of food consumption was unable to be derived and hence a distribution of sodium nitrate, sodium nitrite and total sodium nitrite exposures. As an alternative, the 90th percentile dietary exposures to sodium nitrite, total sodium nitrite and sodium nitrate were estimated using the calculation shown in Equation 5.

Equation 5: 90th percentile dietary exposure estimates for infants aged 9 months

90^{th} percentile dietary exposure = mean dietary exposure x 2

Respondents versus consumers

Estimates of dietary exposures can be calculated for all survey respondents or only for those respondents who consumed a food containing the chemical ('consumers'). This study reports exposure estimates for 'consumers' for all population groups excluding 9 month old infants. Due to the methodology used to calculate sodium nitrate, sodium nitrite and total sodium nitrite dietary exposures, in most population groups all respondents in the 1995 NNS and 2007 KEKP are consumers. The number of respondents, percentage of consumers to respondents, mean and 90^{th} percentile dietary exposures (in mg/day and mg/kg bw/day) are provided in Appendix 5 for sodium nitrate, Appendix 5A for sodium nitrite and Appendix 5B for total sodium nitrite.

Food mapping

Mapping is the process of matching the foods analysed for sodium nitrite and sodium nitrate to the food consumption data from the 1995 NNS and 2007 KEKP survey. Given that all foods available in the food supply could not be analysed, mapping is a major step in the dietary exposure assessment process. Mapping can be based on nutritional considerations and/or the expected presence of a food chemical in a food. There were three main types of mapping:

Direct mapping – where the analysed foods were directly matched to the same food and to similar foods from the 1995 NNS and 2007 KEKP survey (e.g. the analysed food "Apples" was mapped to the 1995 NNS food "Apple, red, raw, unpeeled" and "Quince, stewed, unsweetened", using the assumption that the food chemicals present in apples are the same in all pome fruits).

Mapping using 'factors' – used where the analysed food is in a different form to that consumed in the 1995 NNS or 2007 KEKP survey (e.g. The analysed food "apple, unpeeled" was analysed in its raw form but some respondents in the 1995 NNS reported consuming dried apple). The food consumption amount reported in the 1995 NNS or 2007 KEKP survey is multiplied by a 'factor' to convert the food to the same form as the analysed form (e.g. 10 grams of dried apple was converted to 53 grams of "apple, unpeeled").

Recipes – used where a food consumed in a nutrition survey is composed of more than one analysed food (e.g. the 1995 NNS food "fruit drink, orange, ready-to-drink" is made up of the analysed foods "Water, tap" and "Juice, orange").

There were 52 foods analysed, each for sodium nitrate and sodium nitrite. Using a 'best fit' approach and the methods listed above, the 1995 NNS foods and KEKP foods were matched to the 52 analysed foods or to recipes. Dietary exposure results in this report have been presented in terms of the group of foods that the analysed food represents, rather than in terms of the individual food itself (e.g. the analysed food "apples, unpeeled" is referred to as "pome fruits" in the dietary exposure assessments. Details of the analysed foods and the 1995 NNS and 2007 KEKP foods that they represent are provided in Table A7, Appendix 4B.

Food contribution calculations

Information regarding the major food contributors to the dietary exposure to sodium nitrate, sodium nitrite and total sodium nitrite have been presented in this report. These were calculated as a percentage contribution derived using mean analytical concentrations for the lower end of the range only (i.e. using the assumption that results below the LOR are equal to zero). This assumption is further discussed in the "Assumptions and limitations in dietary exposure assessment" section. Major contributors were defined as those foods that contribute 5% or more to the total dietary exposure.

To obtain an indication of the percentage contribution each food group made to total estimated exposures, the sum of all individuals' exposures from one food group was divided by the sum of all individuals' exposures from all foods containing the food chemicals assessed, and multiplied by 100.

Assumptions and limitations in dietary exposure assessment

The aim of dietary exposure assessments was to make as realistic an estimate of dietary exposure of sodium nitrite, total sodium nitrite and sodium nitrate as possible. FSANZ always ensures the data and methodologies used for dietary exposure assessment are the most up to date and the best available. FSANZ evaluates all data sets prior to dietary exposure assessment for any project and has been proactive in obtaining and using other data and methodologies where applicable and undertaking validation processes where required. FSANZ notes any limitations associated with the dietary exposure assessment so that the results can be interpreted correctly.

Dietary exposure assessment based on 1995 NNS and 2007 KEKP survey food consumption data provides the best estimate of actual consumption of a food and the resulting estimated dietary exposure to a food chemical for the population. FSANZ has undertaken an assessment of changing food consumption patterns across the diet over time and concluded that consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to have changed markedly since 1995 (Cook *et al*., 2001a; Cook *et al*., 2001b). Although improvements have been made to the methods of estimating dietary exposure over time, the introduction of DIAMOND being the main one of these, limitations still exist in the methods as well as in the data.

Limitations relating to the food consumption and chemical concentration data include:

• Data derived from a single 24 hour diet (and in some cases a second (non-consecutive) day of food consumption) is used as a basis for drawing conclusions on lifetime eating patterns, leading to conservative dietary exposure assessments. More comprehensive data on food consumption over multiple days would provide better estimates of food consumption patterns and long-term dietary exposures to food chemicals.

• Due to the age of the 1995 NNS dataset, the data does not include information regarding food products that have been introduced to the market since the 1995 NNS was conducted or for which there may have been changes in food consumption patterns. As discussed above, the consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most peoples' diets, is unlikely to have changed markedly.

• A single food chemical concentration (calculated as the mean analytical result of composite samples) is used in calculating dietary exposure estimates, which does not take into account the level of uncertainty or the variability associated with the concentration used.
• A limitation of estimating dietary exposures over a period of time using information from a recall method is that people may over- or under-report food consumption, particularly for certain types of foods.

• As the 1995 NNS and 2007 KEKP survey do not report on respondents aged below two years, a model diet was used to estimate dietary exposure for infants aged nine months. Mean food consumption amounts in the model diets are used to represent food consumption patterns for an age group as a whole and may not be as accurate as the data derived for other population groups from the 1995 NNS and 2007 KEKP survey that use food consumption data for individuals.

Assumptions made in the dietary exposure assessment for sodium nitrate, sodium nitrite and total sodium nitrite include:

• The food chemical concentration in the analysed foods was a good representation of the concentration of that chemical in all of the other foods to which it was mapped, as shown in Appendix 4B.

• There was no contribution to dietary exposures from medicines (including complementary medicines such as vitamin and mineral supplements).

• The chemical concentration in a particular analysed food was carried over to all of the mixed foods in which it was used as an ingredient (e.g. apple in fruit salad).

• If a food in the infant diet could not be matched exactly to an analysed food, it was matched to an analysed food using the principles outlined in the “Food Mapping” section on page 39. For example, “kiwifruit” was a food in the infant diet but was not analysed for sodium nitrites and sodium nitrates. Therefore the sodium nitrate/ sodium nitrite concentration for “banana” was used for “kiwifruit” as they are both in the same food group of tropical fruits.

• If more than one analysed food could be mapped to a food in the infant diet, the food with the highest sodium nitrite/ sodium nitrate concentration was used (e.g. “peach, canned in natural juice” and “peach, fresh” were both analysed for sodium nitrite and sodium nitrate; “Peach, fresh” was used to represent all peaches in the infant diet since it had the highest concentration of both sodium nitrite and sodium nitrate). The exception to this rule was for the food group “pork (except bacon) and deli meats (except frankfurts and poultry-based)”. “Salami” had the highest concentration of sodium nitrite and sodium nitrate for the foods included in this food group. The assumption was made that “salami” is not suitable for infants aged 9 months. Therefore the next highest sodium nitrate/ sodium nitrite concentration (for “luncheon sausage, uniform texture (Fritz)”) was used.

• Assumptions were also made about the value of analytical results below the limit of quantification (LOR) as described on page 9.

• The amount of ingested nitrate that is converted to nitrite in the salvia varies among individuals, a 5% conversion rate was assumed to be a good representation of the conversion rate of sodium nitrate to sodium nitrite from all foods.

Appendix 4A: Mean Food Consumption and Body Weights

Table A5: Number of respondents and average body weight for each age group

Age	Gender	No. Of Respondents	Average Body Weight (kg)
9 months			8.9
2-5 years¤	All	1,178	18
6-12 years¤	All	2,090	36
13-16 years¤	All	1,219	61
17 years & above*	All	11,129	74
16-44 years*	Female	3,178	66

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey (Day 1 and Day 2 average)

Table A6: Mean consumption amounts for the model diet for infants aged 9 months

ATDS Food	Mean consumption amount (grams per day)
Apples and quinces	17.2
Avocadoes and olives	1.4
Bacon	0.5
Bananas and plantains	13.6
Beetroot	0.2
Berries	2.8
Broccoli and broccoflower	1.5
Cabbages, Brussels sprouts and kohlrabi	0.4
Capsicums, chillies and spices	0.6
Cauliflower	0.9
Cheeses (all types)	4.9
Citrus fruits	6.9
Cucumbers and chokos	1.0
Dried apricots, peel, cherries, ginger and fruit leathers	0.3
Dried grapes/ figs/ dates and prunes	1.7
Dried pulses (except soy beans)	1.8
Fresh beans and bean sprouts	0.8
Fruit juices and ciders	28.1
Grapes	3.3
Infant formulas	544
Leafy vegetables and herbs	0.7
Melons	4.8
Mushrooms	0.4
Non-alcoholic beverages (except milk, waters and juices)	6.8
Onions, garlic, shallots, spring onions and leeks	1.6
Pears and loquats	2.9
Peas (fresh, dried and sprouts)	1.6

Pineapple and jackfruit	1.0
Pizzas (excluding seafood)	0.8
Pork (except bacon) and deli meats (except frankfurts and poultry-based)	3.1
Pumpkins, squash, marrows and zucchini	2.8
Root vegetables (non-starchy)	3.3
Root vegetables (starchy)	11.1
Sausages and frankfurts	2.2
Savoury snacks	0.9
Stalk and stem vegetables	0.5
Stone fruits (furry skinned)	1.4
Stone fruits (smooth skinned) and figs	0.3
Sweetcorn	2.1
Teas	0
Tomatoes/ eggplant/ okra (cooked or processed)	3.5
Tomatoes/ eggplant/ pepino (raw or sun-dried)	2.5
Tropical fruits (rough or furry skin, except pineapples and jackfruit)	0.4
Tropical fruits (smooth-skinned, except bananas, plantains, avocadoes & olives)	0.3
Water (bottled/ plain mineral/ soda)	117
Water (non-bottled)	506
White wines, wine coolers, rice and ginger wines	0

