Risk Assessment Studies
Report No. 40

Chemical Hazard Evaluation

Nitrate and Nitrite in Vegetables Available in Hong Kong

July 2010

Centre for Food Safety
Food and Environmental Hygiene Department
The Government of the Hong Kong Special Administrative Region

Correspondence:
Risk Assessment Section
Centre for Food Safety,
Food and Environmental Hygiene Department,
43/F, Queensway Government Offices,
66 Queensway, Hong Kong.
Email: enquiries@fehd.gov.hk

Table of Contents

Risk Assessment Studies
Report No.40

Nitrate and Nitrite in Vegetables Available in Hong Kong

EXECUTIVE SUMMARY

The Centre for Food Safety (CFS) has conducted a study on nitrate and nitrite in fresh vegetables available in Hong Kong and assessed the associated health risk posed to the local population through dietary exposure to nitrate and nitrite. Based on the results, advice to the public and the trade was formulated.

Vegetables are essential to human health since they are good source of vitamins, minerals and biologically active substances. However, vegetables also contain nitrate and nitrite. Nitrate itself is relatively non-toxic but its metabolites, nitrite, is associated with methaemoglobinaemia (commonly known as Blue Baby Syndrome). Nitrite might also react with amines to form carcinogenic nitrosamines in the stomach.

Nitrates and nitrites occur naturally in the environment. They are important plant nutrients and can be used in fertilisers. In addition, they can be added to some food products as preservatives. Vegetables account for about 70-90% of the total estimated dietary nitrate intake. The concentration of nitrate in vegetables depends on species variation, season, light, temperature, method of growth, and fertiliser used.

The Study

A total of 73 types of different groups of vegetables such as leafy, legumes, root and tuber, and fruiting vegetables were collected in winter and summer for the analysis of nitrate and nitrite concentrations. Laboratory analysis was conducted by the Food Research Laboratory of the CFS.

Results

There was a large variation in mean concentrations of nitrate in different vegetables from a low of 5 mg/kg in oyster mushroom (range <4-9 mg/kg) to a high of 4 800 mg/kg in Chinese spinach (range 3 700-6 300 mg/kg). The nitrate concentrations of different groups of vegetables in descending order were leafy vegetables > root and tuber vegetables > fruiting and legume vegetables. More than 80% of vegetables had mean nitrate concentrations less than 2 000 mg/kg but the mean nitrate concentrations of three types of leafy vegetables namely Chinese spinach, Shanghai cabbage, and petiole Chinese cabbage were over 3 500 mg/kg. Nitrate in vegetables (i.e. Chinese flowering cabbage, Chinese spinach and celery) could be reduced significantly (12-31%) after blanching for 1 to 3 minutes. On the other hand, the nitrite concentrations of vegetable were generally low, with less than 1 mg/kg on average.

Conclusion and recommendation

The levels of nitrate and nitrite found in vegetables in this study were unlikely to pose any immediate health risk to the general population. However, health risk to infants from consuming improperly handled vegetables that contain high nitrite levels cannot be ruled out. Current knowledge and evidence indicated there are strong beneficial effects of consumption of vegetables. In order to maximise the health benefits from eating vegetables, measures have to be taken to reduce the nitrate and nitrite exposure while maintaining the recommended intake of vegetables of the public.

Advice to public

1. Maintain a balanced diet with at least two servings of fruit and three servings of vegetables every day and eat a wide variety of vegetables including leafy vegetables, brassica vegetables, root and tuber vegetables, fruiting vegetables, legume vegetables, etc.

2. Handle and cook vegetables properly (i.e. keep vegetables under refrigeration if they are not being cooked immediately; cook vegetables soon after chopping or mashing; wash, peel, vegetables; blanch high-nitrate vegetables in water and discard the cooking water before consumption.)

3. WHO recommends exclusive breastfeeding for infants up to 6 months of age with appropriate complementary foods afterwards. Generally, infants about 6 months of age are ready for solid foods. Infant foods such as vegetable puree and vegetable congee should be prepared for immediate use. If storage is needed, they should be kept in freezer (at or below -18^{o}C) to avoid accumulation of nitrite due to contamination of bacteria of the food.

Advice to trade

1. Farmers are advised to observe good agriculture practice (GAP) to minimize nitrate concentrations in vegetables.

2. The trade should obtain vegetables from reliable sources and maintain proper records to enable source tracing when required.

3. The trade should store vegetables in either refrigerator or cool and dry places to avoid excessive formulation of nitrite due to spoilage of the vegetables.

Risk Assessment Studies –

Nitrate and Nitrite in Vegetables Available in Hong Kong

OBJECTIVES

The Centre for Food Safety (CFS) has conducted a study on nitrate and nitrite in fresh vegetables available in Hong Kong. The study aims to (i) examine the nitrate and nitrite levels of vegetables on Hong Kong market, (ii) explore the effects of preparation and cooking methods on nitrate levels in vegetables, and (iii) assess the associated health risk posed to the population through dietary exposure to nitrate.

BACKGROUND

2. In 2008, an 8-month-old baby in Hong Kong suffered from methaemoglobinaemia (commonly know as blue baby syndrome) induced by nitrite after consuming congee made of Chinese spinach. Improper handling and storage of vegetables can lead to bacteria contamination. The bacteria can then convert the relatively non-toxic nitrate naturally present in vegetables to the more toxic nitrite. This incident has aroused public awareness about the nitrate and nitrite levels in local vegetables. In this connection, a risk assessment study was conducted to determine the situation in the local scene.

3. Vegetables are essential to human health since they are good source of vitamins, minerals and biologically active substances. The World Health Organization (WHO) recommends intake of a minimum of 400 g (five servings) of fruits and vegetables per day for the prevention of chronic diseases.[1] The Department of Health in Hong Kong also promotes the consumption of at least

two servings of fruit and three servings of vegetables every day (2 plus 3 a day) as part of a balanced diet for optimal health.

