

INGESTED NITRATE AND NITRITE

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This monograph focuses on ingested nitrate and nitrite ions, for which food and water are the major pathways of human exposure. The nitrate and nitrite salts described in Section 1.1 represent some of the potential anthropogenic sources of these compounds in food and water, and their use is presented in relation to exposure by ingestion; however, it is not the intention of this monograph to evaluate the carcinogenicity of any of the individual salts. The highly water-soluble nitrate and nitrite ions are of interest here and the patterns of occurrence and levels of human exposures to nitrate and nitrite ions are described in Sections 1.3 and 1.4.

1. Exposure Data

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle and are ubiquitous in the environment. Both are products of the oxidation of nitrogen (which comprises approximately 78% of the earth's atmosphere) by microorganisms in plants, soil or water. Nitrate is the more stable form of oxidized nitrogen but can be reduced by microbial action to nitrite, which is moderately chemically reactive. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate. Nitrate salts are used widely as inorganic fertilizers, and are also used in explosives, as oxidizing agents in the chemical industry and as food preservatives. Nitrite salts have been also used as food preservatives, especially to cure meats (Environmental Protection Agency, 1987; Health Canada, 1992; Pokorny *et al.*, 2006).

During the second half of the twentieth century, production of nitrogen compounds by humans increased dramatically—intentionally through the use of fertilizers or unintentionally as a by-product of the combustion of fossil fuels. In fact, it has been reported that, since the end of the twentieth century, humans have been adding more nitrogen compounds to the nitrogen cycle than all other sources combined (Fields, 2004).

1.1 Chemical and physical data

1.1.1 Nomenclature, molecular formulae, relative molecular masses and chemical and physical properties

From Chapman and Hall/CRC (2006) and Toxnet (2006), unless otherwise indicated

Nitrate ion

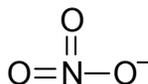
Chem. Abstr. Serv. (CAS) Reg. No.: 14797-55-8

Deleted CAS Reg. Nos: 23746-18-1; 34236-35-6; 73394-83-9

Chem. Abstr. Name: Nitrate

IUPAC Systematic Name: Nitrate

Synonyms: Nitrate (NO_3^-); nitrate(1-); nitrate ion; nitrate ion (NO_3^-); nitrate ion(1-); nitrate; nitric acid, ion(1-)



NO_3^-

Relative molecular mass: 62.005

(a) *Acid:* Conjugated base of the strong acid HNO_3 ; $\text{p}K_a = -1.3$ (WHO, 2003a)

(b) *Reactivity:* Unreactive (WHO, 2003a)

Nitrite ion

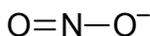
Chem. Abstr. Serv. Reg. No.: 14797-65-0

Deleted CAS Reg. Nos: 12183-96-9; 114466-53-4

Chem. Abstr. Name: Nitrite

IUPAC Systematic Name: Nitrite

Synonyms: Nitrite (NO_2^-); nitrite(1-); nitrite anion; nitrite ion; nitrite ion (NO_2^-); nitrite ion(1-); nitrogen dioxide(1-); nitrogen dioxide ion(1-); nitrogen peroxide ion(1-); nitrous acid, ion(1-)



NO_2^-

Relative molecular mass: 45.995

(a) *Acid:* Conjugated base of the weak acid HNO_2 ; $\text{p}K_a = 3.4$ (WHO, 2003a)

(b) *Reactivity:* Reactive; oxidizes antioxidants, the divalent iron (Fe^{2+}) of haemoglobin to trivalent iron (Fe^{3+}) and primary amines; nitrosates several amines and amides (WHO, 2003a).

Ammonium nitrate

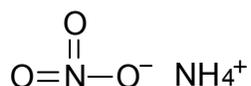
Chem. Abstr. Serv. Reg. No.: 6484-52-2

Deleted CAS Reg. No.: 95255-40-6

Chem. Abstr. Name: Nitric acid, ammonium salt

IUPAC Systematic Name: Nitric acid, ammonium salt

Synonyms: Ammonium nitrate; ammonium nitrate (NH₄NO₃); Emulite; EXP 200; German saltpeter; Norge salpeter; Norway salpeter; Norwegian salpeter; Plenco 12203; Varioform I; ZhVK



NH₄NO₃

Relative molecular mass: 80.043

- (a) *Description:* White hygroscopic crystals; exists in five crystalline forms
- (b) *Boiling-point:* Decomposes at about 210 °C, mostly into water and nitrous oxide (Merck & Co., 2005)
- (c) *Melting-point:* 169.7 °C (Lide, 2005)
- (d) *Density:* 1.725 g/cm³
- (e) *Solubility:* Very soluble in water (118.3 g/100 g at 0 °C; 213 g/100 g at 25 °C; 871 g/100 g at 100 °C) (Lide, 2005); soluble in acetone, ammonia, ethanol (3.8 g/100 g at 20 °C), isopropanol and methanol (17.1 g/100 g at 20 °C); insoluble in diethyl ether (Merck & Co., 2005)
- (f) *pH:* 0.1 M solution in water is 5.43.

Sodium nitrate

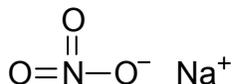
Chem. Abstr. Serv. Reg. No.: 7631-99-4

Deleted CAS Reg. No.: 862599-22-2

Chem. Abstr. Name: Nitric acid, sodium salt

IUPAC Systematic Name: Nitric acid, sodium salt

Synonyms: Chile salpeter; niter; nitric acid sodium salt (1:1); salpeter; soda niter



NaNO₃

Relative molecular mass: 84.995

- (a) *Description:* Colourless trigonal (rhombohedral) crystals; undergoes transition to high-temperature polymorph at ~278 °C
- (b) *Boiling-point:* 380 °C; decomposes on heating to form sodium nitrite (Weast, 1979).
- (c) *Melting-point:* 306 °C
- (d) *Density:* 2.26 g/cm³ (Merck & Co., 2005)

- (e) *Solubility*: Very soluble in water (92 g/100 g at 25 °C; 180 g/100 g at 100 °C); very soluble in ammonia; soluble in ethanol and methanol; very slightly soluble in acetone and glycerol; practically insoluble in dimethyl ether (Weast, 1979)
- (f) *pH*: An aqueous solution is neutral (Merck & Co., 2005).

Sodium nitrite

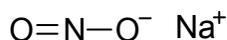
Chem. Abstr. Serv. Reg. No.: 7632-00-0

Deleted CAS Reg. Nos: 32863-15-3; 56227-20-4; 82497-43-6; 82998-40-1

Chem. Abstr. Name: Nitrous acid, sodium salt

IUPAC Systematic Name: Nitrous acid, sodium salt

Synonyms: Nitrous acid soda; nitrous acid sodium salt (1:1)



NaNO_2

Relative molecular mass: 68.985

- (a) *Description*: White to pale yellow, hygroscopic, orthorhombic crystals (Lide, 2005)
- (b) *Boiling-point*: > 320 °C (decomposes) (Lide, 2005); undergoes transition to high-temperature orthorhombic polymorph at 158 °C.
- (c) *Melting-point*: 271 °C
- (d) *Density*: 2.17 g/cm³ (Merck & Co., 2005)
- (e) *Solubility*: Very soluble in water (84.8 g/100 g at 25 °C); very soluble in ammonia; slightly soluble in ethanol (3 g/100 g at 20 °C) (Lide, 2005) and methanol (4.4 g/100 g at 20 °C); sparingly soluble in diethyl ether (0.3 g/100 g at 20 °C) (Weast, 1989)
- (f) *pH*: An aqueous solution is alkaline (~9) (Merck & Co., 2005).

Potassium nitrate

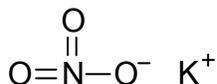
Chem. Abstr. Serv. Reg. No.: 7757-79-1

Deleted CAS Reg. No.: 96193-83-8

Chem. Abstr. Name: Nitric acid, potassium salt

IUPAC Systematic Name: Nitric acid, potassium salt

Synonyms: Niter; nitre; nitric acid potassium salt (1:1); salpeter



KNO_3

Relative molecular mass: 101.103

- (a) *Description*: Colourless, orthorhombic crystals

(b) *Boiling-point*: 400 °C (decomposes) (Lide, 2005); undergoes orthorhombic to rhombohedral form transition at 128 °C; cooling the rhombohedral form gives a metastable rhombohedral polymorph that exists between 128 °C and 109 °C.