Appendix 4B: Food Mapping

Table A7: Infant Diet Food Mapping

Food Group Name	Foods Represented	Analysed Food Concentration Used
Apples and quinces	Apples; quinces	Apples, unpeeled
Avocadoes and olives	Avocado; olives	Avocadoes for sodium nitrite; Olives for sodium nitrate
Bacon	Bacon	Bacon
Bananas and plantains	Bananas; plantains	Banana
Beef, veal and venison∞	Beef; veal; venison and unspecified meat	Not assigned a concentration
Beer, liqueurs and spirits*	Beer; non-cream & non-coffee liqueurs; non-fruit based spirits	Excluded from infant diet
Beetroot	Beetroot	Beetroot, canned
Berries and jams	Berries; jams	Strawberries
Breakfast cereals with mixed grains/ fruits/ nuts*	Mixed grain breakfast cereals and brans; breakfast cereals containing fruits and/or nuts; plain muesli bars	Not assigned a concentration
Broccoli and broccoflower	Broccoli and broccoflower	Broccoli, cooked
Butter and animal fats∞	Butter; ghee; animal fats	Not assigned a concentration
Cabbages, Brussels sprouts and kohlrabi	All green, red and Chinese (e.g. buk choy, Chinese flowering) cabbages; sauerkraut; kohlrabi; Brussels sprouts	Cabbage, cooked
Cakes, muffins and puddings∞	Commercial plain cakes and cake-style muffins (includes iced and uniced); cake-style puddings	Not assigned a concentration
Capsicums, chillies and spices	Capsicum; chillies; capers; spices; curry pastes and powders	Cucumber, raw
Cauliflower	Cauliflower	Broccoli, cooked
Cheeses	Cheeses; dried cheese (recipe mixes)	Dip, cream cheese based
Chocolates, cocoa and fudge∞	Cocoa; chocolate; chocolate bars; chocolate coated-confectionery and fudge (excluding those with fruit, nuts, biscuit or coconut)	Not assigned a concentration
Citrus fruits and kumquats	Citrus fruits; kumquats; marmalade	Oranges, fresh
Coconut and coconut products∞	Coconut; coconut products (e.g. coconut milk)	Not assigned a concentration
Coffee (from ground)*	Ground coffees	Excluded from infant diet
Coffee (from instant) and cereal-based beverages*	Instant coffee; cereal beverages (coffee substitutes)	Excluded from infant diet
Cucumbers and chokos	Cucumbers; cucumber pickles; chokos	Cucumber, raw
Dried apricots, peel, cherries, ginger and fruit leathers	Dried and glace apricots; fruit peel; glace cherries; glace ginger; fruit	Peach, fresh (with a raw equivalence factor

	leathers	of 5.7)
Dried grapes/ figs/ dates and prunes	Dried vine fruits; dried figs; dried dates; prunes	Sultanas
Dried pulses (except soy beans)	All pulses (dried beans and lentils) except soy beans	Baked beans in tomato sauce, canned
Eggs∞	Poultry eggs	Not assigned a concentration
Fancy breads, crumpets and English muffins∞	Flat breads; fruit-, vegetable-, cheese-, or bacon-containing breads; English muffins; crumpets; buns	Not assigned a concentration
Fish (uncrumbed/ unbattered or canned)∞	All uncrumbed or unbattered fish; canned fish	Not assigned a concentration
Flours and single grain cereals∞	Single grain breakfast cereals and brans that do not contain fruit or nuts; cornmeal; tacos; flours and starches; popcorn; bulghur	Not assigned a concentration
Fresh beans and bean sprouts	Pulses (fresh beans); bean sprouts	Beans, green, cooked
Frozen dairy based desserts∞	Ice creams; frozen yoghurt; milk-based ice confections	Not assigned a concentration
Fruit juices and ciders	Fruit juices, including infant juices; cider; fruit based ice-confection	Juice, Orange
Grapes	All raw grapes	Grapes
Hamburgers (all meat types)∞	Hamburgers; chicken burgers; fish burgers	Not assigned a concentration
Honey∞	Honey	Not assigned a concentration
Infant cereals∞	Infant cereal	Not assigned a concentration
Infant custards and yoghurts∞	Infant custards and yoghurts	Not assigned a concentration
Infant dinners∞	Infant dinners	Not assigned a concentration
Infant formulas	Cow's milk formula; soy formula	Water, tap
Lamb, mutton, kangaroo and rabbit∞	Lamb; mutton; kangaroo; rabbit	Not assigned a concentration
Leafy vegetables and herbs	Leafy vegetables; herbs	Spinach, fresh, raw
Margarines and margarine spreads∞	Margarines and margarine spreads, not further specified spreads/fats, vegetable based hard fats	Not assigned a concentration
Melons	Melons	Watermelon
Milks and creams*	All dairy milks (cow and goat), including dried, evaporated, condensed; flavoured milks; cream	Excluded from infant diet
Molluscs and crustacea∞	Molluscs; crustacea	Not assigned a concentration
Multigrain, wholemeal and rye breads∞	Multigrain, wholemeal and rye breads	Not assigned a concentration
Mushrooms	Mushrooms	Mushroom, cooked
Non-alcoholic beverages (except milk, waters and juices)	Soft drinks; flavoured mineral waters; tonic water; fruit-flavoured drinks; sports drinks; water ice confections; sorbet	Soft Drink
Oats∞	Oats	Not assigned a concentration
Oils∞	Oils	Not assigned a concentration

Offal (including pate and liverwurst)∞	Beef, sheep, pig and poultry offal, including pate and liverwurst	Not assigned a concentration
Onions, garlic, shallots, spring onions and leeks	Onions; garlic; shallots; spring onions; leeks	Onions, cooked
Pasta , noodles (except rice) and couscous∞	Pasta; noodles (except rice noodles); couscous	Not assigned a concentration
Peanuts and peanut butter*	Peanuts; peanut butter	Not assigned a concentration
Pears and loquats	Pears; loquats	Apples, unpeeled
Peas (fresh, dried and sprouts)	Peas (fresh, dried and sprouts)	Peas, frozen, cooked
Pineapple and jackfruit	Pineapple; jackfruit	Pineapple, fresh
Pizzas (excluding seafood)	Meat and poultry containing savoury pizzas, excluding seafood	Pizza, meat and vegetable topped, takeaway
Pork (except bacon) and deli meats (except frankfurts and poultry-based)	All plain pork except bacon; deli meats (except bacon, frankfurts, sausages and poultry-based)	Luncheon Sausage, uniform texture (Fritz)
Poultry (excluding skinless)∞	Poultry fillets and pieces with the skin; poultry mince; poultry deli-meats	Not assigned a concentration
Poultry (skinless)∞	Skinless poultry fillets and pieces	Not assigned a concentration
Pumpkins, squash, marrows and zucchini	Pumpkin; squash; marrow; zucchini	Pumpkin, cooked
Red/rose wines, sherry, port and brandy*	Red and rose wines; sherry; port; brandy	Excluded from infant diet
Rice and rice products∞	Cooked and raw rice and rice noodles, rice cakes and rice crackers	Not assigned a concentration
Root vegetables (non-starchy)	Carrots and all other non-starchy root vegetables (radishes, horseradish, water chestnut)	Carrots, cooked
Root vegetables (starchy)	Potatoes, excluding potato crisps; starchy root vegetables (Jerusalem artichokes, parsnips, cassava, turnips, swedes, sweet potatoes); unspecified vegetables	Potatoes, cooked
Sausages and frankfurts	All sausages; frankfurts	Frankfurts
Savoury biscuits and crackers∞	Savoury biscuits (excluding rice crackers); pretzels	Not assigned a concentration
Savoury pastries (containing meat)∞	Meat and chicken pies; cornish pasties; sausage rolls; meat/chicken containing pastries and wontons	Not assigned a concentration
Savoury sauces (excluding tomato)∞	e.g. Soy sauce; oyster sauce; BBQ sauce; Worcerstershire sauce; chilli sauce	Not assigned a concentration
Savoury sauces (tomato)∞	Tomato sauces and salsas (excluding simmer sauces)	Not assigned a concentration
Savoury snacks	Potato crisps, corn chips and extruded savoury snacks	Potato, crisps
Seafood (battered)∞	Battered fish and other seafood	Not assigned a concentration
Seafood (crumbed)∞	Crumbed fish and other seafood	Not assigned a concentration
Seeds and tree nuts (except	All tree nuts except coconut, seeds	Excluded from infant

coconut)*		diet
Soy beverages, soy beans and tofu*	Soy beverages, soy beans and tofu	Excluded from infant diet
Stalk and stem vegetables	All stalk and stem vegetables (celery, celeriac, asparagus, rhubarb, bamboo shoot, fennel, artichoke)	Celery, raw
Stone fruits (furry skinned)	"Furry skinned" stone fruits (peaches, apricots)	Peach, fresh
Stone fruits (smooth skinned) and figs	"Smooth skinned" stone fruits (cherries, nectarines, plums); figs	Peach, fresh
Sugars, confectionery, syrups and icings∞	Sugars, sugar-confectionery, syrups, icing	Not assigned a concentration
Sweet/ plain/ filled biscuits∞	Commercial plain and filled sweet biscuits; meal replacement biscuits; cones for ice cream	Not assigned a concentration
Sweetcorn	Sweetcorn	Sweetcorn, kernels, frozen
Teas*	Teas	Excluded from infant diet
Tomatoes/ eggplant/ okra (cooked or processed)	Canned or cooked tomatoes; tomato paste; tomato juice; cooked eggplant; cooked okra	Tomatoes, raw and canned
Tomatoes/ eggplant/ pepino (raw or sun-dried)	Raw tomatoes and sun-dried tomatoes, pepino, eggplant	Tomatoes, raw and canned
Tropical fruits (rough or furry skin, except pineapples and jackfruit)	Kiwifruit; rambutan; lychees; custard apples; pomegranate	Banana
Tropical fruits (smooth-skinned, except bananas, plantains, avocadoes & olives)	Mango; pawpaw; guavas; tamarillo; feijoa; dates; persimmons; passionfruit; starfruit	Mango
Water (bottled/ plain mineral/ soda)	Bottled water, plain mineral and soda waters	Water, bottles, still
Water (non-bottled)	Tap water	Water, tap
White breads (including high-fibre white)∞	White and high-fibre white breads; croutons	Not assigned a concentration
White wines, wine coolers, rice and ginger wines*	White wines; wine coolers; rice wine; ginger wine	Excluded from infant diet
Yoghurt (except frozen) and dairy desserts (except ice cream)∞	All yoghurts (excluding frozen) and dairy desserts (e.g. mousse, fromage frais)	Not assigned a concentration

∞ Foods that were assigned a zero concentration for sodium nitrite and sodium nitrate and were excluded from the exposure assessment for infants aged 9 months.
* Foods that were removed from the infant diet.