4. While vegetables can impact health positively, their nitrate and nitrite levels should not be overlooked. Nitrate and nitrite are found naturally in the environment, and form part of the nitrogen cycle. Nitrate is formed from oxidation of organic wastes by the action of nitrifying bacteria. The nitrite ion is relatively unstable and is readily oxidized to nitrate.[2] Nitrate and nitrite are chemicals used in fertilisers and can be readily migrate from fertilised soil to groundwater. Nitrate and nitrite has been shown to be produced endogenously in animals and humans. In addition, they are also used as food preservatives in processed foods.[2,3]

5. Nitrates in the soil are a primary source of nitrogen which is essential for plant growth. Nitrate itself is relatively non-toxic but its metabolites, nitrite, is associated with methaemoglobinaemia. Nitrite might also react with amines to form carcinogenic nitrosamines in the stomach.[4]

6. In fact, high nitrate concentrations in vegetables is a worldwide problem. Very high concentration (over 5 000 mg/kg) of nitrate in vegetables especially leafy vegetables has been reported in different places such as Mainland China as well as various countries in Europe.[4,5] Due to the increased use of synthetic nitrogen fertilisers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate than in the past.[6]

Source of nitrate and nitrite exposure

7. Human exposure to nitrate is mainly exogenous through the consumption of vegetables (about 70-90%), and to a lesser extent water and other foods.[2,4,7] Nitrate is also formed endogenously. On the contrary, exposure to nitrite is mainly from endogenous nitrate conversion (about 70-80%).[4,8]

Nitrate and nitrite in vegetable

8. Nitrate plays an important role in the nutrition and function of plants. The nitrate concentration in vegetables depends on a number of factors including species variation, season, light, temperature, method of growth, and fertiliser used. The nitrate and nitrite levels of vegetables after harvesting can be affected by the storage and processing methods.[2,4]

9. Nitrate is mainly to be found in cell vacuoles and is transported in the xylem. The xylem carries water and nutrients from the roots to the leaves, while the phloem carries the products of photosynthesis from the leaves to the growth points of the plant (i.e. storage organs such as seeds or tubers). This means leaf crops such as cabbage, lettuce and spinach have fairly large nitrate concentrations whereas storage organs such as potato, carrot, and pea and beans have relatively small concentrations.[4]

10. Applying nitrogen fertiliser increases nitrate concentrations in the xylem but has virtually no effect on concentrations in the phloem, and therefore leaf crops such as lettuce or cabbage show an increased concentration of nitrate in response to nitrogen fertiliser, except in their very youngest leaves, while storage organs such as peas and beans that are fed by the phloem tend to show little

effect.[4]

11. In Europe, the concentrations of nitrate in vegetables are generally higher in winter owing to the low light intensity and fewer daylight hours.[2,4] However, this difference was not observed in a study conducted in Korea. The author suggested that the disparity in data may due to different environmental conditions and the use of fertilisers in summer by Korean farmers.[9] A study showed that the nitrate concentrations of cold season vegetables in Iran (e.g. cabbage, lettuce, radish, and carrot) was lower in winter than in summer while that of warm season vegetables (e.g. basil and cucumber) was lower in spring than in winter.[10] The Federal Institute for Risk Assessment of Germany (BfR) also opined that when vegetables are in season, they have a lower nitrate concentration as they can mature under optimal growth conditions and less fertiliser is needed.[11]

Influence of storage and food processing

12. The nitrite concentrations in fresh, undamaged vegetables are usually very low but under adverse post-harvest storage conditions nitrite concentration can increase in vegetables as a result of bacterial or endogenous nitrate reductase reducing the nitrate to nitrite.[4,13] Under refrigerated storage, the endogenous nitrate reductase in vegetable is inactivated. However, high levels of nitrite have been found in home-made vegetable purees even after refrigerated storage for only 12 hours or more. Presumably pureeing releases endogenous nitrate reductase causing excessive formation of nitrite. Nitrite accumulation is inhibited under frozen storage.[4]

13. The distribution of nitrate is not even across the product, e.g. removal of

stem and midrib resulted in a decrease of nitrate content by 30-40% in lettuce and spinach. On the other hand, nitrate content was found to decrease by 20 to 62% after peeling of potatoes, bananas, melons and beetroot. Nitrate is soluble in water and washing of leafy vegetables can reduce nitrate levels by 10-15%.[4]

14. Different studies have shown reduction of nitrate levels (16 to 79% loss) when vegetables such as peas, cabbage, beans, carrots, potatoes spinach, endives, and celery leaves are cooked in water. For potatoes, a study found that the greatest decrease in reducing nitrate (36-58%) and nitrite (82-98%) was observed when peeled potatoes were boiled in water compared to steaming.[4]

Toxicity of nitrate and nitrite

Kinetics and metabolism

15. Ingested nitrate is readily and completely absorbed from stomach and the upper small intestine. Nitrate is rapidly distributed throughout the tissues. In humans, about 25% of ingested nitrate is secreted in the saliva and approximately 20% of the secreted salivary nitrate is then converted to nitrite by microorganisms on the tongue and thus for normal individuals about 5-7% of ingested nitrate can be detected as salivary nitrite. However, for individuals with a high rate of conversion this figure maybe up to 20%. The major site for nitrate reduction is at the base of the tongue where a stable, nitrate-reducing mircroflora is present. Oral reduction of nitrate is the most important source of nitrite for humans, and will account for approximately 70-80% of the human total nitrite exposure.[4,8,13]

16. Nitrate can be reduced to nitrite by both enteric bacteria and mammalian nitrate reductase activity. Many species of microorganism resident in the

gastrointestinal tract have nitrate reductase activity. Therefore, bacterial reduction of nitrate may also take place in other parts of the human gastrointestinal tract, but not normally in the stomach; exceptions are reported in humans with low gastric acidity, such as artificially fed infants, certain patient in whom hydrochloric acid secretion is slower than normal, or patients using antacids. Absorbed nitrite is rapidly oxidized to nitrate in blood.[13]

17. In the stomach, under acidic conditions, nitrite will be transformed to nitric oxide and other metabolites. In humans, about 65-70% of the ingested nitrate is eventually excreted in urine as nitrate, ammonia, or urea, and the elimination half-life was estimated to be about 5 hour.[13] Low levels of nitrate and nitrite are excreted in faeces. Nitrate levels in breast milk are low, up to 5 mg/kg have been reported, and they did not exceed the simultaneously measured maternal plasma nitrate levels from lactating women after a normal meal.[8]

Endogenous formation of nitrate and nitrite

18. Nitrate excretion in excess of nitrate intake by humans was reported and further investigation revealed about the endogenous synthesis of nitrate. The major pathway involved the conversion of the amino acid L-arginine by macrophages to nitric oxide. Further oxidation of the nitric oxide will yield nitrite, which is rapidly oxidized to nitrate. Increased endogenous synthesis of nitrate, as reported in animals with induced infections and inflammatory reactions, was also observed in humans.[4,14]

Endogenous formation of N-nitroso compounds and protective effects of a *balanced diet*

19. There is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite. In the presence of amines or amides, endogenous nitrosation takes place in the acidic environment of the human stomach. In a dynamaic *in vitro* gastrointestinal model, the formation of N-nitrosodimethylamine (NDMA) was observed after gradually adding nitrite to food samples (fish). For some of the samples the model produced measurable NDMA levels, and the addition of orange juice or tea (antioxidants) generally decrease the NDMA formation.