(c) *Melting-point*: 334 °C

(d) *Density*: 2.11 g/cm³ (Merck & Co., 2005)

(e) *Solubility*: Very soluble in water (13 g/100 g at 0 °C; 38.3 g/100 g at 25 °C; 247 g/100 g at 100 °C); soluble in ammonia; insoluble in diethyl ether and ethanol (Weast, 1989; Lide, 2005)

(f) *pH*: ~7 (Merck & Co., 2005)

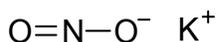
Potassium nitrite

Chem. Abstr. Serv. Reg. No.: 7758-09-0

Chem. Abstr. Name: Nitrous acid, potassium salt

IUPAC Systematic Name: Nitrous acid, potassium salt

Synonyms: Chile salpeter; niter; nitric acid sodium salt (1:1); salpeter; soda niter



KNO₂

Relative molecular mass: 85.093

(a) *Description*: Pale yellow, hexagonal crystals; white, hygroscopic crystals (Lide, 2005)

(b) *Boiling-point*: 537 °C (explodes) (Lide, 2005); undergoes transition from rhombohedral to cubic form at 70 °C; disproportionates on heating in the absence of air to form potassium nitrate (KNO₃), potassium oxide (K₂O) and evolving nitrogen.

(c) *Melting-point*: 440 °C

(d) *Density*: 1.915 g/cm³ (Lide, 2005)

(e) *Solubility*: Very soluble in water (281 g/100 g at 0 °C; 312 g/100 g at 25 °C; 413 g/100 g at 100 °C); very soluble in ammonia; slightly soluble in ethanol (Weast, 1989; Lide, 2005)

1.1.2 Physicochemical properties

Concentrations of nitrate and nitrite can be expressed in different units, as either milligrams per litre (mg/L), milligrams of nitrate nitrogen per litre (mg/L nitrate-N) or milligrams of nitrite nitrogen per litre (mg/L nitrite-N). It can also be expressed in terms of moles (or millimoles); the latter simplifies the comparison of nitrate and nitrite with regard to exposure and their relative contributions to *N*-nitroso compounds. Conversion factors between the different units are given in Table 1.1 (Environmental Protection Agency, 1987; WHO, 2003a).

Table 1.1. Conversion factors between different units

	mM	mg/L NO ₃ ⁻	mg/L NO ₃ ⁻ -N	mg/L NO ₂ ⁻	mg/L NO ₂ ⁻ -N
mM	1	62	14	46	14
mg/L NO ₃ ⁻	0.016	1	0.226	–	–
mg/L NO ₃ ⁻ -N	0.0714	4.429	1	–	–
mg/L NO ₂ ⁻	0.0217	–	–	1	0.304
mg/L NO ₂ ⁻ -N	0.0714	–	–	3.29	1

From Environmental Protection Agency (1987); WHO (2003a)

NO₃⁻, nitrate; NO₃⁻-N, nitrate nitrogen; NO₂⁻, nitrite; NO₂⁻-N, nitrite nitrogen

1.2 Analysis

Spectrometric techniques are used for the determination of nitrate in water. Selected methods for the determination of nitrates and nitrites in various matrices are presented in Table 1.2. A molecular absorption spectrometric method is available for the determination of nitrite in drinking-water, raw water and wastewater (International Standards Organization, 1984b). A continuous flow spectrometric method for the determination of nitrite, nitrate or the sum of both in various types of water is suitable in undiluted samples (International Standards Organization, 1996). Nitrate and nitrite can also be determined in water by liquid chromatography (International Standards Organization, 1992).

WHO (2004a) has reported limits of detection for the following analytical methods: 0.1 mg/L (nitrate) and 0.05 mg/L (nitrite) by liquid chromatography; 0.01–1 mg/L (nitrate) by spectrometric techniques; 0.005–0.01 mg/L (nitrite) by a molecular absorption spectrometric method; and 22 µg/L (nitrate) and 35 µg/L (nitrite) by ion-exchange chromatography.

1.3 Production and use of major nitrate and nitrite salts

1.3.1 *Production and consumption volumes* (from Chemical Information Services (2006), unless otherwise specified)

(a) Ammonium nitrate

Ammonium nitrate was first described in 1659 by the German scientist Glauber, who prepared it by the reaction of ammonium carbonate and nitric acid. He called it 'nitrium flammans' because its yellow flame (from traces of sodium) was different from that of potassium nitrate. Historically, ammonium nitrate was manufactured by a double decomposition method using sodium nitrate and either ammonium sulfate or ammonium chloride. Modern commercial processes, however, rely almost exclusively on the neutralization

Table 1.2. Selected methods for the analysis of nitrate and nitrite in various matrices

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water (drinking-, rain-, ground-, surface)	React with potassium hydrogenphthalate or sodium carbonate/sodium hydrogencarbonate eluent; inject sample; compare peaks with standard solutions.	HPLC/CD-CE	0.1 mg/L (nitrate-N); 0.05 mg/L (nitrite-N)	Ranges of application: 0.1–50 mg/L (nitrate); 0.05–20 mg/L (nitrite)	International Standards Organisation (1992)
Water (drinking-, waste-, aqueous extracts)	Inject sample; compare with standards.	IEC/CD	0.002 mg/L (nitrate-N); 0.004 mg/L (nitrite-N)	NR	Environment Protection Agency (2000)
Water (drinking-, surface, saline; waste- (domestic and industrial))	React with brucine sulfate in sulfuric acid solution; measure absorbance at 410 nm.	Spectrophotometry	0.1 mg/L (nitrate-N)	Applicable range of concentrations: 0.1–2 mg/L nitrate-N	Environment Protection Agency (1997)
Water (waste-)	React with potassium hydrogenphthalate or other appropriate eluent; inject sample; compare peaks with standard solutions.	HPLC/CD-CE or UVD	0.1 mg/L (nitrate-N); 0.05 mg/L (nitrite-N)	Working ranges: 0.1–50 mg/L (nitrate); 0.05–20 mg/L (nitrite)	International Standards Organisation (1995)
Water (raw and potable)	React with sodium salicylate and sulfuric acid; treat with alkali; measure absorbance at 415 nm.	Spectrometry	0.003–0.013 mg/L (nitrate)	Range: up to 0.2 mg/L nitrate-N using max. test portion vol. of 25 mL	International Standards Organisation (1988)

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water (potable, raw, waste-)	React with 4-aminobenzene sulfonamide and orthophosphoric acid at pH 1.9; react diazonium salt formed reacts with <i>N</i> -(1-naphthyl)-1,2-diamino-ethane dihydrochloride; measure absorbance at 540 nm.	Spectrometry	0.001–0.002 mg/L (nitrite)	Range: up to 0.25 mg/L nitrite-N using max. test portion vol. of 40 mL	International Standard Organisation (1984a)
Water (ground-, drinking-, surface, waste-, sea-)	Reduce nitrate to nitrite with cadmium; react with sulfanilamide chloride and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 520-560 nm.	Spectrometry with FIA or CFA	NR (nitrate and nitrite)	Range of application: mass concentration, 0.01–1 mg/L nitrite-N; 0.2–20 mg/L nitrite-/nitrate-N (undiluted samples)	International Standards Organisation (1996)
Water (natural)	Inject sample.	IEC/SCD	0.05 mg/L (nitrate-N); 0.02 mg/L (nitrite-N)	Working ranges: 0.05–150 mg/L nitrate-N; 0.02–70 mg/L nitrite-N	Geological Survey (1987)
Water (low ionic strength)	Inject sample.	IEC/SCD	0.01 mg/L (nitrate-N)	Analytical range: 0.01–0.6 mg/L nitrate-N	Geological Survey (1985)
Water (low ionic strength)	React with sulfanilamide under acidic conditions; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 540 nm.	Colorimetry	0.001 mg/L (nitrite-N)	Application range: 0.001–0.20 mg/L nitrite-N	Geological Survey (1989)