Table A8: Mapping of analysed foods to national nutrition survey foods

Food Analysed	Foods Represented
Apples, unpeeled	Pome fruits (fresh, cooked, dried and juice)
Avocadoes	Avocado
Bacon	Bacon; pancetta; speck
Baked beans in tomato sauce, canned	All pulses (dried beans and lentils); products using pulses e.g. Dhal, falafel soy beans including soy flour and soy based beverage powders; tofu
Banana	Bananas; plantains and tropical fruits including guava, passionfruit, kiwifrui mangosteen, feijoa, pomegranate (fresh, dried and juice); figs (fresh and dried)
Beans, green, cooked	Beans (fresh and cooked); bean sprouts
Beetroot, canned	Beetroot (fresh and canned)
Broccoli, cooked	Broccoli, broccoflower and cauliflower (fresh and cooked)
Cabbage, cooked	All cabbages including green, red and white types; sauerkraut; brussels sprouts
Carrots, cooked	Carrots and all other non-starchy root vegetables (radish, parsnip, horseradish) including ginger
Celery, raw	Stalk and stem vegetables including celery, asparagus, rhubarb, bamboo shoot, fennel, artichoke, lemongrass
Cheese, cheddar, full fat	Semi-soft and hard cheeses (brie, feta, cheddar and parmesan) including cheese powder
Cheese, cottage	Soft cheeses (ricotta, cottage and cream)
Cheese, processed, cheddar type	Processed cheeses including cheese spread and cream cheese
Cucumber, raw	Cucumbers; pickles; chokos; capsicums and chillies (including chilli powde
Grapes	All grapes and lychees
Ham	Unprocessed deli meats; (except bacon, frankfurts, sausages)
Infant dessert, fruit	Infant desserts containing fruit
Juice, orange	Juice and concentrate from citrus fruits including orange, lemon, lime and grapefruit
Lettuce, raw	All lettuce types
Mango	Mango; pawpaw; rambutan (dried, juice and fresh)
Mushroom, cooked	Mushrooms
Olives, preserved	Olives
Onions, cooked	Onions; garlic; shallots; spring onions; leeks (raw, cooked, pickled, dried)
Oranges, fresh	Citrus fruits; kumquats; marmalade
Parsley, fresh	Green leafy herbs e.g. basil, mint, chives (raw and cooked)
Peach, canned in natural juice	Canned stone fruits including peaches, apricots, plums and cherries
Peach, fresh	Stone fruits and persimmon, fresh, dried and juiced including peaches, nectarines, plums, cherries, apricots
Peas, frozen, cooked	Peas (fresh, dried and sprouts)

Pineapple, fresh	Pineapple (fresh, dried, canned and juice)
Pizza, meat & vegetable-containing	Meat, poultry and seafood containing savoury pizzas
Potato crisps	Potato crisps, corn chips and extruded savoury snacks
Potatoes, cooked	Potatoes, excluding potato crisps; starchy root vegetables (parsnips, cassava, turnips, swedes, sweet potatoes); unspecified vegetables
Pumpkin, cooked	Pumpkin; squash; zucchini
Sausage, beef	All sausages and meat patties from pork, beef, poultry and lamb
Soft Drink	Soft drinks including energy drinks; mineral waters including flavoured type sports drink; water ice confections
Spinach, fresh, cooked	Cooked spinach, silverbeet, kale and seaweed
Strawberries	Berries (fresh, dried, cooked and juiced)
Sultanas	Dried vine fruits; dried dates
Sweetcorn, kernels, frozen	Sweetcorn
Tea	Teas including herbal tea; tea flavoured drinks; coffees
Tomatoes, raw and canned	Tomato (raw, cooked, dried, powder, paste and puree)
Water, bottled still	Bottled water
Water, tap	Tap/bore/tank water; intense sweetened liquids (liquid only)
Watermelon	Watermelon; honey dew melon; rockmelon
Wine, white	Red and rose wines; white wines, sherry; port; brandy
Dip, cream cheese based	Cream-based dips
Frankfurts	Frankfurts
Luncheon sausage, uniform texture (Fritz)	Processed meats from beef, poultry and pork; including pate and meat pas
Salami	Salami and cabanossi
Strassburg	Black pudding; brawn; mortadella; bratworst
Spinach, fresh, raw	Raw, spinach, silverbeet, kale and endive; salad greens

Appendix 5: Sodium Nitrate – Dietary Exposure Assessment Results

Table A9: Estimated mean and 90th percentile dietary exposures to sodium nitrate (mg/day)

Chemical	Age Group	No. Respondents	% consumers to respondents			Estimated consumer dietary exposures (mg/day)					
						Mean			**90th percentile**		
			nd*=0	**nd=0.5 LOR**	**nd= LOR**	**nd=0**	**nd=0.5 LOR**	**nd= LOR**	**nd=0**	**nd=0.5 LOR**	**nd=LOR**
Sodium Nitrate	9 months⌘					13	13	13	26	26	27
	2-5 yrs¤	1,178	100	100	100	35	36	36	60	60	60
	6-12 yrs¤	2,090	100	100	100	46	46	47	85	85	86
	13-16 yrs¤	1,219	100	100	100	58	59	59	112	113	113
	17 yrs & above*	11,129	100	100	100	69	70	70	143	143	143
	16-44 yrs female*	3,178	100	100	100	62	63	63	132	133	133

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet
*nd = values below the LOR

Table A10: Estimated mean and 90th percentile dietary exposures to sodium nitrate (mg/kg bw/day)

Chemical	Age Group	Estimated consumer dietary exposures (mg/kg bw/day)					
		Mean (mg/kg bw/day)			**90th percentile (mg/kg bw/day)**		
		nd*=0	**nd=0.5 LOR**	**nd=LOR**	**nd=0**	**nd=0.5 LOR**	**nd=LOR**
Sodium Nitrate	9 months⌘	1.5	1.5	1.5	2.9	3	3
	2-5 yrs¤	2.0	2.0	2.1	3.4	3.5	3.5
	6-12 yrs¤	1.4	1.4	1.4	2.6	2.6	2.6
	13-16 yrs¤	1.0	1.0	1.0	1.9	1.9	1.9
	17 yrs & above*	1.0	1.0	1.0	2.0	2.0	2.0
	16-44 yrs female*	1.0	1.0	1.0	2.1	2.1	2.1

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet
*nd = values below the LOR

Table A11: Major contributing foods to sodium nitrate dietary exposures for population groups aged 2 years and above

Food group	% Contribution				
	2-5 years¤	**6-12 years¤**	**13-16 years¤**	**17 years & above***	**16-44 years females***
Bananas, tropical fruits and figs	13	7	<5	<5	<5
Root vegetables (starchy)	11	12	12	8	7
Pome fruits	7	5	<5	<5	<5
Stalk and stem vegetables	7	7	8	13	12
Green leafy vegetables (cooked)	7	7	9	10	10
Lettuce	5	12	17	16	18
Broccoli, broccoflower and cauliflower	5	5	<5	5	5
Savoury snacks	5	8	7	<5	<5
Berries	5	<5	<5	<5	<5
Fresh beans and bean sprouts	<5	<5	<5	5	<5
Beetroot	<5	<5	<5	7	6
All other foods	27	28	28	26	26

Note: 'nd=0' scenario shown
* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet

Table A12: Major contributing foods to sodium nitrate dietary exposures for infants aged 9 months

Food Group	Contribution (%)
Water (bottled/ plain mineral/ soda)	19
Bananas and plantains	11
Infant formulas	8
Root vegetables (starchy)	8
Water (non-bottled)	8
Leafy vegetables and herbs	6
Stalk and stem vegetables	5
All other foods	35

Appendix 5A: Sodium Nitrite – Dietary Exposure Assessment Results

Table A13: Estimated mean and 90th percentile dietary exposures to sodium nitrite (mg/day)

Chemical	Age Group	No. Respondents	% consumers to respondents			Estimated consumer dietary exposures (mg/day)					
						Mean			90th percentile		
			nd*=0	nd=0.5LOR	nd=LOR	nd=0	nd=0.5LOR	nd=LOR	nd=0	nd=0.5LOR	nd=LOR
Sodium Nitrite	9 months⌘					0.5	1.2	1.9	1.0	2.4	3.8
	2-5 yrs¤	1,178	100	100	100	1.7	3.1	4.5	3.4	5.2	7.2
	6-12 yrs¤	2,090	100	100	100	1.8	3.5	5.2	3.6	5.9	8.4
	13-16 yrs¤	1,219	100	100	100	1.9	3.9	5.8	4.2	6.5	9.3
	17 yrs & above*	11,129	98	100	100	3.0	5.0	7.0	6.9	9.5	12.6
	16-44 yrs female*	3,178	98	100	100	2.6	4.4	6.4	6.3	8.6	11.5

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet
*nd = values below the LOR

Table A14: Estimated mean and 90th percentile dietary exposures to sodium nitrite (mg/kg bw/day)

Chemical	Age Group	Estimated consumer dietary exposures (mg/kg bw/day)					
		Mean (mg/kg bw/day)			**90th percentile (mg/kg bw/day)**		
		nd*=0	**nd=0.5LOR**	**nd=LOR**	**nd=0**	**nd=0.5LOR**	**nd=LOR**
Sodium Nitrate	9 months⌘	0.1	0.1	0.2	0.1	0.3	0.4
	2-5 yrs¤	0.1	0.18	0.26	0.19	0.29	0.4
	6-12 yrs¤	0.06	0.11	0.16	0.12	0.19	0.3
	13-16 yrs¤	0.03	0.07	0.1	0.07	0.11	0.2
	17 yrs & above*	0.04	0.07	0.1	0.1	0.13	0.18
	16-44 yrs female*	0.04	0.07	0.1	0.1	0.14	0.19

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet
*nd = values below the LOR

Table A15: Major contributing foods to sodium nitrite dietary exposures for population groups aged 2 years and above

Food group	% Contribution				
	2-5 years¤	**6-12 years¤**	**13-16 years¤**	**17 years & above***	**16-44 years females***
Deli meats in whole pieces or cuts (except bacon)	13	15	15	10	8
Pumpkin, squash, zucchini	13	12	12	12	11
Bananas, tropical fruits and figs	9	6	<5	<5	<5
Berries	12	7	6	<5	<5
Grapes and lychees	7	5	<5	<5	<5
Pineapple	9	10	11	6	8
Cucumbers, capsicums, chokos and chillies	6	7	8	6	6
Bacon and pancetta	<5	<5	6	<5	<5
Broccoli, broccoflower and cauliflower	<5	5	<5	5	5
Root vegetables (starchy)	<5	<5	5	<5	<5
Smooth processed meats	5	<5	<5	<5	<5
Stone fruits (fresh) and persimmon	<5	5	<5	10	10
Wines and fortified wines	<5	<5	<5	20	22
All other foods	13	16	17	14	12

Note: 'nd=0' scenario shown
* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet

Table A16: Major contributing foods to sodium nitrite dietary exposures for infants aged 9 months

Food Group	Contribution (%)
Pork (except bacon) and deli meats (except frankfurts and poultry-based)	22
Pumpkins, squash, marrows and zucchini	15
Berries and jams	10
Bananas and plantains	10
Dried apricots, peel, cherries, ginger and fruit leathers	7
Grapes	7
All other foods	29

Appendix 5B: Total Sodium Nitrite – Dietary Exposure Assessment Results

Table A17: Estimated mean and 90th percentile dietary exposures to total sodium nitrite (mg/day)