20. A study quoted by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) that concluded that of 120 mg ascorbic acid taken with a meal containing nitrate at 360 mg/day would significantly reduce nitrosamine formation *in vivo*.[15] Another study quoted by the European Food Safety Authority (EFSA) showed that that when nitrate is consumed as part of a normal diet containing vegetables, other bioactive substances concomitantly consumed such as the antioxidant vitamin C can reduce the amount of nitrosamine formed by up to half.[4]

Acute toxicity

21. The acute oral toxicity of nitrate in animals is generally low. It has been observed that the oral lethal dose of nitrate in humans is around 330 mg/kg body weight (bw).[4] Nitrite is a more toxic than nitrate. Various oral lethal doses of nitrite in human adults have been reported.[16]

Methaemoglobinaemia

22. The major acute toxic effect of nitrite poisoning is methaemoglobinaemia.

Blood is the target organ.[16] Blood contains an iron-based compound called haemoglobin (Hb), which carries oxygen. When nitrite is present, Hb can be converted to methaemoglobin (metHb), which cannot carry oxygen. The normal metHb level in humans is less than 2% and in infant under 3 month of age is less than 3%. The low level of metHb is maintained through a system of enzymatic functions that continually convert metHb back to Hb.[8,14] When metHb concentrations reach 10% of normal Hb and above, symptoms of cyanosis (a bluish colour of skin and lips) usually appear. At higher concentrations, asphyxia may occur. Methylene blue is the specific antidote indicated in case of methaemoglobinaemia induced by nitrates and nitrites.[16]

23. Young infants less than 3 months of age are more susceptible to nitrite-induced methaemoglobinaemia because of the higher reduction of nitrate to nitrite by gastric bacteria due to the low production of gastric acid, the relatively easy oxidation of foetal Hb, and the immaturity of the methaemoglobin reductase system.[3,14] However, the risk of nitrite-induced methaemoglobinaemia of infants about 6 months of age should not be overlooked since solid food including vegetables are usually introduced around this age. Some clinical data showed that consumption of silver beets (mean nitrate concentration=3 200 mg/kg) and incorrect storage of homemade purees of mixed vegetables were identified as potential causes of acquired infantile methaemoglobinaemia; age at risk is not limited to the first 4 to 6 months of life.[17] Another study from Mainland China also found methaemoglobinaemia cases due to consumption of vegetable congees and soups that had been stored overnight in infants older than 6 months of age.[18] Other groups especially susceptible to metHb formation include pregnant women and people deficient in glucose-6-phosophate dehydrogenase or metHb reductase

and probably the elderly.[14]

24. Nitrate has to be converted to nitrite before it can cause acute toxicity. At present, JECFA has not set acute reference doses for nitrate and nitrite that cause methaemoglobinaemia.

25. JECFA in 2002 considered the results of studies in humans on the potential of a high nitrate intake to cause methaemoglobinaemia were equivocal. Some of the studies showed an association between a high nitrate concentration in drinking-water and methaemoglobinaemia, and others indicated that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors in infantile methaemoglobinaemia.[13]

26. EFSA in 2008 reviewed new epidemiological studies that have been published from 2002 onwards and considered the findings also raise questions about the role of diarrhoeal disease in the development of methaemoglobinaemia. It stated that a number of factors are critical to metHb formation including the presence of increased nitrite, intestinal infection together with inflammation of the stomach lining and a lower activity of metHb reductase which converts metHb back to Hb.[4]

<u>Genotoxicity</u>

27. Sodium nitrate was not mutagenic in *in vitro* test. Sodium nitrite was mutagenic in *in vitro* test but gave negative results on mutagenic activities in *in vivo* tests. JECFA concluded that there was no evidence for the reclassification of either nitrate or nitrite as genotoxic compounds.[13,15]

Carcinogenicity

28. Nitrite was shown to react with nitrosatable compounds in the human stomach to form N-nitroso compounds. Many of these N-nitroso compounds have been found to be carcinogenic in all the animal species tested, although some of the most readily formed compounds, such as N-nitrosoproline, are not carcinogenic in humans.[14]

29. Several studies had found association between high nitrite intake and gastric and/or oesophageal cancer; however, other studies, particularly prospective cohort studies, revealed no such association. On the other hand, case-control studies revealed a negative correlation between nitrate intake and gastric cancer. Most likely this is due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer.[6,14] Overall, the epidemiological studies reviewed by JECFA in 2002 and EFSA in 2008 did not provide evidence that nitrate and nitrite are carcinogenic to humans.[4,13,15,]

Safety reference value

30. JECFA allocated an acceptable daily intake of 0-5 mg/kg bw, expressed as sodium nitrate, or 0-3.7 mg/kg bw, expressed as nitrate ion 3.7 mg/kg bw/day for nitrate, on the basis of the no-observed-effect-level (NOEL) for growth depression in a short-term study in dog and a long-term study in rat. The ADI does not apply to infants below the age of 3 months.[8]

31. JECFA stated that it took a cautious position in deriving an ADI for nitrate. It was aware that vegetables are an important potential source of intake of nitrate. However, in view of the well-known benefits of vegetables and the

lack of data on the possible effects of vegetable matrices on the bio-availability of nitrate, the JECFA considered it to be **inappropriate** to compare exposure to nitrate from vegetables directly with the ADI and hence to derive limits for nitrate in vegetables directly from it.[8]