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water (surface, domestic, industrial, brine)	React with sulfanilamide under acidic conditions; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 540 nm.	Colorimetry	0.01 mg/L (nitrite-N)	Application range: 0.01–1.0 mg/L nitrite-N	Geological Survey (1990)
Water (surface, ground-, waste-, drinking-)	Inject sample.	IEC/CSEC	0.003 mg/L (nitrate-N); 0.004 mg/L (nitrite-N)	NR	APHA/ AWWA/WEF (2005a)
Water (untreated and treated drinking-)	Inject sample.	IEC/DCD	0.017 mg/L (nitrate-N); 0.015 mg/L (nitrite-N)	NR	APHA/ AWWA/WEF (2005b)
Water	Dilute sample with reagent water; remove suspended solids by filtration; measure absorbance at 254 nm.	CIE/UVD	0.1 mg/L	NR	APHA/ AWWA/WEF (2005c)
Water (potable, uncontaminated natural)	Remove suspended solids by filtration; acidify with hydrochloric acid; measure absorbance at 220 nm (screening method).	UVS	NR (nitrate-N)	NR	APHA/ AWWA/WEF (2005d) Method B

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water (potable)	Remove suspended solids by filtration; acidify with sulfuric or hydrochloric acid; measure absorbance of second derivative at 230–210 nm.	UVS	0.05 mg/L (nitrate-N)	Applicable range: 0.05–2 mg/L nitrate-N	APHA/AWWA/WEF (2005d) Method C
Water	Immerse electrode tips in sample; record potential reading when stable; compare with standards.	NIE	0.14 mg/L (nitrate-N)	Applicable range: 0.14–1400 mg/L nitrate-N	APHA/AWWA/WEF (2005d) Method D
Water	Reduce nitrate to nitrite with cadmium–copper complex; react with sulfanilamide; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 543 nm [cadmium reduction method].	Spectrophotometry	0.01 mg/L (nitrate-N and nitrite-N)	Applicable range: 0.01–1.0 mg/L nitrate-N	APHA/AWWA/WEF (2005d) Method E

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water (potable, surface and saline; domestic and industrial waste-)	Reduce nitrate to nitrite with cadmium–copper complex; react with sulfanilamide; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance [automated cadmium reduction method].	Colorimetry	0.5 mg/L (nitrate-N)	Applicable range: 0.1–10 mg/L nitrate-N	APHA/ AWWA/WEF (2005d) Method F
Water (potable and surface; domestic and industrial waste-)	Reduce nitrate to nitrite with hydrazine sulfate; react with sulfanilamide; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 520 nm [automated hydrazine reduction method].	Colorimetry	0.01 mg/L (nitrate-N)	Applicable range: 0.1–10 mg/L nitrate-N	APHA/ AWWA/WEF (2005d) Method H
Water	Reduce nitrate to nitrite with cadmium–copper complex; react with sulfanilamide; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 540 nm [cadmium reduction flow injection method].	Colorimetry with FIA	0.025 mg/L (nitrogen)	Applicable range: 0.00025–10 mg/L nitrate-N	APHA/ AWWA/WEF (2005d) Method I

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water	React with sulfanilamide under acidic conditions; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 543 nm.	Spectro-photometry	0.005 mg/L (nitrite-N)	Applicable range: 10–1000 mg/L nitrite-N	APHA/AWWA/WEF (2005e) Method B
Water (drinking- and waste-)	Inject sample.	IEC	0.5 mg/L (nitrate-N); 0.4 mg/L (nitrite-N)	Application concentration range: 0.3–100 µg/L (range differs for each analyte)	AOAC International (2000) Method 993.30
Water	Inject sample.	IEC/CSEC	0.42 mg/L (nitrate); 0.36 mg/L (nitrite)	Application concentration range: differs for each analyte	ASTM International (1997)
Water (high purity)	Inject sample; compare with standards.	IEC/CD-CE	0.0002 mg/L	Application concentration range: 0.02–100 µg/L	ASTM International (2000a)

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Water (drinking-, waste-, other aqueous matrices)	Inject sample; measure absorbance at 254 nm.	CIE/UVD	0.08 mg/L (nitrate); 0.1 mg/L (nitrite)	Application concentration range: 0.1–50 mg/L	ASTM International (2000b)
Water (surface, saline, waste-, ground-)	Reduce nitrate to nitrite with cadmium–copper complex; react with sulfanilamide; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 540 nm [automated and manual cadmium reduction method].	Spectrophotometry	0.05 mg/L (nitrate-N and/or nitrite-N)	Application range: 0.05–1.0 mg/L nitrogen	ASTM International (2004)
Water (surface and saline; domestic and industrial waste-)	React with brucine in sulfuric acid; measure absorbance at 410 nm.	Colorimetry	0.1 mg/L (nitrate-N)	Application range: 0.1–2 mg/L	AOAC International (2000) Method 973.50

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Meat and meat products	Extract with hot water; precipitate proteins; filter; reduce extracted nitrates to nitrites with metallic cadmium; add sulfanilamide chloride and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 538 nm.	Photoelectric colorimetry or spectrophotometry	NR (nitrate)	NR	International Standards Organisation (1975)
Cured meat	Mix with water; add bromocresol green indicator; oxidize nitrites to nitrates; add sulfuric acid and phosphotungstic acid; add silver ammonium hydroxide; add sulfuric acid; add <i>meta</i> -xylenol; compare colour to standards.	Colorimetry	NR (nitrate and nitrite)	NR	AOAC International (2000) Method 935.48
Cured meat	React with sulfanilamide; react couple diazo compound formed with <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 540 nm.	Colorimetry	NR (nitrites)	NR	AOAC International (2000) Method 973.31

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Curing preparations	Mix potassium iodide and starch solution; bubble in carbon dioxide; add sulfuric acid; titrate with sodium thiosulfate.	Titration (nitrites)	NR (nitrites)	NR	AOAC International (2000) Method 964.13
Fruit, vegetables and derived products	Extract with hot water; precipitate proteins by addition of potassium hexacyanoferrate(II) and zinc acetate; filter precipitate; reduce nitrates to nitrites with metallic cadmium; add sulfanilamide chloride and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 538 nm.	Spectrometry	NR (nitrates and nitrites)	NR	International Standards Organisation (1984b)

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Dried milk	Dissolve in water; precipitate fat and proteins; filter; reduce nitrate to nitrite with zinc powder and cadmium ion; add sulfanilamide chloride and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 538 nm.	Spectrometry	NR (nitrate)	NR	International Standards Organisation (1987)
Milk and milk products ^b	Dissolve in water; precipitate fat and proteins; filter; reduce nitrate to nitrite with copperized cadmium; react with sulfanilamide chloride and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 538 nm.	Spectrometry with FIA or SFA	NR (nitrate and nitrite)	NR	International Standards Organisation (2004a)
Milk and milk products ^c	Suspend sample in water; reduce nitrate to nitrite with cadmium; measure absorbance (nitrate determination); suspend sample in ammonium/sodium chloride solution (nitrite determination); measure absorbance at 550 nm.	Spectrometry with SFA (routine method)	NR (nitrate and nitrite)	NR	International Standards Organisation (2004b)

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Milk and milk products ^d	Suspend sample in warm extraction buffer solution; separate fat by centrifuging and rapid cooling; reduce nitrate to nitrite with cadmium; react with sulfanilamide and <i>N</i> -(1-naphthyl)ethylenediamine dihydrochloride; measure absorbance at 540 nm.	Spectrometry with FIA	0.5 mg/kg (nitrate ions); 1.0 mg/kg (nitrite ions)	NR	International Standards Organisation (2004c)
Cheese (very hard, hard, semisoft, soft)	Mix cheese–water slurry; add zinc sulfate and sodium hydroxide; heat; cool; filter; reduce nitrate to nitrite; measure absorbance at 522 nm.	Spectrophotometry	≥1 µg/g nitrate	NR	AOAC International (2000) Method 976.14
Flour	Mix with water; react with sulfanilic acid; add α -naphthylamine hydrochloride solution; compare with standards.	Digestion	NR (nitrite-N)	NR	AOAC International (2000) Method 951.03
Fertilizer	Precipitate nitrate ions in acid solution as a complex with nitron reagent; filter precipitate; dry; weigh.	Nitron gravimetry	NR (nitrate)	NR	International Standards Organisation (1981)

Table 1.2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Limit of quantitation	Reference
Fertilizer	Mix with hot water; add sulfuric acid; add iron sulfate solution; assess colour change (purple, brown, reddish).	Colorimetry	NR (nitrates)	NR	AOAC International (2000) Method 920.01
Animal feed	Extract with cadmium and barium chloride solution; precipitate proteins in alkaline solution; reduce nitrates to nitrites with cadmium; measure absorbance at 540 nm.	Colorimetry	NR (nitrate-N and nitrite-N)	NR	AOAC International (2000) Method 968.07
Forages	Extract nitrate from sample into aluminum sulfate solution; compare with standards.	Potentiometric titration	0.50% potassium nitrate	NR	AOAC International (2000) Method 986.31
Infant food (containing meat)	Reduce nitrate to nitrite with spongy cadmium; measure absorbance at 530 nm	Spectro-photometry	100–300 mg/kg (as sodium nitrate)	NR	AOAC International (2000) Method 993.03

CD, conductivity detection; CFA, continuous flow analysis; CIE, capillary ion electrophoresis; CSEC, chemical suppression of eluant conductivity; DCD, direct conductivity detection; FIA, flow injection analysis; HPLC/CD-CE, high-performance liquid chromatography with conductivity detection and cation exchanger; IEC, ion-exchange chromatography; max., maximum; NIE, nitrate ion electrode; NR, not reported; SCD, specific conductivity detection; SFA, segmented flow analysis; UVD, ultraviolet detection; UVS, ultraviolet spectrophotometry; vol., volume

^a The Working Group noted the limited validity of the methods that lack a limit of detection and/or quantitation.