Chemical	Age Group	No. Respondents	% consumers to respondents			Estimated consumer dietary exposures (mg/day)					
						Mean			**90th percentile**		
			nd=0	**nd=0.5 LOR**	**nd=LOR**	**nd=0**	**nd=0.5 LOR**	**nd=LOR**	**nd=0**	**nd=0.5 LOR**	**nd=LOR**
Sodium Nitrite	9 months⌘					1.1	1.9	2.6	2.3	3.7	5.2
	2-5 yrs¤	1,178	100	100	100	3.4	4.9	6.3	6.2	7.9	10
	6-12 yrs¤	2,090	100	100	100	4.1	5.8	7.5	7.5	9.6	12
	13-16 yrs¤	1,219	100	100	100	4.8	6.8	8.8	8.8	12	15
	17 yrs & above*	11,129	98	100	100	6.4	8.5	10.5	13.1	15.9	18.9
	16-44 yrs female*	3,178	98	100	100	5.6	7.6	9.5	11.8	14.5	17.4

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet
*nd = values below the LOR

Table A18: Estimated mean and 90th percentile dietary exposures to total sodium nitrite (mg/kg bw/day)

Chemical	Age Group	Estimated consumer dietary exposures (mg/kg bw/day)					
		Mean (mg/kg bw/day)			90th percentile (mg/kg bw/day)		
		nd*=0	nd=0.5 LOR	nd=LOR	nd=0	nd=0.5 LOR	nd=LOR
Sodium Nitrite	9 months⌘	0.1	0.2	0.3	0.3	0.4	0.6
	2-5 yrs¤	0.2	0.28	0.36	0.35	0.47	0.58
	6-12 yrs¤	0.12	0.18	0.23	0.24	0.31	0.39
	13-16 yrs¤	0.08	0.12	0.15	0.16	0.21	0.25
	17 yrs & above*	0.09	0.12	0.15	0.18	0.23	0.27
	16-44 yrs female*	0.09	0.12	0.15	0.19	0.23	0.28

* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet
*nd = values below the LOR

Table A19: Major contributing foods to sodium nitrite dietary exposures for population groups aged 2 years and above

Food group	% Contribution				
	2-5 years¤	**6-12 years¤**	**13-16 years¤**	**17 years & above***	**16-44 years females***
Deli meats in whole pieces or cuts (except bacon)	7	7	6	5	<5
Pumpkin, squash, zucchini	8	7	6	7	6
Bananas, tropical fruits and figs	11	6	<5	<5	<5
Berries	8	5	<5	<5	<5
Pineapple	<5	5	<5	<5	<5
Cucumbers, capsicums, chokos and chillies	5	6	6	5	5
Broccoli, broccoflower and cauliflower	5	5	<5	5	5
Root vegetables (starchy)	7	8	9	5	5
Stalk and stem vegetables	<5	<5	5	7	7
Lettuce	<5	6	11	9	10
Green leafy vegetables (cooked)	<5	<5	5	5	6
Stone fruits (fresh) and persimmon	<5	<5	<5	5	5
Wines and fortified wines	<5	<5	<5	10	11
All other foods	32	35	35	27	27

Note: 'nd=0' scenario shown
* derived using the Australian 1995 National Nutrition Survey
¤ derived using the Australian 2007 Children's National Nutrition and Physical Activity Survey
⌘ derived using a model diet

Table A20: Major contributing foods to total sodium nitrate dietary exposures for infants aged 9 months

Food Group	Contribution (%)
Bananas and plantains	11
Water (bottled/ plain mineral/ soda)	11
Pork (except bacon) and deli meats (except frankfurts and poultry-based)	10
Pumpkin, squash, marrow and zucchini	9
Berries and jams	7
Root vegetables (starchy)	6
Infant formulas	5
Water (non-bottled)	5
All other foods	36

Appendix 6: Pharmacokinetics and toxicology of nitrate and nitrite

A 6.1 Pharmacokinetics

Summary

Nitrate is rapidly absorbed from the upper gastrointestinal tract (GIT) in humans. Maximum concentrations are observed in the plasma at around 90 minutes, and the half-life of nitrate in plasma is about 5-7 h. When ingested in nitrate-rich vegetables, nitrate is virtually completely bioavailable.

Nitrite administered to human volunteers in drinking water is rapidly absorbed from the GIT with maximum plasma levels seen at around 15 minutes. The plasma half-life is around 30 minutes. Nitrite is rapidly metabolised to nitrate following oral and IV administration. Maximum plasma nitrate concentrations are seen at around 1.5 h.

Nitrite reacts with haemoglobin (Hb) to form methaemoglobin (MetHb). In adult volunteers orally administered average sodium nitrite doses of around 2.4 mg/kg bw, the maximum concentration of MetHb was achieved at 0.8 h, and the half-life of MetHb in the blood was 1.2 h.

Pharmacokinetic studies

van Velzen et al, (2008) investigated the oral bioavailability of nitrate in human adult volunteers following IV administration of sodium nitrate and ingestion of nitrate-rich vegetables. Volunteers (12 subjects; 21-28 years of age; 61.5-91 kg) were treated IV with 500 mg sodium nitrate or orally with 300 g cooked spinach, 300 g raw lettuce or 300 g cooked beetroot. Nitrate and nitrite concentrations were analysed in plasma and pharmacokinetic parameters determined. Two subjects complained of headaches after intravenous infusion of sodium nitrate. No other clinical signs were reported. Pharmacokinetic parameters are shown in Table A21. Nitrate was estimated to be completely bioavailable following ingestion in vegetables. Maximum plasma concentration was observed at 90-100 minutes and the plasma half-life was around 5-7 h. Nitrite concentration was below the limit of detection. Methaemoglobin levels were not determined.

Table A21: Pharmacokinetic parameters (mean ± standard deviation) in human volunteers administered nitrate by IV infusion and in cooked and raw vegetables.

Parameter	IV infusion 365 mg nitrate	Cooked spinach 564 mg nitrate	Raw lettuce 1014 mg nitrate	Cooked beetroot 643 mg nitrate
Dose (mg/kg)	4.9±0.7	7.6±1.1	13.7±2.0	8.7±1.3
C_{max} (mg/kg)	-	19.8±2.2	41.2±6.1	24.8±3.3
T_{max} (h)	-	1.8±0.4	1.5±0.5	1.7±0.5
$T_{1/2}$ (h)	5.0±1.5	5.7±1.5	6.7±1.1	6.1±0.9
V_d (L/kg)	0.30±0.04			
AUC_{0-24} (mg h/kg)	112±20[a]	173±23[a]	353±58	208±32
F (%)	100	98±12[b]	114±14[a]	106±15[a]

[a] n=11, [b] n=10

A recently published study by Hunault et al, (2009) investigated the oral bioavailability of nitrite from drinking water and MetHb formation in adult human volunteers. Nine subjects (20-25 years of age; 62-71 kg) were administered single oral doses of sodium nitrite (0.12

and 0.06 mmol $NaNO_2$/mmol Hb) and an intravenous dose of sodium nitrite (0.12 mmol $NaNO_2$/mmol Hb) in a three-way cross-over trial. Nitrite levels were analysed in the plasma and pharmacokinetic parameters were determined. Nitrate and MetHb concentrations were also determined. Absolute nitrite bioavailability was reported as 98% and 95% at 0.12 and 0.06 mmol $NaNO_2$/mmol Hb, respectively. Clinical signs and symptoms included nausea and headache in some volunteers at the high-dose while only headache (4 subjects) was reported at the low-dose. Notably, the study was not blinded which may have led to over-reporting of clinical signs and symptoms. MetHb concentrations were about 3-4% at the low dose and around 9-10% following the high-dose. Median arterial blood pressure was decreased 14 mmHg and an increase in heart rate of 18 beats per minute was reported after IV infusion of sodium nitrite. Similar results were observed following oral administration of 310 mg sodium nitrite, while no clear treatment-related effects on blood pressure or heart rate were seen at 160 mg sodium nitrite. Pharmacokinetic parameters are summarised in Table A22.

Table A22: Pharmacokinetic parameters in human volunteers administered nitrite by IV infusion or orally in solution

Dose $NaNO_2$	**0.12 IV***	**0.12 Oral***	**0.06 Oral***
	310 mg (290-380)	310 mg (290-380)	160 mg (140-190)
Plasma nitrite			
C_{max} (mg/kg)	-	2.7 (1.3-3.6)	1.6 (0.8-2.4)
T_{max}(min)	-	15 (15-45)	15 (15-30)
$T_{1/2}$ (min)	21 (23-40)	27 (22-42)	35 (18-54)
$AUC_{0-\infty}$(mg h/L)	2.7 (2-3)	2.5 (2-4)	1.3 (0.8-2.0)
Plasma nitrate			
C_{max} (mg/kg)	13.9 (11-17)	13.9 (9–17)	6.7 (6–8)
T_{max}(min)	1.8 (1.2–2.5)	1.5 (1–2)	1.5 (1–3)
$T_{1/2}$ (h)	8.1 (6–12)	8.8 (6–10)	13.3 (4–19)
$AUC_{0-\infty}$(mg h/L)	135 (110–281)	167 (107–204)	119 (38–184)
Methaemoglobin			
C_{max} (mg/kg)	9.8 (8–12)	8.9 (8–11)	3.8 (3–4)
T_{max}(h)	1.2 (1.0–1.3)	1.0 (0.8–1.5)	0.8 (0.5–1)
$T_{1/2}$ (h)	1.8 (0.9–2)	1.6 (0.8–2)	1.2 (0.6–2)
$AUC_{0-\infty}$(mg h/L)	33 (26–42)	27 (21–36)	8.9 (7–11)

*0.12 and 0.06 mmol $NaNO_2$/mmol Hb
Results expressed as median and range (in parentheses)

Adult volunteers (14 subjects) were administered beetroot juice (500 mL) containing nitrate at around 2.1-2.8 g/L. Nitrite in the beetroot juice was below the level of detection (< 50 nmol/L). Plasma nitrate levels increased around 16-fold with maximum concentrations (*ca* 400 µmol/L) seen at 1.5 h. Levels of nitrite in plasma were also significantly increased (2-fold) to approximately 0.6 µmol/L after ingestion of beetroot juice with a maximum observed at 3 h. Systolic blood pressure was decreased by 10 mm Hg at 2.5 h. Peak differences in diastolic (*ca* 8 mm Hg) and mean arterial pressure (*ca* 8 mm Hg) were seen 3 h after ingestion. Spitting blocked the rise in plasma nitrite and the decrease in systolic blood pressure (Webb et al, 2008).