32. JECFA established an ADI of 0-0.07 mg/ kg bw for nitrite, expressed as nitrite ion, on the basis of the NOEL for effects on heart and lung in a 2-year study in rat.[15]

Potential beneficial effects of nitrate

33. There is evidence linking nitrate and nitrite to the prevention of microbial infections, reduction of hypertension and cardiovascular diseases.[6] Nitric oxide and solutions of acidified nitrite, mimicking gastric conditions, have been shown to have antimicrobial activity against a wide range of organisms, in particular, gastrointestinal pathogens such as *Yersinia* and *Salmonella*. Thus nitrate in the form of nitric oxide may play a role in host defence.[4,13]

34. Nitric oxide is also known to have vasodilatory properties and to modulate platelet function. A recent study quoted by ESFA hypothesized that a high nitrate content of beetroot juice represented a vasoprotective nitric oxide via bioactivation. In healthy volunteers, approximately 3 hours after ingestion of 500 ml of beetroot juice, a dietary nitrate load of 2.9 g/L, a significant reduction of blood pressure was observed (-10.4/8 mm Hg) and this effect was correlated with peak increases in plasma nitrite concentration.[4] The potential beneficial effects of nitrate and nitrite subject to further study.

Regulatory control of nitrate and nitrite

As food additive

35. Currently, no provisions on nitrates and nitrites have been established under the Codex General Standard for Food Additives. However, Codex has established maximum permitted levels for nitrates and nitrites in some commodity standards for various cheese products and cured meat products respectively. Nitrates and nitrites have also been permitted to be used as food additives in some specified food in countries such as Australia, Canada, Mainland China, member countries in the European Union (EU) and the USA.

36. As stipulated in the Preservatives in Food Regulation (Cap. 132BD) in Hong Kong, nitrates and nitrites are permitted in specified food categories with maximum permitted levels of nitrates, expressed as sodium nitrate, ranged from 50 mg/kg (in various cheese products) to 500 mg/kg (in certain cured meat product). For nitrites, the maximum permitted levels of nitrites, expressed as sodium nitrite, ranged from 10 mg/kg (in various cheese products) to 200 mg/kg (in certain cured meat product).

As contaminant in food

37. Codex has not discussed or established any food safety standard for nitrate as food contaminant. Overseas food authorities in Australia, Canada, Mainland China, and the USA have also not established any legal standard for nitrate as contaminant in food. However, according to the Commission Regulation (EC) No. 1881/2006, maximum levels for nitrate as contaminants in certain foodstuffs have been established in EU. Because of widely varying climatic conditions, production methods and eating habits in different parts of the EU,

maximum levels for fresh spinach and fresh lettuce are set depending on the season (see Table 1).[19]

Table 1: Maximum levels for nitrate in certain foodstuffs in EU.

Foodstuffs	**Maximum level (mg nitrate/kg)**	
Fresh spinach (*Spinacia oleracea*)	Harvested 1 October to 31 March Harvested 1 April to 30 September	3 000 2 500
Preserved, deep-frozen or frozen spinach		2 000
Fresh Lettuce (*Lactuca sativa L.*) (protected and open-grown lettuce) excluding lettuce below	Harvested 1 October to 31 March: Lettuce grown under cover Lettuce grown in the open air Harvested 1 April to 30 September Lettuce grown under cover Lettuce grown in the open air	 4 500 4 000 3 500 2 500
Iceberg-type lettuce	Lettuce grown under cover Lettuce grown in the open air	2 500 2 000
Processed cereal-based foods and baby foods for infants and young children		200

SCOPE OF STUDY

38. To estimate the nitrate levels of fresh vegetables the dietary exposure to nitrate, this study covered a total of 73 types of commonly consumed vegetables. For better estimation of exposure, 5 samples of each types were collected in winter (December 2008 to January 2009) and in summer (from June to July 2009), respectively.

METHODS

Laboratory analysis

39. Laboratory analysis was conducted by the Food Research Laboratory of the CFS. Fresh vegetable samples (edible portion) will be analysed for both nitrate and nitrite as purchased. The analysis of nitrate in vegetables is conducted according to the standard method BS EN 12014-2:1997, Foodstuffs - Determination of nitrate and /or nitrite content - Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products. The determination of nitrite content was referenced to Part 7 of the same BS standard: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction, followed by flow injection analysis. The limit of detection (LOD) of nitrate ions is 4 mg/kg and for nitrite ions is 0.8 mg/kg.

Setting analytical value for results below limit of detection

40. According to the WHO's recommendation on evaluation of low-level contamination of food, half of LOD is used for all results less than LOD when less than or equal to 60% of results are below LOD. When greater than 60% but

smaller and equal to 80% of results are below LOD and with at least 25 results quantified, two estimates using 0 and LOD for all the results less than LOD are produced.[20] In this study, 3% of results for nitrate and 66% of results for nitrite were below LOD. Therefore, the dietary exposure estimated for nitrate was calculated by setting analytical value below LOD to half LOD. As for nitrite, the estimated exposure was presented in range. The upper bound was calculated by setting analytical values below LOD to LOD while the lower bound was calculated by setting analytical value below LOD to zero.

Experiments on soaking and blanching effects

41. Three vegetables, namely Chinese flowering cabbage, Chinese spinach, celery were selected. All vegetables were washed under tap water for about 30 seconds to remove soil and dirt after removal of the non-edible parts. Whole pieces of Chinese flowering cabbage and Chinese spinach were used. As for celery, each stalk was cut into 6 inch long for soaking and 3 inch long for cooking.

Soaking

42. 300 g of each vegetable sample were soaked in 1.5 litre tap water for 2 hours respectively. The nitrate concentrations of water were measured at 0 hour, 1 hour and 2 hours and that for the sample was measure at 2 hours. The change in nitrate proportion in the sample during soaking was then calculated. There were 4 replicates for each test.

Blanching

43. 300 g of each vegetable sample were cooked in 1.5 litre boiling tap water for 1 minute, 3 minutes, 5 minutes, and 10 minutes in separated tests. The

nitrateconcentration of both the water and sample were measured after each test to determine the change in nitrate proportion in each sample during blanching. There were 4 replicates for each test.