^b Includes whole and partly skimmed and skimmed dry milk, hard, semi-hard and soft cheeses, processed cheese, whey cheese, caseins and caseinates and dried whey.

^c Includes milk, cheese, liquid and dried milk products and baby products.

^d Hard, semi-hard and soft cheeses of various ages, processed cheese, whey powder, milk powder and milk-based infant food

of nitric acid, produced from ammonia through catalysed oxidation, with ammonia. The predominant commercial form has been the solid product that is used as a fertilizer. However, the use of ammonium nitrate as a component in urea–ammonium nitrate liquid fertilizer has grown to the extent that approximately half of the ammonium nitrate produced is actually marketed as a solution (Weston *et al.*, 2003).

World production of ammonium nitrate peaked in 1988 and then declined by 21% between 1986 and 2002 to 13.6 million tonnes (see Table 1.3). Eastern and western Europe recorded large declines while modest increases occurred in Africa and the USA. The former Soviet Union, the USA and Western Europe were the main producers. Together, these regions accounted for 72% of total world production in 2002; eastern Europe contributed approximately 7% and Africa contributed approximately 6%.

Table 1.3. World production and apparent consumption of ammonium nitrate in 1986 and 2002 (thousands of tonnes)

Geographical region	1986		2002	
	Production	Consumption	Production	Consumption
Former Soviet Union	4800	4684	3917	2185
Western Europe	5175	5295	3451	3674
Eastern Europe	2538	2337	1005	1124
Africa	621	747	800	811
Middle East	323	432	407	691
Eastern Asia ^a	715	708	405	754
Central and South America	250	264	314	620
North America				
Canada	403	270	392	257
Mexico	35	37	27	65
USA	2111	2256	2439	2727
Southwest Asia ^b	198	198	199	258
Oceania	85	94	158	167
Southeast Asia ^c	8	7	82	137
Japan	29	26	12	17
Total	17 291	17 355	13 608	13 487

From Suresh (2004a)

^a Eastern Asia: Cambodia, China, Democratic People's Republic of Korea, Lao People's Democratic Republic, Mongolia, Viet Nam

^b Southwest Asia: Afghanistan, Bangladesh, Bhutan, India, Nepal, Pakistan, Sri Lanka

^c Southeast Asia: China (province of Taiwan), Indonesia, Malaysia, Myanmar, Philippines, Republic of Korea, Singapore, Thailand

In 2002, the USA and western Europe were the major consumers and accounted for 47% of world consumption. Eastern Europe and the former Soviet Union combined accounted for an additional 25% and Africa accounted for 5%. Apparent world consumption peaked at 17.7 million tonnes of nitrogen in 1988, and then declined by about 23% to 13.6 million tonnes in 2002. This decline in consumption is primarily attributed to the increased use of urea in the developing world and, more recently, because of safety concerns. It also results from a major reduction in consumption and production in eastern Europe and the former Soviet Union after 1988; consumption in this region decreased by 3.7 million tonnes of nitrogen between 1986 and 2002. In addition, consumption in western Europe fell because of a general decline in the use of nitrogen fertilizers following changes in the European Union agricultural subsidy policy (Suresh, 2004a).

In 2002, production of ammonium nitrate and calcium ammonium nitrate combined amounted to about 14.4 million tonnes of nitrogen. Western Europe is a major producer of calcium ammonium nitrate and accounted for approximately 55% of the combined production of ammonium nitrate and calcium ammonium nitrate (Suresh, 2004a).

Between 80 and 85% of ammonium nitrate is used worldwide as material for nitrogen fertilizers; explosives account for most of the remainder (see below) (Suresh, 2004a).

(b) *Sodium nitrate*

Sodium nitrate is produced worldwide primarily by leaching ores that contain nitrate with brine, followed by fractional crystallization. Alternatively, sodium nitrate can be obtained synthetically by the absorption of nitrous gases or by the neutralization of nitric acid.

The maximum world production of sodium nitrate of 3 million tonnes per year occurred around 1930, at which time the Chilean nitrate industry produced ~2.9 million tonnes. Production of synthetic sodium nitrate also peaked in the mid-1930s (730 000 tonnes per year). In 2004, total world production of sodium nitrate had declined to about 63 000 tonnes, of which 98% was produced in Chile from natural deposits. The remainder was manufactured in Germany and Mexico, generally as a by-product of the manufacture of nitric acid. In addition, China has been reported to manufacture unknown but significant volumes of sodium nitrate for domestic use (Pokorny *et al.*, 2006).

(c) *Sodium nitrite*

Sodium nitrite has been synthesized by several chemical reactions that involve the reduction of sodium nitrate. Industrial production of sodium nitrite is primarily by the absorption of nitrogen oxides into aqueous sodium carbonate or sodium hydroxide (Pokorny *et al.*, 2006).

(d) *Potassium nitrate*

Potassium nitrate is produced commercially by the reaction of potassium chloride and nitric acid and is the second largest source of non-chloride potassium fertilizer.

1.3.2 *Uses*

(a) *Fertilizers*

Ammonium nitrate is used primarily as a nitrogen fertilizer (Weston *et al.*, 2003). Its use as a fertilizer grew rapidly after the Second World War and accounted for about 90% of production in 1975, after which it was gradually replaced by urea (Weston *et al.*, 2003; Suresh, 2004a).

Sodium nitrate is also used as a fertilizer. From 1880 to 1910, it accounted for 60% of world production of nitrogen fertilizers. In the 1990s, it accounted for only 0.1%, although it was still preferred for some specific crops and soil conditions. This decline resulted from an enormous growth in the manufacture of other types of fertilizer and the use of less expensive nitrogen fertilizers that were produced from synthetic ammonia, such as urea, ammonium nitrate, ammonium phosphates, ammonium sulfate and ammonia itself (Pokorny *et al.*, 2006).

Potassium nitrate is more soluble than potassium sulfate and is used as a fully soluble fertilizer in applications such as fertigation [application of a soluble fertilizer via the irrigation system] and interior landscaping.

(b) *Cured meat*

Sodium nitrite has been used extensively in curing meat and meat products, particularly pork products such as ham, bacon and frankfurters; certain fish and poultry products are also cured with brines that contain sodium nitrite. The process may include dry curing, immersion curing, or direct addition or injection of the curing ingredients. Curing mixtures are typically composed of salt (sodium chloride), sodium or potassium salts of nitrite and nitrate and seasonings. Sodium nitrite acts as a colour fixative and inhibits the growth of bacteria, including *Clostridium botulinum*, which is the source of botulism toxin. Nitrite is a relatively strong reducing agent that has antibacterial properties; however, the preservation of foodstuffs can be attributed to a large degree to the high concentration of salts (including nitrate) that are employed during the curing process. In addition, nitrate can act as a reservoir from which nitrite may be formed by microbiological reduction (Pokorny *et al.*, 2006).