In a separate study, sodium nitrite was infused, sequentially to human volunteers at doses of 0, 7, 14, 28, 55 and 110 µg/kg per minute for 5 minutes. After each dose was infused, blood was taken to measure nitrite and nitrate in plasma and whole blood. Blood pressure, heart rate and MetHb were also determined. Then, the next dose of nitrite was initiated. Plasma nitrite and intraerythrocytic nitrite increased from 0.13 to 26.1 µmol/L and 0.29 to 34.9 µmol/L respectively during nitrite infusion. Iron nitrosyl haemoglobin increased from 0.14 to 7.9 µmol/L following infusion at 110 µg/kg. Decreases in mean arterial blood pressure from 97 to

86 mm Hg were observed. Heart rate was increased slightly but not significantly (68 to 76 bpm) following exposure at the high-dose. Plasma nitrate increased from approximately 18 µmol/L to 64 µmol/L at the end of the infusion period then decayed with an estimated half-life of 6 h. Whole blood MetHb levels increased from baseline of 0.7% to 3.2% with highest levels observed about 20 minutes after cessation of infusion {Dejam et al, 2007). A total calculated dose of 1.07 mg/kg bw sodium nitrite over a 30 minute period administered IV resulted in maximum MetHb levels of around 3%.

A 6.2 *Reduction of nitrate to nitrite in saliva*

Summary

A number of studies have shown high levels of nitrate in the saliva following administration of nitrate to human volunteers. High salivary nitrate concentrations occur via active uptake of systemically absorbed nitrate by the salivary glands and secretion into saliva. A quantitative estimate is that about 25% of absorbed nitrate is secreted back into the saliva (Eisenbrand et al, 1980; Spiegelhalder et al, 1976; Tannenbaum et al, 1976).

A proportion of nitrate in the saliva is reduced to nitrite by commensal bacteria located mainly at the base of the tongue (reviewed in McKnight et al., 1999). There appears to be a direct relationship between salivary nitrate and nitrite concentrations, and nitrate ingested by individuals in the diet, such that about 20% of salivary nitrate is reduced to nitrite in the oral cavity (Eisenbrand et al, 1980; Spiegelhalder et al, 1976; Tannenbaum et al, 1976).

Therefore, overall, about 6.3% of ingested nitrate on a molar basis, or 5% by mass, of ingested nitrate is converted to nitrite in the saliva of humans (National Academy of Sciences, 1981). In many cases, endogenous nitrate reduction to saliva may be the primary source of nitrite exposure in humans, because dietary nitrate exposure in the diet far exceeds that of nitrite.

Studies investigating reduction of nitrate to nitrite in saliva

Spiegelhalder et al, (1976) measured concentrations of nitrate and nitrite in saliva of 11 adult volunteers for up to 7 h following administration of 30-550 mg of nitrate in vegetable juice or spinach. Mean nitrate concentrations in saliva increased from approximately 70 ppm to around 450 ppm 1 h following ingestion of 450 mg nitrate in red-beet juice. Nitrite concentrations increased from around 9 ppm to 100 ppm at the same time point. There was a direct correlation between the amount of nitrate ingested and the amount of nitrate and nitrite in saliva. It was estimated that about 20% of nitrate in the oral cavity is reduced to nitrite.

Cortas and Wakid, (1991) administered sodium nitrate in distilled water orally to five subjects at a dose of 40 mg/kg bw. Blood and saliva were collected at 10, 20, 40, 60 and 180 minutes and measured for nitrate and nitrite concentration. In addition, urine was obtained and renal clearance estimated. No clinical signs were observed. Nitrate was rapidly absorbed with a peak plasma concentration of 1.8 mM observed at about 40 minutes. Erythrocyte nitrate concentrations were approximately 2/3 of the levels observed in the plasma. In saliva, nitrate and nitrite concentrations prior to dosing were 23 and 50 µmol/L respectively. Nitrate concentrations increased to approximately 15 mmol/L with a peak observed within 30-60 minutes, while salivary nitrite concentrations were continuing to increase at 3 h at which time concentrations were about 5 mmol/L (graphed data). Nitrate concentrations in saliva were estimated to be approximately 9 times that observed in plasma suggesting that salivary glands can actively secrete against a concentration gradient. Mean clearance in the urine was 26 mL/min. Nitrite was below the reported limit of detection (0.1 µmol/L) in all fluids except saliva.

In human volunteers (five subjects), salivary nitrate and nitrite concentrations reached a maximum concentration 30-60 minutes after consuming oral doses of potassium nitrate of 25, 50, 100 or 170 mg. Not all doses were examined in each subject but the data presented

suggest a wide variability between individuals. Urinary nitrate concentration increased to reach a maximum value 4-6 h after dosing, thereafter declining to baseline levels within 24 h. About 70% of the administered nitrate dose was recovered in the urine within 24 h of the nitrate challenge. Faecal samples contained low levels of nitrate (<5 mg) (Bartholomew and Hill, 1984).

In 14 volunteers administered 240 mg nitrate (expressed as sodium nitrate) in celery juice, salivary nitrite concentration reached a maximum concentration of between 300 and 400 ppm at 2 h and then decreased over 24 h to reach baseline levels. Doses of 60 mg sodium nitrate were also generally sufficient to cause large increases in salivary nitrite concentrations (Tannenbaum et al, 1976).

Hart and Waters, (1983) investigated the formation of nitrite in saliva *in vitro* and *in vivo*. Saliva from 10 subjects was incubated at 37 ˚C after the addition of 16 mM-sodium nitrate solution and aliquots were removed at 0, 20, 40, 60 and 80 minutes for determination of nitrite. Incubation of sodium nitrate with saliva led to a linear increase in nitrite concentration for about 80 minutes. A 25-fold difference was observed between the highest and lowest rates of nitrite production from the ten subjects. In *in vivo* studies, samples of saliva were given by volunteers immediately before and 1 h after ingesting 16 or 64 mg sodium nitrate in 250 mL of water. There was no clear increase in the rate of nitrite formation in volunteers that ingested 16 mg sodium nitrate in drinking water. Subjects given 64 mg sodium nitrate showed definite increases in the rate of nitrite formation. An approximate 5-fold variation was observed between volunteers. Nitrate concentration in the saliva was not determined and other time points were not investigated.

The effect of a nitrate-rich meal on nitrate and nitrite concentrations in saliva, plasma and urine was investigated in human subjects by Pannala et al, (2003). After a low nitrate diet on Day 1, healthy volunteers (15 males and 14 females; 20-50 years of age) were given a meal that included about 120 g of a commercially grown greenhouse lettuce on 3 consecutive days. The dietary exposure to nitrate was estimated to be about 222 mg per meal while dietary nitrite exposure was stated to be less than 1 mg. Salivary nitrate concentrations increased markedly (from 0.3 to 2.6 mmol/L) 2 h after ingestion of a nitrate-rich meal. Nitrite concentrations in saliva increased from *ca* 0.3 mmol/L to 1.8 mmol at 2 h. Levels of nitrate and nitrite remained relatively constant for at least 5 h after meal ingestion. Plasma nitrate concentration was increased about 7-fold at (*ca* 26 umol/L *cf* 179 umol/L) at 1 h after ingestion of nitrate-rich meals, however there was no significant change in plasma nitrite levels (78 nmol/L *cf* 90 nmol/L). Urinary nitrate excretion increased from approximately 50 mg on Day 1 to around 220-250 mg on other study days. The rate of elimination of nitrate in urine peaked between 4-6 h after exposure and returned to baseline within 24 h. A small (approximately 2-fold) but significant increase was also seen in urinary nitrite elimination on days 2 to 4.

Eisenbrand et al, (1980) investigated the nitrite concentrations in saliva of humans of different age groups. It was found that nitrite concentrations increased with age being 0.6, 1.2, 3.7, 3.7, 4.2 and 6.8 ppm for 1 week to 6 month old infants, 7-12 month infants, children 1-6 years, children 7-9 years, adolescents 10-15 years and adults, respectively. The number of samples in each group ranged between 14 to 117. The authors noted that despite low nitrite concentrations in the two youngest groups, the saliva of these groups consistently contained nitrate, in some cases at concentrations of up to 250 ppm.

Lundberg et al, (2004) observed an increase in plasma nitrite levels in a study in which 10 mg/kg bw nitrate in 100 mL water was orally administered to nine volunteers (26-46 years, 5 males and 4 females). No clinical signs were reported. At 30 minutes, nitrate concentrations in saliva increased from 0.19 to 8.2 mM while nitrite concentration had increased from 104 µM to 713 µM. At the same time point, plasma nitrate had increased from 30 to 432 µM. Plasma nitrite concentrations also increased from 123 nM to 392 nM over a 90 minute period. Avoiding swallowing clearly inhibited the increase in plasma nitrite concentrations.

Nitrate levels increased in urine but there were no significant effects on urinary nitrite concentration.

An oral dose of 3.5 mmol ^{15}N-labelled sodium nitrate in distilled water was administered to human volunteers and samples of urine, saliva, plasma were collected for 48 h. Faecal samples were collected for 3 d. An average of 60% of the radiolabel was excreted in the urine within 48 h as nitrate, while less than 0.1% appeared in the faeces. The radiolabel was also recovered in the urine (3%) and faeces (0.2%) as ammonia or nitrate. Approximately 35% of the administered radiolabel remained unaccounted for. The half-life for nitrate was estimated to be 5 h and the volume distribution was approximately 30% of body weight. It was estimated that the endogenous synthesis of nitrate was about 1 mmol/day (Wagner et al, 1983).

In a human subject administered radiolabelled $^{13}NO_3$ orally after a meal, approximately 50% of the label was removed from the stomach within 30 minutes. In a subject that had not eaten for about 10 hours approximately half of the label was removed from the stomach within 10 minutes. At 30-40 minutes 2-3% of the radiolabel was found in the bladder and mouth and salivary glands. Similar results (3-4% of the radiolabel) were seen after intravenous administration (Witter et al, 1979).

McKnight et al, (1997) administered a 50 mL solution containing 2 mmol of potassium nitrate to ten healthy volunteers (six male; four female; mean body weight 69 kg; 21-43 years) who had been fasted overnight. Subjects were observed for 6 h. No adverse effects were reported. Plasma, salivary and gastric nitrate, salivary nitrite and gastric headspace NO concentration were increased. Gastric nitrite concentrations remained low although analytical difficulties were reported. The maximum concentration of NO was seen at approximately 60 minutes (*ca* 90 ppm). The authors suggested that the high concentrations of NO produced in the stomach are likely to inhibit intestinal pathogens.

A 6.3 Species differences in MetHb formation and reduction

A 6.3.1 Species differences in MetHb formation

The rate of formation of MetHb has been shown to vary considerably between animal species *in vitro*. In partially purified haemoglobin solutions incubated with freshly prepared sodium nitrite the rate of MetHb was faster for ruminants (sheep, goats, cows) than in humans, horses and pigs (Smith and Beutler, 1966).

Methaemoglobin formation was investigated in erythrocytes from rabbit, cat, dog, ox and man for 60 minutes following incubation with 2.5 mmol sodium nitrite. Results were presented in the form of graphs. At 60 minutes, the percentage of MetHb ranged from approximately 35-55% in erythrocytes from cat, dog, ox and man, while the MetHb levels in rabbit erythrocytes were only approximately 10%. Methaemoglobin formation was also observed in rats, rabbits and cats following an IV dose of 0.3 mmol/kg sodium nitrite. A maxima of 18% was seen in rats at 30 minutes, while respective values were 8% in rabbits at 10 minutes, and 48% in cats at 90 minutes (Klimmek et al, 1988).