Food consumption data

44. The food consumption data used in the present study was extracted from the Hong Kong Population-Based Food Consumption Survey 2005-2007 commissioned by the Food and Environmental Hygiene Department. This survey investigated the food consumption of a population-based sample of 5 008 Hong Kong adults aged 20 to 84 years, selected through an anonymous and scientific household address sampling procedure. Food consumption data were collected by 24-hour dietary recalls. The survey results have been age- and gender- weighted and represent a population of about 5 394 000 Hong Kong residents aged 20-84.

Estimation of dietary exposure

45. Daily dietary exposure to nitrate and nitrite from an individual vegetable were obtained by combining the consumption data and the levels of nitrate and nitrite of that vegetable. Total exposure for each adult was obtained by summing exposures from all vegetable. The mean and the 95th percentile of the daily exposure levels were used to represent the dietary exposure for average and high consumers respectively.

RESULTS

Concentrations of nitrate and nitrite in vegetables

46. A total of 73 types of vegetables were tested for nitrate and nitrite. The

test results for each are presented in Annex I. There was a large variation in mean concentrations of nitrate in different vegetables from a low of 5 mg/kg in oyster mushroom (range <4-9 mg/kg) to a high of 4 800 mg/kg in Chinese spinach (range 3 700-6 300 mg/kg). The mean concentrations of nitrate in different groups of vegetables in descending order were leafy vegetables (2 100 mg/kg) > root and tuber vegetables (720 mg/kg) > fruiting (14-370 mg/kg) and legume vegetables (140 mg/kg). This trend was in agreement with those reported in other studies.[4,5,12,21]

47. The mean nitrate concentrations of some common vegetables in this study were similar to those reported in other places (Table 2). Certain leafy vegetables namely Chinese spinach, petiole Chinese cabbage, and Shanghai cabbage, had mean nitrate concentrations higher than 3 500 mg/kg but there was no international data available for comparison. Nevertheless, a study showed that six out of the 12 types of leafy vegetables including petiole Chinese cabbage and Shanghai cabbage sampled in Beijing also had mean nitrate concentrations higher than 3 500 mg/kg.[5]

Table 2. Comparison of Nitrate Mean Values in Vegetables of Different Places.

Vegetables	Mean concentration of nitrate (mg/kg)					
	Hong Kong, China	**Beijing, China[5]**	**Xiamen, China[2]**	**European Countries[4]**	**New Zealand[22]**	**Korea[9]**
Aubergine/ Eggplant	350	371	250	314	--	--
Beetroot	3 000	--	--	1 379	635	--
Bell pepper	77	218	--	--	--	76
Broccoli	420	--	416	279	111	--
Cabbage	1 200	1 978	630	311	275	725
Carrot	220	457	352	296	48	316
Cauliflower	250	--	237	148	--	--
Celery	1 700	--	--	1 103	1 339	--
Chili pepper	33	203	178	67	--	--
Chinese cabbage	1 300	2 533	1 764	933	--	1 740
Coriander	3 200	3 400	--	2 445	--	--
Cucumber	110	256	186	185	--	212
Garlic	18	--	--	69	--	124
Common beans	470	--	300	323	--	--
Lettuce	950	2 419	--	1 324	1 323	2 430
Mushroom	43	--	--	61	--	--
Onions	13	--	--	164	--	23
Potato	170	351	335	168	107	452
Pumpkin	260	--	--	894	55	639
Spinach	3 100	3 177	2 824	1 066	824	4 259
String bean	190	523	151	618	--	--
Tomato	57	35	58	43	--	--
Watercress	1 300	--	688	136	1 364	--
White radish	1 400	2 078	936	1 416	--	1 878

Notes: - "—" denotes data not available.

-Results of Beijing and Xiamen are rounded to whole figure.

48. The nitrite concentrations in vegetables were generally low with average mean nitrite concentration less than 1 mg/kg. The nitrite concentrations in 66% of vegetable samples were below the limit of detection. Beet root was found to have the highest mean nitrite concentration 7.6 mg/kg (range 3.1-8.9 mg/kg) and one garlic spear sample had nitrite concentration up to 21 mg/kg.

49. Due to the different climates of origin of vegetables and limited sample size for each type of vegetables, it is difficult to conclude whether the difference, if any, in nitrate concentrations between vegetables sampled in summer and winter was caused by seasonal variation or other factors.

Effects on soaking and blanching on reducing nitrate in vegetables

50. The change in nitrate proportion of Chinese flowering cabbage, Chinese spinach and celery during soaking was shown in Table 3. There were 97 to 100% of nitrate reminded in the three vegetables after soaking in water for 2 hours.

Table 3: Nitrate proportion in vegetables (%) during soaking.

	0 h	1 h	2 h
Chinese flowering cabbage	100	99	99
Chinese spinach	100	98	97
Celery	100	100	100

51. The change in nitrate proportion in vegetables during cooking was shown in Table 4. The nitrate proportion in vegetables decreased with the blanching time. After 10 minutes of blanching, only 45% and 53% of nitrate remained in Chinese flowering cabbage and Chinese spinach, respectively, and 73% of nitrate

remained in celery. However, the these vegetables after blanched for 10 minutes or longer became overly soft and lost their original crunchy texture. Nevertheless, nitrate concentrations were reduced significantly in vegetables blanched for 1 to 3 minutes of which up to 31% of nitrate in Chinese flowering cabbage were leached into water after 3 minutes of blanching. It should be noted that nitrate will not be decomposed or evaporated during blanching; therefore, the intake of nitrate will not be reduced if the cooking water or soup was consumed as well.

Table 4: Nitrate proportion in vegetables (%) during blanching.

	0 min	1 min	3 min	5min	10 min
Chinese flowering cabbage	100	86	69	61	45
Chinese spinach	100	88	75	64	53
Celery	100	88	83	81	73

Consumption pattern of vegetables

52. According to the Hong Kong Population-Based Food Consumption Survey 2005-2007, Hong Kong adults in average consumed 195 g vegetables a day. Leafy vegetables accounted for 56% of total vegetables consumed while fruiting vegetables accounted for 19%. For other groups of vegetables, each only contributed less than 10% to the total vegetable consumption. The food consumption data for different groups of vegetables are given in Table 5.

Table 5: Consumption pattern of vegetables of Hong Kong adults.