In addition to the preservation of meats, curing is a process that develops and maintains a stable red colour in cured and smoked meats, for which sodium or potassium nitrate or nitrite is responsible. When nitrate is used as the curing agent, the conversion (reduction) of nitrate to nitrite by bacteria in the meat or poultry is a necessary step in the development of the cured colour. The amount of nitrate that is reduced to nitrite is dependent upon the number of nitrate-reducing bacteria and several environmental

conditions such as temperature, moisture content, salt content and pH. Hence, the conversion rate and subsequent amount of nitrite that is formed is difficult to control. Similarly, the further reduction of nitrite to nitric oxide, which reacts with myoglobin (muscle pigment) to produce the cured colour, is affected by the same environmental conditions. When nitrite is used as the curing agent, there is no need for the nitrate reduction step, and the development of the cured colour is much more rapid. When the cured meat is heated, exposed to a more acid environment or left for a sufficient length of time under normal conditions, the nitric oxide–myoglobin is converted into a stable red pigment called nitrosohaemochrome. The time required for a cured colour to develop may be shortened with the use of cure accelerators, such as ascorbic acid and erythorbic acid or their derivatives, sodium ascorbate and sodium erythorbate. Cure accelerators speed up the chemical conversion of nitrous acid to nitric oxide, help to keep myoglobin in a reduced state and also serve as oxygen scavengers to prevent the fading of the colour of cured meat in the presence of sunlight and oxygen (Anon., 2003).

(c) *Other uses*

(i) *Sodium nitrate*

Sodium nitrate is used in several industrial processes, in most of which it acts primarily as an oxidizing agent. A major use is in the manufacture of medium- and high-quality glass, such as optical and artistic glass, television and computer screens and fibreglass. In the manufacture of explosives, sodium nitrate is used mainly in blasting agents. Another large application is as an ingredient in the production of charcoal briquettes. Sodium nitrate is also used in the manufacture of enamels and porcelain as an oxidizing and fluxing agent, in formulations of heat-transfer salts for heat-treatment baths for alloys and metals, in rubber vulcanization and in petrochemical industries. Other uses of sodium nitrate include the treatment of water, the melting of ice, in adhesives, cleaning compounds and pyrotechnics, in the nitration of organic compounds, in certain types of pharmaceutical production, in the refining of some alloys, for the recovery of lead, in the production of uranium and in the leaching of copper ore (Pokorny *et al.*, 2006). Nitrate is used for the treatment of angina pectoris (Parker & Parker, 1998).

(ii) *Potassium nitrate*

The major industrial use of potassium nitrate is as a component of specialty glasses, especially for cathode-ray tubes for television sets and computer monitors. Other important uses include fireworks, steel manufacture and in a eutectic mixture with sodium nitrite as a heat-transfer agent (Suresh, 2004b; Freilich & Petersen, 2005).

(iii) *Sodium and potassium nitrites*

Most of the industrial uses of sodium nitrite are based on its oxidizing properties or its liberation of nitrous acid in acidic solutions. Sodium nitrite is a convenient source of nitrous acid in the nitrosation and diazotation of aromatic amines and the production of azo dyes (Pokorny *et al.*, 2006).

Other applications of sodium nitrite include the syntheses of saccharin, synthetic caffeine, fluoroaromatics and other pharmaceutical products, pesticides and organic substances; as an inhibitor of polymerization; in the production of foam blowing agents; in the removal of hydrogen sulfide from natural gas; in textile dyeing; and as an analytical reagent (Pokorny *et al.*, 2006). Sodium and potassium nitrites are listed in the European and US Pharmacopeiae, which would indicate that they can be used in pharmaceutical preparations (Thomson Reuters, 2008). Sodium nitrite can be used as an antidote for cyanide poisoning and as a vasodilator (Lundberg & Weitzberg, 2005).

1.4 Occurrence

1.4.1 Food

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle and are ubiquitous in the environment. In addition, since the early 1900s, nitrate has been used extensively in agricultural activities, mainly as a fertilizer. The content of nitrate and nitrite in vegetables depends on the type of vegetable, the method of production, the use of fertilizer, the season and light. Nitrate and nitrite are also used as food additives in processed food as preservatives and colour fixatives in meat, poultry, fish and cheese (European Commission, 1995). Levels of nitrate and nitrite may be determined in raw commodities or in food as consumed. Also, two types of data are available: national surveys on food that is ready to eat and randomized samples that aim to control agricultural raw commodities. A national survey on nitrates and nitrites in food exists only for the United Kingdom and is based on the Total Diet Study method (Food Standards Agency, 1998a). Every year, 24 different towns throughout the United Kingdom are selected from which the food samples are bought. Every 2 weeks, instructions and equipment are dispatched to trained shoppers. Once bought, the foods are packed up and collected for delivery to the laboratory. At the laboratory, the food samples are prepared and, where necessary, cooked according to normal domestic practice. A portion of each food, relative to its contribution to the United Kingdom diet, is then combined with similar foods into 21 groups. A homogenous composite sample is then prepared for each group, and these are subdivided according to analytical requirements. The resulting samples are stored for 5 years and analysed for a range of contaminants such as heavy metals, dioxins, pesticides and nutrients as required, which allows the Food Standards Agency to estimate the average exposure of the population to these constituents. When available, these data are presented for each food category because they relate to average concentrations (pooled samples) of nitrate and nitrite in food as consumed. Such an approach provides a more realistic estimate of the concentrations that can be used to estimate long-term exposure.

(a) *Nitrate*

(i) *Vegetables and fruit*

Many data exist on the concentration of nitrate in vegetables but most of them are more than 20 years old. Reported values range from 30 to 6000 mg/kg (for reviews, see Walker, 1990; Gangolli *et al.*, 1994; European Commission, 1997). On the basis of published data, vegetables can be divided into three groups according to their nitrate content: low nitrate (< 100 mg/kg), medium nitrate (100–1000 mg/kg) and high nitrate (> 1000 mg/kg) (Chilvers *et al.*, 1984). Leafy vegetables such as lettuce, spinach, celery leaf and beetroot leaf have the highest nitrate concentrations (above 1000 mg/kg). In the Republic of Korea (Chung *et al.*, 2003), the concentrations of nitrate were found to be 4259 mg/kg in spinach while an average level of 1800 mg/kg was reported in radishes and Chinese cabbage. In Singapore (Dutt *et al.*, 1987), the concentrations of nitrate in salad, lettuce and spinach were 1360, 1470 and 4570 mg/kg, respectively. In Italy (De Martin & Restani, 2003), the concentration of nitrate in leafy vegetables was reported to range between 80 and 6250 mg/kg, with a higher mean concentration in those obtained by organic farming both for green salad (1680 versus 3009 mg/kg) and chicory (3232 versus 4629 mg/kg). In France (Malmauret *et al.*, 2002), the median concentration of nitrate in conventional and organic products was found to be 1591 and 1135 mg/kg, respectively, in spinach and 804 and 1221 mg/kg, respectively, in lettuce. In Poland, the concentration of nitrate in lettuce was found to be up to 3500 mg/kg (Nabrzyski & Gajewska, 1994). In the United Kingdom between 1996 and 1998 (Ysart *et al.*, 1999a), the average concentration of nitrate in lettuce produced in glasshouses was 2382 and 3124 mg/kg in the summer and winter, respectively, whereas that in lettuce produced outdoors was 1085 mg/kg. In the same study, the concentration of nitrate in spinach was 1900 mg/kg. In a more recent survey in the same country (Food Standards Agency, 2004), the average concentration of nitrate in lettuce produced in glasshouses in summer was 2999 mg/kg (range, 676–4382 mg/kg; $n = 18$); in winter, the average was 3617 mg/kg (range, 1945–5720 mg/kg; $n = 33$). In lettuce produced outdoors, the concentrations were lower both in summer (average, 1140 mg/kg; range, 181–2656 mg/kg) and in winter (average, 1997 mg/kg; range, 810–3100 mg/kg); the average concentration of nitrate in spinach was 1815 mg/kg (range, 141–3909 mg/kg).

A second category of vegetables includes potatoes, cabbage and spring greens, which have concentrations that range between 100 and 1000 mg/kg. In Singapore (Dutt *et al.*, 1987), the concentrations of nitrate were 930, 340, 210 and 140 mg/kg, respectively, in cabbage, green beans, aubergines, ladies finger [okra] and potatoes. In Poland, green beans contained up to 800 mg/kg (Nabrzyski & Gajewska, 1994). In France, concentrations of nitrate in carrots were 113 and 394 mg/kg, respectively, for conventional and organic products (Malmauret *et al.*, 2002). In the same study, French beans were found to contain 711 and 561 mg/kg nitrate, respectively, in conventional and organic products.