A 6.3.2 Species differences in MetHb reduction

The rate of reduction, catalyzed by MetHb reductase, also varied between species, with some correlation observed between MetHb formation and reduction rates. That is, while MetHb formation was faster in ruminants the rate of MetHb reductions was also highest in these species (Smith & Beutler, 1966).

Rockwood et al, (2003) investigated methaemoglobin reductase activity in seven non-human primate species as well as dogs and ferrets. In comparison to humans, MetHb reductase

activities were generally slightly higher in blood samples from non-human primates and ferrets, and lower in dogs. Rhesus monkey was the most similar to humans.

Power et al, (2007) investigated methaemoglobin reductase activity in intact fetal and adult sheep and in human blood. MetHb disappearance followed first order kinetics with specific rate constants of 12.9 ± 1.3 min^{-1} for fetal sheep, 5.88 ± 0.26 min^{-1} for adult sheep, 4.27 ± 0.34 min^{-1} for adult humans, and 3.30 ± 0.15 min^{-1} for newborn cord blood. The half-life of methaemoglobin was about 54, 135, 201 and 162 minutes in blood of fetal sheep, adult sheep, newborn infants and adult humans, respectively.

A 6.4 Toxicity

A 6.4.1 MetHb levels in adults and infants following administration of nitrate in solution, or in food.

A pilot study was conducted in eight healthy volunteers that received a single oral dose of 10 mg/kg bw sodium nitrate. Samples of gastric juice, plasma, saliva and urine were analysed for nitrate and nitrite and the percentage methaemoglobin was also measured. No clinical signs were reported and blood pressure and methaemoglobin were unaffected. The half-life of nitrate was estimated to be 6.5 h and 70% of the nitrate dose was eliminated in the urine with 10 h of dosing. The amount of nitrate excreted in the saliva was estimated to be 28% of the dose over 24 h. Approximately 8% of the nitrate administered was estimated to be present in saliva as nitrite. Nitrite concentration in the plasma was not increased after nitrate administration. Omeprazole (which increases gastric pH) did not affect nitrate concentrations in plasma, saliva or gastric juice, the nitrite concentrations in plasma or saliva, the per cent methaemoglobin or blood pressure (Colbers et al, 1996; abstract only).

Sodium nitrate was administered by oral solution to healthy volunteers (10 subjects; 20-30 years maintained on low nitrate diets) at 15 mg/kg bw for 28 days. Blood samples were taken on days 0, 7, 28 and at pre- and post-study screening for MetHb analyses. For nitrate and nitrite analyses, blood samples were taken at the pre- and post-study screening and twice weekly during the study. No clear treatment-related effects were reported. Plasma nitrate concentrations were generally slightly increased compared to day 0 values and controls. A clear relationship to nitrate intake was not evident for plasma nitrite though this may have been influenced by the quantification limit for nitrite which was 0.2 mg/kg. There was no elevation in the percentage of MetHb. Blood sampling times were not reported (Lambers et al, 2000).

In an early study, Cornblath and Hartmann, (1948) reported that oral treatment of four infants, aged 11 days to 11 months, for periods of two to eighteen days with 50 mg/kg bw/day nitrate ion increased MetHb levels to a maximum of 5.3% in the absence of cyanosis. When the dose was doubled to 100 mg/kg bw/day and fed to four infants ranging from 2 days to 6 months, of age over six to nine days, the maximum level of MetHb observed was 7.5% in one infant (10 days of age, after administration of nitrate solution for eight days). No cyanosis was evident. In 6-7 week old infants previously cyanotic due to ingestion of well water, the maximum MetHb concentration was 11% following administration of 100 mg nitrate/kg bw/day, and there was evidence of cyanosis though it was not marked. Individual details including the number of infants and days of treatment were not provided. A number of other experiments were carried out in attempt to determine the role of well water bacteria in inducing methaemoglobinaemia in infants. The authors concluded that bacteria from well water, both pathogenic and non-pathogenic, which can survive in the stomach and upper small intestine can convert nitrate to nitrite. The characteristic low acidity of gastric juice in neonates may favour such conditions and contribute to the sensitivity of infants to nitrate.

Infants (aged 3.5 to 8 months) fed spinach (200 g) with about 680 mg/kg nitrate to provide an intake of between 16.5 to 21 mg/kg bw/day nitrate, showed no clear treatment related

effects on MetHb levels compared to infants who ingested low nitrate vegetables (14 mg/kg) for one week. Six of the infants showed no increase in MetHb levels while one subject showed a slight increase, but this infant had the highest levels prior to the consumption of the nitrate-rich spinach (Kubler, 1958). Results are shown in Table A23.

Table A23: MetHb concentration in infants that consumed low and high nitrate containing vegetables for one week.

Subject	Age in months	Body weight (kg)	MetHb % before treatment	MetHb % High nitrate	MetHb % Low nitrate
1	5	7.3	0.6	0.2	1.2
2	8	8.0	0.2	0.2	0.4
3	8	7.2	0.1	0.6	0.8
4	4	8.2	0.8	0.8	0.8
5	6	7.3	0.3	0.05	1.0
6	3.5	5.9	2.6	3.4	1.0
7	5	6.5	0	0.2	0
Mean	**5.7**	**6.7**	**0.7**	**0.8**	**0.7**

A 6.4.2 MetHb formation following exposure to nitrate in drinking water

No significant difference in MetHb levels were seen between 62 children (aged 1-8 years) consuming high nitrate well water (*ca* 100–500 mg/L nitrate ion) and 37 children consuming low-nitrate water (43 mg/L nitrate ion). Mean MetHb levels for the low- and high-exposure groups were 0.98 and 1.13%, respectively (Craun et al, 1981). Water consumption was reported to be around 1.2 L per day which corresponds to a mean group nitrate exposure of approximately 2.6 mg/kg bw/day in the low exposure group and 6-30 mg/kg bw/day (average 18 mg/kg bw/day) at the high-exposure assuming a mean body weight of approximately 20 kg.

Simon et al. (1964) summarised 745 cases of infant methaemoglobinaemia due to nitrate-contaminated water in Germany between 1956 and 1964. Virtually all cases (97%) of methaemoglobinaemia were associated with water from private wells, which in 84% of cases contained nitrate concentrations over 100 mg/mL. Infants were almost all under the age of 3 months (98%), 53% had diarrhoea and 8.6% died. Infants above the age of 3 months had low MetHb levels irrespective of the concentration of nitrate in the drinking water. Average MetHb levels were 0.8, 0.7, and 0.7% in groups of infants aged greater than 3 months that received water containing 0 (considered to be nitrate free by the authors), 50-100 and > 100 mg/L nitrate (Simon et al, 1964).

Shuval et al, (1972) studied infants in communities with low (5 mg/L) and high concentrations (50–90 mg/L) of nitrate in drinking water in Israel. There were no significant differences in MetHb levels for infants aged more than 3 months of age in the low nitrate area (MetHb 0.97%) or high-nitrate area (0.99%). In addition, there were no significant differences in MetHb levels in other age groups including infants aged 1-60 days. Assuming a water intake of 150 mL/kg bw per day, nitrate exposures in the two groups were estimated to be about 0.75 and 10 mg/kg bw/day (using a mean of 70 mg/L nitrate in the high exposure group).

Toussaint and Selenka, (1970) supplied healthy infants (age 1-3 months) with infant formula prepared with water containing 150 mg nitrate/L (corresponding to about 22.5 mg/kg bw/day

nitrate), or 2, 8, 15, 25 and 35 mg/L nitrite (corresponding to about 0.3, 1.2, 2.2, 3.7 and 5.2 mg/kg bw/day nitrite), for 10 days. In nitrate treated infants, MetHb levels increased to 2-3% within 1-2 days and remained elevated over the 10 day period. Exposure to nitrite in infant formula at 2 and 8 mg/L was not associated with increased MetHb levels. At 25 mg/L and 35 mg/L nitrite, MetHb levels increased to 3-4% and remained relatively steady throughout the dosing period.

Hegesh and Shiloah, (1982) argued that acute diarrhoea could be the determining factor in many cases of methaemoglobinaemia in infants rather than nitrate exposure after observing high nitrate levels occur in blood and urine as a regular part of the syndrome. In a study of 58 infants, MetHb levels 0.4 to greater than 8% were observed in infants with acute diarrhoea with levels generally correlated to blood nitrate levels. Results are shown in Table A24.

Table A24: MetHb and nitrate concentrations in blood of infants with acute diarrhoea

MetHb %	Infants (n)	Mean and 95% confidence interval for nitrate levels in blood (µmol/L)
<0.4	3	71 (36-97)
0.4-0.8	15	130 (31-250)
0.8-2.0	17	142 (39-343)
2.0-4.0	6	244 (162-398)
4.0-8.0	5	286 (135-459)
>8.0	12	604 (234-1270)

A 6.4.3 Case reports of methaemoglobinaemia following exposure to nitrate or nitrite

Bradberry et al, (1994) reported an unusual case of methaemoglobinaemia caused by the accidental contamination of drinking-water with sodium nitrite. The patient had a MetHb concentration of 49%. The amount of sodium nitrite ingested was estimated to be 0.7 g.

Ten adults suffered moderate to severe methaemoglobinaemia following accidental ingestion of a nitrite salt, rather than table salt. All patients were cyanotic and tachypnoeic while other clinical signs included altered level of consciousness, hypotension and tachycardia. MetHb levels in 2 of the most severe cases were 79 and 71%. One patient died in Casualty while others recovered fully following treatment with methylene blue and ascorbic acid. No exposure estimates were available (Kaplan et al, 1990).

Five cases of methaemoglobinaemia (21 %-57 %) after ingestion of sodium nitrite occurred in Sydney in 2006. All cases were unintentional poisonings. Blood levels of nitrite were not reported, and no estimate of exposure was available (Maric et al, 2008).

Keating et al, (1973) reported cyanosis in a 2-week old male infant following consumption of 500 mL of carrot juice estimated to result in an exposure of approximately 104 mg/kg bw nitrite and 70 mg/kg bw nitrate. MetHb was estimated to represent about 60% of total haemoglobin.

Three cases of methaemoglobinaemia were reported in 18-41 year old adults (two male, one female) following the ingestion of meat containing 15,000 ppm nitrite or 10,000 ppm nitrite. MetHb concentrations ranged from 7.7% to 66%. Patients were treated with oxygen and in one case, methylene blue. All patients recovered. No exposure estimates were made (Walley and Flanagan, 1987).