Type of vegetables	Mean consumption (g/day)	Percentage (%)
Leafy vegetables	110	56
Brassica vegetables	9	4
Stalk and Stem vegetables	5	2
Bulb vegetables	7	3
Legume vegetables	14	7
Root and tuber vegetables	15	8
Fruiting vegetables	36	19
Others (not specified)	1	<1

Note: Data are rounded to whole figure.

Exposure to nitrate and nitrite from vegetables

53. If all vegetables consumed were raw, the dietary exposure to nitrate from vegetables for average consumers was estimated to be 4.4 mg/kg bw/day and for high consumers was estimated to be 13 mg/kg bw/day which are about 120% and 350% of ADI, respectively.. If all vegetables consumed were cooked, the dietary exposure to nitrate from vegetables for the average consumer of adults was estimated to be 3.5 mg/kg bw/day and for high consumer was estimated to be 10 mg/kg bw/day which are about 95% and 270% of ADI, respectively. A conservative assumption was made that there was a 20% reduction of nitrate levels in cooked vegetables in general based on the results of the cooking experiment in the present study and the data from EFSA's report.[4]

54. The dietary exposure to nitrite from vegetables for average consumers was estimated to be 0.0038 mg/kg bw/day (lower bound) to 0.0051 mg/kg bw/day

(upper bound) and for high consumers was estimated to be 0.012 mg/kg bw/day (lower bound) to 0.015 mg/kg bw/day (upper bound). The estimated exposures to nitrite were well below the ADI.

DISCUSSION

55. The WHO/FAO expert consultation report on diet, nutrition and prevention of chronic diseases, sets population nutrient goals. The report states that there is convincing evidence that fruits and vegetables decrease the risk for cardiovascular disease and obesity, and evidence that they probably decrease the risk of diabetes.[1] In addition, the IARC stated that vegetables provide biologically active substances as well as nutrients like pro-vitamin A, vitamin C, calcium, iron, folate, potassium, magnesium, fibre, etc. and concluded that eating fruits and vegetables may lower the risk of cancer, particularly cancers of the gastrointestinal tract.[23]

56. EFSA stated that a toxicological endpoint of concern for nitrate is nitrosamine formation and the potential for tumour formation. However, when nitrate is consumed in a normal diet containing vegetables, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, may inhibit the endogenous formation of nitrosamines. Epidemiological studies relating to nitrate and human cancer risk do not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk. EFSA considered that the beneficial effects of eating vegetables and fruit outweigh potential risk to human health from exposure to nitrate through vegetables.[4]

57. When assessing risk from nitrate in vegetable, both the potential risk of

nitrate and the benefits of eating vegetables have to be considered. According to JECFA, it is **inappropriate** to compare exposure to nitrate from vegetables directly with the ADI because of well-known benefits of vegetables and the lack of data on the possible effects of vegetable matrices on the bio-availability of nitrate.[8] In addition, negative correlation between nitrate intake and gastric cancer was found by some studies which may due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer.[6]

58. The French Food Safety Agency commented that based on present toxicological studies, the nitrate and nitrite effects on human health are still not agreed upon, it is therefore important to keep conducting toxicological studies to establish more accurate ADIs.[24]

59. Nevertheless, a reduce nitrate contamination in vegetables can represent added value for vegetable already rich in essential nutrients. Furthermore, it is prudent to reduce dietary nitrate and nitrite intake by maintaining a balance diet and avoiding over-indulgent in foods that contain nitrate or nitrite as food additives as well as vegetables containing high levels of nitrate.

60. Incorrect storage of homemade purees of mixed vegetables were potential causes of acquired infantile methaemoglobinaemia and age at risk is not limited to the first 4 to 6 months of life.[17] This indicates health risk to young infants from consuming improperly handled vegetables resulting from nitrite accumulation cannot be ruled out.

LIMITATIONS

61. The nitrate concentrations varied greatly among vegetables. Although over 700 individual samples from 73 types of vegetables were taken in this study, increasing the number of sample for each vegetable for laboratory analysis could provide a more precise estimate of the average concentrations of nitrate and nitrite for each vegetable.

62. There are uncertainties in this study. Firstly, the study did not cover all nitrate and nitrite containing foods. The estimated dietary exposures of nitrate and nitrite were based on vegetables consumption only. Secondly, raw vegetables were analysed for nitrate and nitrite while the consumption data were based on vegetables as consumed and they were mostly eaten cooked. The nitrate and nitrite concentrations of cooked vegetables could be lower from their raw counterparts.

CONCLUSION AND RECOMMEDATIONS

63. The levels of nitrate and nitrite found in vegetables in this study were unlikely to pose any immediate health risk to the general population. However, health risk to infants from consuming improperly handled vegetables that contain high nitrite levels cannot be ruled out. Current knowledge and evidence indicated there are strong beneficial effects of consumption of vegetables. In order to maximise the health benefits from eating vegetables, measures have to be taken to reduce the nitrate and nitrite exposure while maintaining the recommended intake of vegetables of the public.

Advice to public

1. Maintain a balanced diet with at least two servings of fruit and three servings of vegetables every day and eat a wide variety of vegetables including leafy vegetables, brassica vegetables, root and tuber vegetables, fruiting vegetables, legume vegetables, etc.

2. Handle and cook vegetables properly (i.e. keep vegetables under refrigeration if they are not being cooked immediately; cook vegetables soon after chopping or mashing; wash, peel, vegetables; blanch high-nitrate vegetables in water and discard the cooking water before consumption.)

3. WHO recommends exclusive breastfeeding for infants up to 6 months of age with appropriate complementary foods afterwards. Generally, infants about 6 months of age are ready for solid foods. Infant foods such as vegetable puree and vegetable congee should be prepared for immediate use. If storage is needed, they should be kept in freezer (at or below -18^{o}C) to avoid accumulation of nitrite due to contamination of bacteria of the food.

Advice to trade

1. Farmers are advised to observe good agriculture practice (GAP) to minimize nitrate concentrations in vegetables. These include:

   -reduce the use of chemical fertilizers (particularly nitrogen source fertilizer, e.g. urea) to avoid excess nitrate build up in soil or vegetables;

-conduct regular laboratory check (e.g. at least once a year) on nitrate/ammonium level in irrigation water; and

-follow good soil management practices (e.g. crop rotation, organic matter application) to enhance growth of soil microbes hence facilitating a balanced nitrogen cycle.