In a third category, vegetables such as asparagus or onions and fresh fruit, including tomatoes, had the lowest concentrations (less than 100 mg/kg). The average concentration of nitrate in fresh fruit in the United Kingdom (Food Standards Agency, 1998a) was found to be 27 mg/kg (range, 12–46 mg/kg). In Singapore (Dutt *et al.*, 1987), the concentrations of nitrate in tomatoes, asparagus, onions and mushrooms were found to be 60, 55, 35 and 15 mg/kg, respectively. In the Republic of Korea (Chung *et al.*, 2003), the concentrations of nitrate in onions, soya bean sprouts and green peppers were found to range between 23 and 76 mg/kg. In France (Malmauret *et al.*, 2002), the concentration of nitrate in tomatoes was found to be 19 and 1 mg/kg in conventional and organic products, respectively. In Poland (Nabrzyski & Gajewska, 1994), the concentration of nitrate in various fruit (currants, gooseberries, raspberries and cherries) ranged between 1.3 and 36 mg/kg, while a concentration of 58.7 mg/kg was found in strawberries.

(ii) *Cereal grains and their products*

The mean concentration of nitrate in bread and miscellaneous cereals obtained in total diet studies in the United Kingdom (Food Standards Agency, 1998a) was 7.2–11 mg/kg (range, undetected–20 mg/kg).

The mean nitrate contents reported by Walker (1990) and Gangolli *et al.* (1994) were between 0.5 and 16 mg/kg in cereals and cereal products worldwide. The variability of these results is easily explained by the various locations of production, seasons of sampling and type of products analysed. The concentration of nitrate increased after baking; darker breads or breads that contained rye had slightly higher levels. In bread crumbs and pasta, the concentrations were reported by the same authors to range between 15 and 24 mg/kg.

(iii) *Milk and dairy products*

In the Total Diet Study in the United Kingdom, the average concentration of nitrate in dairy products was 27 mg/kg and that in milk was 3.9–5.3 mg/kg (Food Standards Agency, 1998a).

The concentration of nitrates in cows' milk is generally below 5 mg/L (Walker, 1990; Gangolli *et al.*, 1994). In cheese without nitrate additives, the concentration of nitrates was found to be in the range of 1–8 mg/kg (Walker, 1990).

(iv) *Eggs*

The average concentration of nitrates in eggs was estimated in the United Kingdom Total Diet Study (Food Standards Agency, 1998a) to range between 4.4 and 5.4 mg/kg (range, undetected–12 mg/kg).

(v) *Cured meat*

A survey of nitrate and nitrite preservative samples obtained from supermarkets and other retail outlets in England and Wales has recently been completed. A total of 200 samples, 40 each from Wales, northern England, South East England, South West England and the Midlands, were purchased and analysed between December 1996 and

February 1997. The sampling programme was designed to reflect recent changes in shopping patterns towards freshly sliced produce available from supermarket delicatessen counters or local butchers' shops. Samples were transported to the laboratory and processed immediately (Food Standards Agency, 1998b).

Concentrations of nitrate in meat products in the United Kingdom Total Diet Study (Food Standards Agency, 1998a) were found to be 45 mg/kg (range, 14–101 mg/kg). In a more specific study on nitrate in 200 samples of cured meat (Food Standards Agency, 1998b), levels ranging from 1.4 to 445 mg/kg were found in bacon and ham and those ranging from 0.2 to 450 mg/kg were found in other cured meat products. The mean value of nitrate from all samples was found to be 62 mg/kg and was similar to that obtained in a previous survey. The difference between the two series of results can be explained by the inherent variability of nitrate content in processed meats when treated with additives and by the fact that the second study targeted meat products that were known to contain nitrates whereas the objective of the Total Diet Study was to sample various preserved meat available for consumers and analyse these for several chemicals.

(vi) *Fish*

In the United Kingdom Total Diet Study (Food Standards Agency, 1998a), the average concentration of nitrate in fish was estimated to be 11 mg/kg (range, 5–19 mg/kg).

The concentration of nitrate in fish was generally found to be below 2.5 mg/kg. It should be noted that, because nitrate and nitrite are authorized as food additives in certain countries, higher concentrations, ranging up to 380 mg/kg, were also reported (Walker, 1990).

(vii) *Beer*

In the United Kingdom, the Food Standards Agency (1998a) reported a mean concentration of nitrate in beers of 30 mg/kg (range, 10–100 mg/kg) in one survey. In a later survey, the mean concentration was found to be 16 mg/kg (range, 0.2–140 mg/kg) (Gangolli *et al.*, 1994).

(viii) *Breast milk*

The human mammary gland does not appear to concentrate nitrate, and, although human breast milk can contain nitrate, it has not been shown to be a significant source of exposure of infants (Greer *et al.*, 2005).

(b) *Nitrite*

(i) *Vegetables*

The average concentration of nitrite in vegetables is generally below 2 mg/kg. A mean concentration of 0.5 mg/kg was found in the United Kingdom Total Diet Study (Food Standards Agency, 1998a). In Korean vegetables (Chung *et al.*, 2003), an average concentration of 0.6 mg/kg was found.

(ii) *Cereal grains and cereal products*

The average concentration of nitrite in cereal products was reported to be 5 mg/kg in one study in the United Kingdom and 4 mg/kg in another study in the USA (Walker, 1990). In the 1997 Total Diet Study in the United Kingdom (Food Standards Agency, 1998a), the concentration of nitrite was found to range between undetected and 1.8 mg/kg.

(iii) *Fish*

The concentration of nitrite in fish was found to be below 0.6 mg/kg in several reports (Walker, 1990; Food Standards Agency, 1998a).

(iv) *Cured meat*

‘Cured meat’ generally means meat that has been preserved using curing ingredients consisting of food-grade salt and sodium or potassium nitrite, and includes ham, bacon and sausages; it may also include dry-cured meat or meat cured by other processes that do not use nitrite. From a practical point of view, analytical surveys, and in particular the Total Diet Study, are not designed to determine nitrite content only and investigate preserved meat in general. It is therefore difficult to rule out the fact that some samples of meat analysed may have been preserved or cured without the use of nitrites, which could lead to a ‘dilution’ of the reported average concentration. Nevertheless, such sampling is representative of the exposure of consumers to various preserved meats. In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study, cured meat mostly meant nitrite-preserved meat, although some other meat preparations were included as cured meat (Linseisen *et al.*, 2002; Bingham & Riboli, 2004). In the USA and western Europe, but not in China, processed meat is a more abundant commodity than processed fish and is the main source of dietary nitrite. It is therefore essential that the effects of cured meat be distinguished from those of fresh meat.

In the United Kingdom Total Diet Study, the mean concentration of nitrite in cured meat was 4.1 mg/kg (range, 1.5–8.4 mg/kg), while, in a more specific study conducted in the United Kingdom, the average residue level of nitrite was found to be 24 mg/kg (range, 0.2–170 mg/kg) (Food Standards Agency, 1998a,b). [Such a result is probably related to the inclusion in the Total Diet Study of samples of meat products that did not contain nitrate or nitrite as additives.] A survey conducted in the USA (Cassens, 1997) of residual nitrite in 164 samples of cured meat in three trials showed average concentrations of 5–10 mg/kg (range, 0–48 mg/kg). Results reported in the 1970s showed average residual nitrite to be 52.5 mg/kg (range, 0–195 mg/kg).

In a survey from 1981, sausages (e.g. hot dogs) had a mean content of about 100 mg/kg nitrite and fried bacon and fried ham contained about 35 mg/kg nitrite (National Research Council, 1981). In a report that compiled 85 studies conducted between 1970 and 1991 in Canada and the USA of nitrite levels in cured meat, modelization of the results suggested some reduction in nitrite levels during the study period in most types of meat studied, except for frankfurters (Pogoda & Preston-Martin, 2001).

(c) *Transformation of nitrate to nitrite and vice versa during storage*

Transformation of nitrate to nitrite during storage, especially in home-prepared food, is known to occur. Evidence from studies on methaemoglobinaemia support the contention that the nitrate contained in vegetables is converted to nitrite before consumption (Bruning-Fann & Kaneene, 1993). Levels of nitrite up to 400 mg/kg have been reported in vegetables that have been damaged, poorly stored or stored for extended periods and in pickled or fermented vegetables (WHO, 1995, 2002a).