Sanchez-Echaniz et al, (2001) retrospectively reviewed data from 7 infants diagnosed with acquired methaemoglobinaemia attributed to consumption of mixed vegetables between 1993 and 1998 in Spain. The infants (mean age *ca* 8 months) were fed homemade purees of mixed vegetables that were prepared in advance and kept in the refrigerator for 12 to 27 h. All cases were associated with silver beets; other vegetables included green beans, pumpkin, potatoes, carrot, spinach and leek. Patients presented with cyanosis, irritability, vomiting and in one case tachypnoea. MetHb ranged from between 10-58%. No actual nitrate or nitrite intake data were available, however the authors noted that silverbeets in the area had particularly high levels of nitrate (mean 3200 mg/kg).

Finan et al, (1998) reported life threatening methaemoglobinaemia associated with sodium nitrite exposure in 4 year old twin boys and a two year old girl. The children had consumed milky tea contaminated with sodium nitrite at concentrations of 4940 – 5100 mg/L. The volume of tea consumed was not reported. MetHb concentration for two cases were reported as 38% and 77%. All three children were deeply cyanotic and recovered fully following treated with methylene blue.

A 6.5 *Acute toxicity*

A 6.5.1 Nitrate

The acute oral toxicity of nitrate in laboratory animals is generally low with LD_{50} values in the range of 2500-6250 mg/kg bw in mice, 3300-9000 mg/kg bw in rats and 1900-2680 mg/kg bw in rabbits. A lower oral LD_{50} of 300 mg/kg bw was reported in pigs (Walker, 1990).

Estimates of human oral lethal doses ranged from 67 – 833 mg nitrate ion/kg bw however results in the literature contained little experimental detail and are sometimes contradictory (WHO, 1995a). JECFA considered that the oral lethal dose of nitrate in adult humans is probably around 20 g nitrate ion or 330 mg nitrate ion/kg bw, most similar to the pig (WHO, 1995a).

A 6.5.2 Nitrite

The oral LD_{50} for sodium nitrite in mice was between 175-220 mg sodium nitrite/kg bw and 85 mg/kg bw for rats (WHO, 1995b).The oral lethal dose for humans was estimated to be between 2-9 g representing 33 to 250 mg nitrite/kg bw, the lower doses applying to children and elderly people (Corre and Breimer 1979).

A 6.6 Short term toxicity in laboratory animals

A 6.6.1 Nitrate

A number of early short term toxicity studies in nitrate exposed mice, rats, rabbits and dogs were reviewed by JECFA (Speijers, and van den Brandt, 2002; WHO, 1995a). The main findings were consistent with elevated MetHb levels including reduced body weight gain, clinical signs (tachycardia and weakness) and an abnormal colour of the blood and spleen.

A 6.6.2 Nitrite

A 6.6.2.1 Mice

Groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite (equivalent to average daily doses of approximately 90, 190, 345, 750, or 990 mg/kg to males and 120, 240, 445, 840, or 1,230 mg/kg to females) in drinking water for 14 weeks. No treatment-related mortality or clinical signs were reported. Body weights of males exposed to 5,000 ppm were significantly less (*ca* 10%; $p \leq 0.01$) than those of the controls; body weight gain of males was significantly decreased at 3,000 and 5,000 ppm (17-28%; $p < 0.05$).

In males, absolute and relative spleen weight was increased at 5000 ppm and relative spleen weight was increased at 3000 ppm. Spleen weight (absolute and relative) was also increased in females at 3000 and 5000 ppm. In females, liver weight (absolute and relative) was increased at 3000 ppm and 5000 ppm. Absolute and relative heart weights were significantly increased at 5000 ppm in females. Absolute kidney weight was increased at 3000 and 5000 ppm, relative kidney weight was increased at 5000 ppm in females.

Percentage sperm motility in 5,000 ppm males was significantly lower (*ca* 47%) than controls and estrous cycle length was significantly increased in females at 1,500 (11%) and 5000 ppm (15%). There were no significant effects on these parameters at 375 ppm (effects on these parameters at 750 ppm were not investigated).

Microscopic examination of the forestomach showed increased incidences of minimal to mild squamous cell hyperplasia (focal) at the limiting ridge in 5,000 ppm males and females. Incidences of extramedullary haematopoiesis were increased in the spleen at 3000 and 5000 ppm in males and at ≥1500 ppm in females. An increased incidence of degeneration of the testes characterised by an increase in the size of residual bodies within the seminiferous tubules was seen at 3000 and 5000 ppm. The residual bodies were large and spherical with glassy eosinophilic staining and a basophilic core.

On the basis of this study, sodium nitrite exposure concentrations selected for use in the subsequent 2-year drinking water study in mice were 750, 1,500, and 3,000 ppm (National Toxicology Program, 2001).

A 6.6.2.2 Rats

Male and female F344/N rats (10 sex/group) were exposed to 0, 375, 750, 1,500, 3,000, or 5,000 ppm sodium nitrite (equivalent to average daily doses of approximately 30, 55, 115, 200, or 310 mg sodium nitrite/kg body weight to males and 40, 80, 130, 225, or 345 mg/kg to females) in drinking water for 14 weeks. One female in the 3,000 ppm group died. Treatment-related clinical findings included brown discoloration in the eyes and cyanosis of the mouth, tongue, ears, and feet of males exposed to 3,000 or 5,000 ppm and of females exposed to 1,500 ppm or greater. Body weight was significantly decreased at ≥ 3,000 ppm in males (6-15%) and 5000 ppm in females (5%). Body weight gain was significantly decreased at ≥3,000 in both males (14-25%) and females (16-25%).

Absolute (15-23%) and relative (23-38%) spleen weight was increased in females at 3,000 and 5,000 ppm. MetHb concentrations were significantly increased in all exposed groups of male and female rats. The percentage of methaemoglobin ranged from 0.2 – 1.7% (control), 0.3-1.3% (375 ppm), 0.8-32.1% (750 ppm), 0.6-12.9% (1,500 ppm), 4.5-26.6% (3,000 ppm) and 2.8-47.9% (5000 ppm) dependent upon sampling day and time (Tables A24 and A25). Individual animal data were not available and the large variation in results for MetHb at 750 ppm was not explained.

Table A24: MetHb concentration at Days 5 and 19, and Week 14 (values in parentheses are methaemoglobin as a percentage of haemoglobin)

MetHb (g/dL)	0	375 ppm	750 ppm	1,500 ppm	3,000 ppm	5,000 ppm
			Male			
Day 5	0.03 ± 0.02 (0.21%)	0.04 ± 0.02 (0.28%)	4.36 ± 0.78**[c] (32.06%)	0.08 ± 0.03** (0.58%)	1.37 ± 0.41**[e] (9.65%)	3.97 ± 0.75** (28.77%)
Day 19	0.09 ± 0.05 (0.64%)	0.06 ± 0.02 (0.4%)	0.28 ± 0.12* (1.85%)	0.38 ± 0.12** (2.59%)	1.25 ± 0.37** (8.17%)	3.26 ± 0.36** (26.08%)
Week 14	0.03 ± 0.01 (0.20%)	0.08 ± 0.01** (0.5%)	0.12 ± 0.02** (0.8%)	0.25 ± 0.07** (1.70%)	0.71 ± 0.20** (4.52%)	3.38 ± 0.80** (21.13%)
			Female			
Day 5	0.02 ± 0.01 (0.13%)	0.10 ± 0.05* (0.68%)	0.05 ± 0.01 (0.32%)	0.21 ± 0.08** (1.36%)	2.41 ± 0.76** (15.75%)	4.95 ± 0.92** (32.57%)
Day 19	0.03 ± 0.01 (0.19%)	0.11 ± 0.03* (0.72%)	0.18 ± 0.04** (1.18%)	2.01 ± 0.49** (12.88%)	3.78 ± 0.79** (23.77%)	6.66 ± 0.36** (47.91%)
Week 14	0.06 ± 0.02 (0.39%)	0.14 ± 0.02** (0.93%)	0.16 ± 0.02** (1.09%)	0.48 ± 0.05**[c] (3.2%)	0.99 ± 0.20** (6.11%)	2.27 ± 0.54** (12.97%)

Values represent mean ± standard deviation
*p ≤ 0.05, **p ≤ 0.01

Table A25: MetHb at Days 70 and 71, sampled at different timepoints, of a 14-week drinking water study in rats (values in parenthesis are methaemoglobin as a percentage of haemoglobin)

MetHb (g/dL)	0	375 ppm	750 ppm	1,500 ppm	3,000 ppm	5,000 ppm
			Male			
Day 70 (at 2000 hours)	0.05 ± 0.02 (0.36%)	0.09 ± 0.02[a] (0.6%)	0.12 ± 0.02* (0.81%)	0.44 ± 0.17** (2.9%)	0.47 ± 0.27** (2.92%)	0.54 ± 0.09** (3.70%)
Day 70 (at 2200 hours)	0.02 ± 0.01 (0.13%)	0.05 ± 0.01 (0.32%)	0.07 ± 0.01** (0.46%)	0.14 ± 0.00**[b] (0.92%)	0.70 ± 0.48** (4.19%)	0.64 ± 0.23** (4.21%)
Day 71 (at 0900 hours)	0.03 ± 0.01 (0.19%)	0.09 ± 0.01** (0.58%)	0.10 ± 0.03* (0.65%)	0.54 ± 0.23** (3.6%)	3.50 ± 0.68**[a] (22.01%)	5.13 ± 1.40** (35.6%)
			Female			
Day 70 (at 2000 hours)	0.04 ± 0.02 (0.25%)	0.21 ± 0.08* (1.36%)	0.39 ± 0.12** (2.58%)	0.60 ± 0.11** (3.80%)	0.57 ± 0.20** (3.26%)	2.76 ± 0.75**[a] (17.81%)
Day 70 (at 2200 hours)	0.27 ± 0.09 (1.72%)	0.12 ± 0.02[a] (0.77%)	0.26 ± 0.06[a] (1.69%)	0.26 ± 0.05 (1.65%)	0.50 ± 0.10 (2.98%)	0.44 ± 0.07[b] (2.84%)
Day 71 (at 0900 hours)	0.09 ± 0.02 (0.57%)	0.19 ± 0.05* (1.25%)	1.16 ± 0.30** (7.34%)	1.06 ± 0.09** (6.97%)	4.39 ± 0.79** (26.61%)	3.32 ± 1.78**[a] (19.30%)

Values represent mean ± standard deviation
*p ≤ 0.05, **p ≤ 0.01
[a] n=4, [b] n=3

Other red blood cell changes generally indicative of regenerative anaemia were observed at higher doses. An apparent decrease in the erythron was evident at early time points. Haemoglobin was significantly decreased at 5000 ppm at Day 19 in males and females. There were also decreases in haematocrit (*ca* 10%) at 5000 ppm in males and females at the same time point but the results were not statistically significant. Reticulocytes were increased at ≥ 3,000 ppm in males and from 1500 ppm in females, and increases in nucleated erythrocyte counts were seen in 3,000 and 5,000 ppm males and 5,000 ppm females.