2. The trade should obtain vegetables from reliable sources and maintain proper records to enable source tracing when required.

3. The trade should store vegetables in either refrigerator or cool and dry places to avoid excessive formulation of nitrite due to spoilage of the vegetables.

REFERENCES

[1] WHO. Diet, Nutrition and the Prevention of Chronic Diseases. Report of the joint WHO/FAO expert consultation. Technical Report Series 916. 2003.
Available from: URL: http://www.fao.org/docrep/005/AC911E/AC911E00.HTM

[2] WHO. Nitrate and nitrite – intake assessment. In: Safety evaluation of certain food additives (Food additives Series 50). Geneva: WHO; 2003. Available from: URL: http://www.inchem.org/documents/jecfa/jecmono/v50je07.htm

[3] US EPA. Nitrates and nitrites – Teach Chemical Summary; 2007. Available from URL:http://www.epa.gov/teach/chem_summ/Nitrates_summary.pdf

[4] EFSA. Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. The EFSA Journal 2008; 69: 1-79. Available from: URL:
http://www.efsa.europa.eu/EFSA/Scientific_Opinion/contam_ej_689_nitrate_en.pdf

[5] FENG J. et al. Assessment of nitrate exposure in Beijing residents via consumption of vegetables. Chinese Journal of Food Hygiene 2006; 18(6): 514-516. [Article in Chinese]

[6] Santamaria P. Review – Nitrate in vegetables: toxicity content, intake and EC regulation. Journal of Food Agriculture 2006; 86:10-17.

[7] US EPA. Intergrated Risk Information System – Nitrate (CASRN 14797-55-8). 1991. Available from: URL: http://www.epa.gov/iris/subst/0076.htm

[8] WHO. Nitrate. In: Safety evaluation of certain food additives (Food additives Series 35). Geneva: WHO; 1996. Available from :URL :
http://www.inchem.org/documents/jecfa/jecmono/v35je14.htm

[9] Chung S.Y. et al. Survey of nitrate and nitrite contents of vegetables grown in Korea. Food Additives and Contaminants 2003; 20(7):621-628.

[10] Shahlaei A. et al. Evaluation of nitrate and nitrite content of Iran Southern (Ahwaz) vegetables During Winter and Spring of 2006. Asian Journal of Plant Sciences 2007; 6(8):1197-1203.

[11] BfR. Nitrate in rocket lettuce, spinach and other lettuces. Upated BfR opinion no. 032/2009. 2009. Available from: URL: http://www.bfr.bund.de/cm/245/nitrate_in_rocket_lettuce_spinach_and_other_lettuces.pdf

[12] Ayaz A. et al. Survey of nitrate and nitrite levels of fresh vegetables in Turkey. Journal of Food technology 2007; 5(2): 177-179.

[13] WHO. Nitrate (and potential endogenous formation of *N*-nitroso compounds). In: Safety evaluation of certain food additives (Food additives Series 50). Geneva: WHO; 2003. Available from: URL:http://www.inchem.org/documents/jecfa/jecmono/v50je06.htm

[14] WHO. Nitrate and nitrite in drinking water – background document for development of WHO Guidelines for Drinking-water Quality. Geneva: WHO; 2007. Available from URL:http://www.who.int/water_sanitation_health/dwq/chemicals/nitratenitrite2ndadd.pdf

[15] WHO. Nitrite (and potential endogenous formation of N-nitroso compounds). In: Safety evaluation of certain food additives (Food additives Series 50). Geneva: WHO; 2003. Available from: URL:http://www.inchem.org/documents/jecfa/jecmono/v50je05.htm

[16] IPCS. Nitrates and nitrites – Poisons Information Monograph G016. Geneva: WHO; 1999. Available from:URL:http://www.inchem.org/documents/pims/chemical/pimg016.htm

[17] Echaniz J. et al. Methemoglobinemia and Consumption of Vegetables in Infants. Pediatrics 2009; 107(5) 1024-1028. Available from URL: http://www.pediatrics.org/cgi/conent/full/107/5/1024

[18] Zhang et al. Clinical analysis of 22 cases of methemoglobinemia in children. Journal of Jinggangshan University (Science and Technology) 2009; 30(2) 92-94. [Article in Chinese]

[19] European Commission. Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. 2006. Available from: URL: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_364/l_36420061220en00050024.pdf

[20] WHO. GEMS/Food-EURO Second Workshop on reliable evaluation of low-level contamination of food – report of a workshop in the frame of GEMS/Food-EURO. WHO; 1995. Available from: URL: http://www.who.int/foodsafety/publications/chem/en/lowlevel_may1995.pdf

[21] Tang H.H. et. al. Survey and evaluation of heavy metals, nitrate and nitrite contamination in vegetables in Xiamen's market. Food Science 2007; 28 (8):327-331. [Article in Chinese]

[22] Thomson B. Nitrates and nitrites dietary exposure and risk assessment: prepared as part of a New Zealand Food Safety Authority contract for scientific services. Institute of Environmental Science & Research Limited. Christchurch Science Centre. 2004. Available from: URL:http://www.nzfsa.govt.nz/consumers/food-safety-topics/chemicals-in-food/residues-in-food/consumer-research/nitrite-nitrate-report.pdf

[23] IARC. Handbooks of Cancer Prevention: Fruit and Vegetables. International Agency for Research on Cancer, Lyon, France; 2003: 8:1-375.

[24] Menard C. et al. Assessment of dietary exposure of nitrate and nitrite in France. Food Additives and Contaminants 2008; 25 (8):971-988.