In contrast, levels of nitrite in cured meat appear to decrease during storage as it is converted into nitric oxide. The decrease is mainly due to its reactivity, and may average 70% (National Research Council, 1981).

(d) *Formation of nitrosamines*

The formation of nitrosamines from nitrite within preserved food is possible, and the formation of *N*-nitroso compounds in nitrite-preserved meat and fish has been reviewed (Tricker, 1997). The main sources of *N*-nitroso compounds in the diet are nitrite-preserved meat products (Tricker, 1997; Haorah *et al.*, 2001). Haorah *et al.* (2001) reported a mean concentration of 5.5 $\mu\text{mol/kg}$ *N*-nitroso compounds in frankfurters, but only 0.5 $\mu\text{mol/kg}$ *N*-nitroso compounds in fresh meat.

1.4.2 *Water*

The natural occurrence of nitrate and nitrite in soil results from microbial oxidation of ammonia which is derived from organic nitrogenous material such as plant proteins, animals and animal excreta (WHO, 1978). The background concentration of nitrate in sources of water in the USA is very low, and is generally less than 2 mg/L nitrate-N in groundwater and less than 0.6 mg/L in streams. Higher levels of nitrate are found in contaminated sources, and especially in groundwater (Mueller & Helsel, 1996).

Intensive use of nitrogen fertilizers in crop culture is usually the main source of water contamination, but manure and slurries from concentrated animal feeding operations may be a significant source in some areas. Other anthropogenic sources are disposal of municipal effluents by sludge spreading on fields and deposition of airborne nitrogen compounds emitted from the combustion of fossil fuels by industry and automobiles (Puckett, 1994; Mueller & Helsel, 1996). Point sources include septic tanks, old landfills and leaking sewage systems (WHO, 2003a).

As it is not bound to the soil and is very soluble, nitrate that is not taken up by plants leaches into groundwater or rivers. This occurs especially when the transit of water through soil is rapid, due to its geological composition (e.g. sandy soils). The main diffuse sources of contamination of surface water are agricultural run-off, drainage from roads and suburban lawns, and nitrogen transported by rain (Graffy *et al.*, 1996). Nitrite is a relatively unstable form of nitrogen that is rapidly converted to nitrate by bacteria; thus, the concentration of nitrite in environmental media such as water is usually very low, even when the concentration of nitrate is high (WHO, 1978; Health Canada, 1992).

Public water supplies are rarely heavily contaminated by nitrate because they are usually more closely protected and water authorities are obliged to comply with certain water quality standards. Private wells, the quality of which is not usually regulated, are at greater risk of contamination, in particular shallow wells that are located in agricultural areas. Nitrate is one of the major causes of eutrophication in surface water which leads to abundant growth of algae and aquatic plants. As a result, the concentration of nitrate in surface water is usually lower than that in groundwater (WHO, 1978; Health Canada, 1992).

The treatment of contaminated groundwater is very expensive and natural groundwater recharge can often take many years or decades. Despite a reduction in the use of commercial nitrogen fertilizers in Europe in the 1990s, no substantial reduction in the pollution of groundwater by nitrate has been observed in recent years (European Environment Agency, 2003).

(a) *Nitrate*

Many studies have been carried out on the occurrence of nitrate in sources of water as part of the characterization of water quality throughout the world; most of these have focused on special areas where waters were affected by specific sources of contamination, primarily agricultural activity. In most cases, the water studied was used for multiple purposes and the focus of the study was the impact of environmental sources of contamination.

(i) *Levels and sources of contamination of groundwater*

Groundwater is particularly vulnerable to contamination by nitrate. Data from several continents on the concentrations of nitrate in groundwater are summarized in Table 1.4.

Africa

Two studies were conducted in Morocco. Mourabit *et al.* (2002) evaluated 53 wells and springs located in the Loukkos area where agricultural activity was substantial. Wells were selected in arable areas where fertilizers were used extensively. Concentrations of nitrate-N ranged from 0 to 32.6 mg/L, and 23% of the samples (12/53) contained more than 11.3 mg/L. Shallow wells were more heavily contaminated than deep wells or springs, and extensive use of fertilizers was identified as the main cause of the contamination. Koukal *et al.* (2004) studied the quality of groundwater in the Fez area. Seven shallow wells that were located near rivers polluted by industrial discharges were sampled once; concentrations of nitrate-N ranged from undetectable to 9.4 mg/L.

Chippaux *et al.* (2002) studied representative samples of groundwater aquifers in Niamey, Niger. Seventeen shallow wells and 20 boreholes were monitored during 1998–99. The mean of concentrations in superficial aquifers (shallow wells) varied from 0.2 to 7.7 mg/L nitrate-N; those in borehole wells (depth, 50–70 m) were lower, and varied from 0.05 to 1.6 mg/L nitrate-N. The concentration of nitrate increased slightly during the dry

Table 1.4. Concentrations of nitrate^a in groundwater of different regions of the world

Country, region	Type of water/identification	Population covered	Nitrate-N (mg/L)	Main activities or sources of pollution	Reference
Africa					
Morocco, Loukkos region	Shallow and deep wells	500 000	0–32.6	Mainly agricultural	Mourabit <i>et al.</i> (2002)
Morocco, Fez region	Shallow wells	850 000	<0.2–9.4	Extensive industrial development	Koukal <i>et al.</i> (2004)
Niger, Niamey	Shallow wells and boreholes	700 000	0.05–7.7	Large urbanized area	Chippaux <i>et al.</i> (2002)
Nigeria, Lagos and Ibadan	Shallow and deep boreholes	~8.2 million	1.4–13.4	Some wells near dumpsites	Sridhar (2000)
Senegal, Dakar	Dakar aquifer	2 million	9.7–81.1 (mean, 42.9)	Lack of sanitation, faulty latrines	Tandia <i>et al.</i> (2000)
Asia					
India–Pakistan	Superficial aquifer (Hudiara drain)	37 villages	2.2–14.6	Agriculture, discharge of untreated industrial and sewage waste	Afzal <i>et al.</i> (2000)
Japan, near Nagoya City	Kakamigahara aquifer	135 000	0.5–26.8 (mean, 9.1)	Agricultural, industrial and urbanized areas	Mohamed <i>et al.</i> (2003); Babiker <i>et al.</i> (2004)
Lebanon, Akkar region	Groundwater	NR	7.5–36.8	Agricultural area, salt-water intrusion	Halwani <i>et al.</i> (1999)
Philippines, Iloco Norte	Shallow wells		0–27	Intensively cropped (mostly rice–sweet pepper sequence)	Shrestha & Ladha (2002)
Turkey, Konya	Groundwater aquifers	850 000	Min., 0.4–3.0; max., 32–110; mean, 2.2–16	Rapidly growing urban area, multiple sources of contamination including agricultural and industrial activities	Nas & Bertkay (2006)

Table 1.4 (contd)

Country, region	Type of water/identification	Population covered	Nitrate-N (mg/L)	Main activities or sources of pollution	Reference
Europe					
France, Brittany, Pyrenees and Allier regions	Wells and boreholes, 3 hydrological contexts		Averages, 6.8–24; range, <0.05–55.4	Animal manure, mineral fertilizers and wastewater discharges	Widory <i>et al.</i> (2005)
United Kingdom, England and Wales	Groundwater vulnerable to nitrate pollution (boreholes)		3.4–10.4	Agricultural areas	Lake <i>et al.</i> (2003)
United Kingdom, mid-Wales	Groundwater (boreholes)		0.0–1.3 (mean, 0.2)	Moorland and forested catchments	Neal <i>et al.</i> (2003)
North America					
USA, nationwide	Groundwater from 23 national water-quality assessment units (through 1992)		Median: forest land, 0.1; agricultural land, 3.4; urban land, 1.8	Forest, agricultural and urban land	Mueller <i>et al.</i> (1995)
USA, nationwide	Groundwater from major aquifers at 20 national water-quality units (1992–95)		Median: agricultural land use, 3.4; urban land use, 1.6; major aquifers, 0.5	Agricultural and urban land use	Nolan & Stoner (2000)
USA, California	Community wells operated by cities of Fresno and Clovis		2% >10.2; 29% 4.5–10.1	Sewage plants, septic tanks, agricultural use of fertilizer and wineries	Kloos (1997)
USA, Michigan	Groundwater (domestic wells)		0.0–48.6 (mean, 1.3)	Agricultural land use	Chowdhury <i>et al.</i> (2003)