The decrease in the erythron appeared transient and was replaced by an increased erythron at week 14 at which times increased haemoglobin concentration was seen for 3000 and 5000 ppm males and females. Increased haematocrit was seen for 3000 ppm females and 5000 ppm males and females, and erythrocyte counts were increased in 5000 ppm females. Reticulocyte counts were increased at Week 14 at 3000 and 5000 ppm for males and at 5000 ppm for females.

Percentage sperm motility was significantly decreased at 1500 ppm (7%) and 5000 ppm (18%). No effects on sperm motility were seen at 375 ppm – other doses were not assessed.

No abnormal microscopic changes were seen in the spleen. Bone marrow showed increased incidences of erythropoietic activity in males (5/10, 5/10, 5/10, 8/10, 8/10, 9/10) and females (1/10, 0/10, 1/10, 3/10, 7/10, 10/10) consistent with the hematologic findings of regenerative anaemia. The incidence of squamous cell hyperplasia of the forestomach, characterised by thickening of the squamous epithelium of the forestomach and hyperkeratosis, was increased at 5000 ppm in males and females. The severity was graded as minimal in males and mild in females. The total nitrosamine concentrations in the blood were not significantly different from controls.

On the basis of this study, doses selected for the subsequent 2-year study of sodium nitrite in drinking water were 750, 1,500 and 3,000 ppm. The NOAEL was 375 ppm equivalent to 40 mg/kg bw/day on the basis of elevated MetHb levels at 750 ppm (National Toxicology Program, 2001).

A 6.7 Chronic toxicity/Carcinogenicity

A 6.7.1 Nitrate

There was no evidence that nitrate is carcinogenic in mice or rats when administered in the feed or drinking water (WHO 35). JECFA concluded that overall, epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate (WHO 50). IARC also concluded that for nitrate in food or drinking water there is “inadequate evidence of carcinogenicity (Grosse et al., 2006). The European Food Safety Authority CONTAM Panel concurred with these conclusions noting that the evidence does not support that nitrate intake from drinking water or diet, is associated with increased cancer risk (EFSA, 2008).

A 6.7.2 Nitrite

Recently performed National Toxicology Program carcinogenicity studies on nitrite are summarised in Sections A 6.3.3.2.1 and A 6.3.3.2.2. In mice, there was equivocal evidence of an increase in squamous cell papilloma and carcinoma in the forestomach of female mice at 165 mg/kg bw/day. Nitrite was not carcinogenic in male mice at doses of up to 220 mg/kg bw/day or in male or female rats at doses of up to 150 mg/kg bw/day. JECFA considered that results of epidemiological studies with nitrite did not provide evidence that nitrite is carcinogenic to humans. The IARC opinion concluded that there is ‘limited evidence for carcinogenicity’ for nitrite in food on the basis of an association with stomach cancer (Grosse et al., 2006). In reviewing available epidemiological data since the JECFA evaluation, the EFSA CONTAM Panel concluded that the evidence that high nitrite intake might be associated with increased cancer risk is equivocal.

A 6.7.2.1 Mice

In an NTP study, male and female mice were administered 0, 750, 1,500, or 3,000 ppm sodium nitrite in drinking water for two years, approximately equivalent to doses of 60, 120, or 220 mg/kg bw/day for males and 45, 90, or 165 mg/kg bw/day for females. There was no treatment-related effect on survival rate and there were no clinical signs attributable to treatment. Body weight was decreased at 3000 ppm in female mice. Blood MetHb concentration was similar to controls in all treated groups at 12 months. Hyperplasia of the squamous epithelium of the forestomach was observed more frequently in 3,000 ppm females than in the controls. The incidences of squamous cell papilloma (0/50, 0/50, 1/50, 3/50) and squamous cell carcinoma (0/50, 0/50, 0/50 and 2/50) were increased at 3000 ppm in females. The combined (squamous cell papilloma and squamous cell carcinoma)

incidence of 10% was outside the historical control range (up to 4%) for forestomach neoplasms. Hyperplasia of the glandular stomach was seen at 1500 (2/50; p>0.05) and 3000 ppm (10/50; p<0.05) in male mice. There were no neoplasms in the forestomach or glandular stomach of male mice (National Toxicology Program, 2001).

A 6.7.2.2 Rats

In an a 2-year NTP study, male and female rats (50 sex/group) were administered sodium nitrite in the drinking water at 750, 1500 and 3000 ppm, approximately equivalent to average daily doses of 35, 70 and 130 mg/kg bw/day to males and 40, 80 and 150 mg/kg for females. There was no treatment-related effect on survival rate and no treatment-related clinical signs were reported. Mean body weight was decreased at 3000 ppm throughout the study period. At 2-weeks and 3 months blood MetHb concentration was significantly increased at 1500 and 3000 ppm in male and female rats. No significant increase in blood MetHb was seen at 750 ppm. The incidence of hyperplasia of the squamous epithelium of the forestomach was significantly increased in males and females at 3,000 ppm. The severity was generally minimal affecting the epithelium of the limiting ridge at the junction of the forestomach and glandular stomach. In a few cases severe hyperplasia was seen characterised by folding of the squamous epithelium and hyerkeratosis (National Toxicology Program, 2001).

A 6.7.3 Carcinogenicity of N-nitrosamines

It is known that nitrite and dietary amines can react to form nitrosamines, however, whether endogenous nitrosation takes place under actual food intake conditions in sufficient amounts to pose a risk to human health is uncertain. In considering this issue, the IARC Working Group concluded that “ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (group 2A)”. However the Working Group did not perform a separate assessment for nitrate or nitrite because nitrite is produced endogenously from nitrate, and the conditions leading to endogenous formation of N-nitroso compounds are often found in a healthy human stomach. Similarly JECFA expressed the view that a quantitative risk assessment was not appropriate for nitrite on the basis of N-nitroso compounds, due to a lack of evidence of the endogenous formation of carcinogenic N-nitroso compounds at intake levels achievable in the diet.

A 6.8 Reproductive/developmental toxicity

A 6.8.1 Nitrate

The reproductive and developmental effects of sodium nitrate and potassium nitrate were investigated in mice, rats, hamsters and rabbits. Doses (expressed as mg nitrate-nitrogen) ranged from 0.6-66 mg/kg/day for mice, from 0.3-41 mg/kg/day for rats, from 0.4-66 mg/kg/day for hamsters and from 0.3-41 mg/kg/day for rabbits. There were no significant effects on maternal reproductive parameters, fetotoxicity or malformations at any dose tested. The reproductive and developmental NOAEL was 66 mg nitrate-nitrogen/kg/day for mice and hamsters and 41 mg nitrate-nitrogen/kg/day for rats and rabbits (US EPA, 1991).

A 6.8.2 Nitrite

Sodium nitrite was administered to Swiss mice at concentrations of 0.06%, 0.12% and 0.24% (w/v) in drinking-water (equivalent to daily doses of 125, 260 and 425 mg/kg bw/day, respectively) for a continuous cohabitation phase according to the RACB protocol. Water consumption was decreased 10 to 17% at the high-dose. There were no treatment-related effects on reproductive parameters or pups nursed to weaning. A decrease in pup weight at the high-dose was attributed to decreased milk production in dams associated with a

decrease in water consumption. High-dose and control mice were retained after weaning and examined for reproductive toxicity. F1 fertility and reproductive success was unaffected by sodium nitrite exposure. The NOAEL for reproductive toxicity was 425 mg/kg bw/day sodium nitrite (Chapin et al, 1997).

Rats were given sodium nitrite in drinking water at concentrations of 2000 mg/L or 3000 mg/L equivalent to doses of around 220-420 and 300 – 514 mg/kg throughout gestation and lactation, respectively. Body weight was decreased in dams at both doses at day 20 of lactation. Water intake was decreased in treated dams during gestation and lactation. No significant differences in litter size, mean pup weights or sex ratio was seen in offspring of treated groups. Body weight was lower in pups from treated dams compared to controls from days 6-20 after birth. Pups from treated groups were described as severely anaemic and showed decreased Hb, red blood cell counts and mean corpuscular volume (MCV) compared to controls on Days 9 and 16. MetHb levels were not significantly increased. In a second experiment, rats were administered sodium nitrite in drinking water at 500, 1000 and 2000 mg/L, equivalent to doses of around 72-118, 119-222, and 200-304 mg/kg bw/day, respectively, during gestation and lactation. There were no significant effects on maternal weight gain and no clinical signs were reported in the dams. Water consumption was decreased at the high-dose. There were no significant differences in litter size, birth weight or sex ratio. Pup weight was significantly lower at 2000 mg/L at lactation day 17 and 20. Haemoglobin and MCV were decreased in the pups at 2000 mg/L from lactation day 7 to 20. Methaemoglobin levels were not determined (Roth et al, 1987).

Sodium nitrite was given to pregnant ICR-mice in the drinking water at concentrations of 0, 100 or 1000 mg/litre on days 7-18 of gestation. No teratogenic effects were observed at the doses tested (Shimada et al*.,* 1989).

Pregnant rats were administered 2.5 to 50 mg/kg of sodium nitrite either orally or intraperitoneally and the concentrations of nitrite and MetHb measured in the dam and the foetus for around 2 h. After a single oral dose of 30 mg/kg sodium nitrite, MetHb levels in dams were around 15% at 40 minutes and declined to around 7% by 2 h. MetHb levels were lower in the foetal blood reaching a maximum of around 5% at 60 minutes and declining to around 2% at 2 h (Gruener et al, 1973).

Potassium nitrate was administered to male and female guinea pigs (3-6 per group) in the drinking water at concentrations of 300, 2500,10000 and 30000 mg/L (equivalent to approximately 89, 742, 3679 and 8190 mg/kg bw/day) for 143-204 days. At the high-dose, one female died and the number of litters produced and live births was decreased at 30000 ppm. There was no apparent effect in fertility at other doses (Sleight and Atallah, 1968).

6.9 Genotoxicity

Nitrate is not mutagenic in *in vitro* studies or genotoxic *in vivo* (Walker, 1990; WHO, 1995a). Sodium nitrite was mutagenic in *Salmonella typhimurium* strain TA100, with and without Aroclor 1254-induced hamster and rat liver enzymes; no mutagenicity was observed in strain TA98. Intraperitoneal injection of sodium nitrite to male mice and rats did not induce micronucleus formation in bone marrow, and a test for micronuclei in peripheral blood from mice in a 14-week also gave negative results (National Toxicology Program, 2001).

6.10 Evaluation by JECFA

JECFA evaluated nitrate and nitrite most recently in 2002. The ADI values for both compounds were established on the basis of studies in rats. The ADI for nitrate was 0-5 mg/kg bw for nitrate, expressed as sodium nitrate based on a NOAEL of 500 mg/kg bw/day

for decreased body weight gain in a 2 year study. For nitrite, JECFA established an ADI of 0.1 mg/kg bw based on a NOAEL of 10 mg/kg bw/day nitrite expressed as sodium nitrite. At higher doses effects included dilated bronchi with infiltration of lymphocytes, alveolar infiltration and thin dilated coronary arteries.