Annex

Nitrate and Nitrite Concentrations in Vegetables (mg/kg)

Vegetables	Number of samples	Nitrate Mean	Nitrate Range	Nitrite Mean	Nitrite Range
Leafy Vegetables (including Brassica Leafy Vegetables)	**20 (types)**	**2 100**	**79-6 300**	**1.2**	**ND-9.1**
Baby Chinese cabbage	10	2 100	540-2 800	0.6	ND-1.2
Bean shoot[a]	5	260	120-430	0.5	ND-0.9
Ceylon spinach[b]	5	1 100	79-2 300	ND	ND
Chinese spinach/Chinese amaranth[b]	5	4 800	3 700-6 300	1.7	0.9-2.6
Chinese cabbage/ Tienntsin cabbage	10	1 300	480-2 900	0.6	ND-1.2
Chinese flowering cabbage	10	2 400	1 200-3 500	3.3	ND-7.8
Chinese kale	10	1 600	340-2 700	1.6	ND-5.9
Chinese lettuce	10	1 300	670-1 800	0.5	ND-1.3
Chinese wolfberry	10	2 400	1 400-3 000	1.1	ND-1.7
Coriander	10	3 200	1 800-5 000	0.6	ND-1.1
European lettuce	10	950	600-1 400	0.5	ND-1.0
Green water spinach	10	870	190-2 500	0.5	ND-1.2
Indian lettuce	10	1 300	510-1 900	0.5	ND-0.9
Leaf mustard	10	3 300	2 100-4 500	1.2	ND-2.2
Petiole Chinese cabbage/Petiole bok choi	10	4 100	2 300-5 400	2.6	ND-9.1
Red Chinese spinach[b]	5	2 000	94-3 900	1.3	0.8-1.9
Shanghai cabbage	10	3 600	2 300-5 100	2.2	ND-5.0
Spinach	10	3 100	1 100-4 700	2.3	ND-5.7
Watercress	10	1 300	580-2 200	0.7	ND-1.9
White water spinach[b]	5	1 200	290-2 400	0.6	ND-1.6
Brassica (Cole or Cabbage) Vegetables, Head Cabbages, Flowerhead Cabbages	**3 (types)**	**620**	**16-2 800**	**0.5**	**ND-0.9**
Broccoli	10	420	280-670	0.5	ND-0.9
Cauliflower	10	250	25-720	0.5	ND-1.1
European variety cabbage	10	1 200	16-2 800	0.5	ND-0.9

Stalk and Stem Vegetables	**6 (types)**	**830**	**8-4 600**	**0.6**	**ND-4.4**
Asparagus	10	21	14-37	0.5	ND-1.3
Bamboo shoot[b]	5	130	54-240	ND	ND
Celery	10	1 700	390-3 200	ND	ND
Chinese celery	10	3 100	1 800-4 600	0.6	ND-1.2
Mung bean sprouts	10	18	8-37	1.3	ND-4.4
Soybean sprouts	10	20	10-32	0.5	ND-1.6
Bulb Vegetables	**7 (types)**	**520**	**5-2 300**	**1.3**	**ND-21**
Blanching chives	10	320	76-620	0.7	ND-1.8
Bud chives	10	310	37-540	0.9	ND-2.1
Chinese chives	10	1 500	120-2 300	1.2	ND-4.0
Garlic bulb	10	18	9-33	0.8	ND-1.8
Garlic spears	10	780	81-2 300	4.0	ND-21
Onion	10	13	5-36	0.5	ND-1.1
Spring onion	10	680	100-1 400	0.7	ND-1.4
Legumes Vegetables	**5 (types)**	**140**	**ND-830**	**0.5**	**ND-1.4**
Common bean	23	470	170-830	0.5	ND-1.0
Green soybean	10	18	5-34	0.6	ND-1.2
Green String beans	10	190	23-420	ND	ND
Snow pea	10	13	ND-26	0.6	ND-1.2
Sugar snap pea	10	10	7-13	0.6	ND-1.4
Root and Tuber Vegetables	**13 (types)**	**720**	**ND-4 100**	**1.1**	**ND-8.9**
Beetroot[b]	5	3 000	1 600-4100	7.6	3.1-8.9
Carrot	10	220	43-490	0.5	ND-1.1
Ginger	10	1 300	790-1 800	0.8	ND-3.7
Kudzu	10	230	120-390	0.5	ND-1.2
Lotus root	10	33	9-60	1.3	ND-2.6
Potato	10	180	100-270	0.8	ND-1.7
Radish, Chinese green	10	1 900	1 400-2 600	0.4	ND-0.8
Radish, Chinese red	10	300	60-740	ND	ND
Radish, Chinese white	10	1 400	630-2 200	0.5	ND-0.9
Sweet potato	10	43	ND-220	ND	ND
Taro	10	570	49-1 300	0.5	ND-1.1

Water chestnuts	10	20	11-36	0.7	ND-1.5
Yam beans	10	170	39-400	0.5	ND-1.1
Fruiting Vegetables, Cucurbits	**7 (types)**	**370**	**11-1 400**	**0.6**	**ND-2.2**
Bitter gourd	10	380	99-730	0.7	ND-1.7
Cucumber	10	110	28-260	0.5	ND-0.9
Hairy gourd	10	250	190-340	0.6	ND-1.2
Pumpkin	10	260	11-810	0.8	ND-2.2
Sponge gourd	10	260	30-470	ND	ND
Wax gourd/Winter melon	10	520	260-1 000	0.5	ND-1.0
Zucchini/Jade melon	10	840	480-1 400	0.7	ND-1.3
Fruiting Vegetables, Mushrooms	**6 (types)**	**14**	**ND-140**	**0.7**	**ND-2.5**
Chicken-leg mushroom	10	5	ND-11	0.5	ND-0.9
Gold-needle mushroom	10	6	ND-12	0.8	ND-1.5
Oyster mushroom	10	5	ND-9	1.1	ND-2.5
Shiitake mushroom	10	6	ND-13	ND	ND
Straw mushroom	10	16	11-29	0.5	ND-1.0
White button mushroom	10	43	10-140	1.0	ND-2.2
Fruiting Vegetables, Other than Cucurbits and Mushrooms	**6 (types)**	**93**	**ND-470**	**0.9**	**ND-2.9**
Eggplant	10	350	250-470	0.8	ND-1.5
Bell pepper	10	77	9-180	1.1	ND-1.7
Long pepper	10	57	9-150	0.9	ND-1.6
Tomato	10	57	ND-180	0.5	ND-0.9
Small Red pepper (hot)	10	9	ND-24	1.3	ND-2.9
Sweet corn	10	7	ND-16	0.5	ND-1.1

Notes: -ND denotes not detected.
-Limits of detection (LOD) for nitrate ions is 4 mg/kg and for nitrite ions is 0.8 mg/kg.
-Mean values are calculated by assigning ND results as half LOD.
-Results are rounded to 1 or 2 significant figures depends on the detection limit.
-[a] winter samples only.
-[b] summer samples only.