Table 1.4 (contd)

Country, region	Type of water/identification	Population covered	Nitrate-N (mg/L)	Main activities or sources of pollution	Reference
USA, Ohio	Groundwater (subsurface flow)		2.0–25.5	Experimental watershed, surface run-off and subsurface flow; some agricultural activity	Owens & Bonta (2004)
USA, Oklahoma and Texas	Groundwater near Lake Texoma		Medians, 0.1–3.1; max., 12	Agriculture, oil production and residential areas	Kampbell <i>et al.</i> (2003) An <i>et al.</i> (2005)
USA, Texas	Groundwater from Gulf Coast aquifer	5 million	0.1–5.2 (median, 0.1)	Oil/gas production and agriculture; salt-water intrusion	Hudak & Wachal (2001)
USA, Texas	Bolson aquifers/El Paso		<0.1–11.7 (median, 0.4–1.7)	Farming, ranching and recreational land use	Hudak (2003)
USA, Texas	Nine counties, northeast (well water)	4.7 million	<0.07–23.0 (median, <0.07)	Urban and agriculture land use	Hudak & Sanmanee (2003)
USA, Washington State	Groundwater; shallow and deep wells (northwestern)		0–32.4	Excessive regional agricultural practices	Mitchell <i>et al.</i> (2003)
USA, West Virginia	Appalachian region (karst terrain)		0.1–3.5 (medians)	Agricultural activities	Boyer & Pasquarell (1995)
South America					
Venezuela, Maracaibo area	Groundwater	~2 million	0.2–9.5	Rural and urbanized areas	Morales <i>et al.</i> (2000)
Oceania					
New Zealand, Waikato region	Shallow aquifer		0.02–25 (average, 4.8)	Intensive grazing, gardening, pastoral land use and livestock	McLay <i>et al.</i> (2001)

max., maximum; min., minimum; NR, not reported

^a All original values have been converted into mg/L nitrate-N.

season. Lack of sanitation and seepage from animal and human excreta into the superficial layers were postulated to be the source of pollution.

Sridhar (2000) studied 114 shallow and deep borehole wells in different major cities in Nigeria. Concentrations ranged from 1.4 to 13.4 mg/L nitrate-N. Lack of sanitation and proximity of waste dumpsites were identified as the main sources of contamination.

Tandia *et al.* (2000) studied the groundwater supply of Dakar, Senegal. Dug wells and piezometers were sampled in a vulnerable area [exact number of wells and samples not detailed]. The average concentration of nitrate-N was 42.9 mg/L with a range of 9.7–81.1 mg/L. A large increase in contamination was noted compared with previous samplings. The concentration of nitrate increased with proximity to latrines. Rapid demographic expansion and lack of adequate sanitation practices were described as the main causes of the contamination.

Asia and Oceania

In an industrial area of India and Pakistan, Afzal *et al.* (2000) studied the quality of water that received large quantities of untreated waste from factories. Twenty-one groundwater samples from superficial wells (depth, 1–2 m) were collected along the Hudiara drain; concentrations of nitrate-N ranged from 2.2 to 14.6 mg/L.

The quality of water was evaluated in Japan (Mohamed *et al.*, 2003; Babiker *et al.*, 2004) in the Kakamigahara groundwater basin that was previously known to be contaminated with nitrate due to agricultural activities. Fifty-seven samples were taken from domestic, farm, monitoring and public supply boreholes; 22% of the samples contained more than 9.9 mg/L N-nitrate and 90% contained more than 2.9 mg/L; the maximum level was 26.8 mg/L. A geographical information system analysis demonstrated an association between contamination with nitrate and intensive cultivation of vegetables. The concentration of nitrate was correlated with the depth of the groundwater and inversely correlated with the amount of precipitation. Some contamination was found in the urban area and was associated with nearby vegetable fields, with some urban sources or with sites of animal husbandry.

Halwani *et al.* (1999) studied 15 shallow wells in the northern region of Lebanon (Akkar plain) which was known to be susceptible to contamination by nitrate from agricultural activities. Concentrations of nitrate-N ranged from 7.5 to 36.8 mg/L, and all but one well had levels higher than 11.3 mg/L. Extensive fertilization and lack of vegetation during the winter were identified as the main causes of pollution of the groundwater by nitrate.

In the Philippines, Shrestha and Ladha (2002) monitored 21 water sources (eight from hand pumps on farms) that were located in a watershed where agriculture had been practiced for 1 year. Twelve (57%) had a concentration of nitrate-N above 10 mg/L with a maximum of 27 mg/L. Concentrations of nitrate were maximal in wells that were highest above sea level and increased slightly during the rainy season. The effect of high elevation might be due to a type of agriculture that needs greater amounts of fertilizer.

Nas and Berktaý (2006) studied the contamination by nitrate of the groundwater source of the city of Konya in Turkey. Water samples were taken from 139 wells in 1998 and 156 wells in 2001. Average concentrations of nitrate-N increased from 2.2 mg/L in 1998 to 16.1 mg/L in 2001. The maximum concentration increased from 32 mg/L in 1998 to 110 mg/L in 2001. Several factors were postulated as possible causes of the contamination: run-off or seepage from fertilized agricultural land, municipal and industrial wastewater, refuse dumps, animal feedlots, septic tanks and private sewage disposal systems.

In New Zealand, McLay *et al.* (2001) analysed data from the Groundwater Nitrate Monitoring Project that was conducted in the Waikato region—the most intensely farmed area of this country. Water samples were taken twice a year (early autumn and spring) for 3 years (1995–98) from 88 borewells that covered diverse land uses. The concentrations of nitrate in groundwater, averaged over the 3 years, varied from 0.02 to nearly 25 mg/L nitrate-N. About 9% of the wells exceeded the 11.3-mg/L standard and 56% of the sites had a mean level higher than 3 mg/L. The concentration of nitrate was higher in areas where market garden crops were cultivated. High values were also detected in other farm areas.

Europe

In France, Widory *et al.* (2005) studied sources of contamination of groundwater by nitrate using an isotopic approach in wells and boreholes of three watersheds that had different hydrogeological contexts: fractured bedrock (Arguenon, Brittany), deep alluvial groundwater (Pia, Pyrenees) and subsurface alluvial groundwater (Ile du Chambon, Allier). In the first aquifer, nine sites were sampled five times over 1.5 years, and the mean concentration of nitrate-N was 24.0 mg/L (range, 0.7–55.4 mg/L). The main source of contamination, as suggested by analysis of ^{15}N and ^{11}B isotopes, was animal manure (especially pig farms). Wastewater sewage was also identified as a possible contributor. In the second watershed, eight sites were sampled twice at a 6-month interval, and the average concentration of nitrate-N was 11.5 mg/L (range, 2.3–31.4 mg/L). Mineral fertilizers were identified as the main contributor to contamination by nitrate, and wastewater discharge was also considered to be a possible source. In the third watershed, 16 sites were sampled twice at a 6-month interval, and the average concentration of nitrate-N was 6.8 mg/L (range, undetected–12.0 mg/L). Wastewater was identified as the main source of pollution of the groundwater.

Lake *et al.* (2003) monitored concentrations of nitrate in groundwater from agricultural areas of England and Wales using the results from more than 3700 sites. A geographical information system was used to identify areas that were susceptible to pollution by nitrate. Mean concentrations of nitrate-N ranged from 3.4 to 10.4 mg/L and declined with increasing depth, but class of vulnerability (based on surface leaching, soil characteristics, drift cover and type of aquifer) was a more important factor.

Neal *et al.* (2003) studied groundwater contamination by nitrates in mooreland and forested catchments in mid-Wales (United Kingdom). Twenty boreholes were studied,

most of which were monitored monthly over 1 year. Four boreholes were monitored every 2 weeks over 4 years after tree felling. The mean concentration of nitrate-N in boreholes before felling was 0.2 mg/L with a maximum of 0.5 mg/L. After tree felling, the concentration increased to a maximum of 1.3 mg/L. These increases lasted for at least 3 years after felling. Shallow depths contained higher concentrations of nitrate.

North America

Several studies have been conducted on the concentration of nitrate in groundwater in the USA. Major nationwide studies were carried out by the Geological Survey, and some states conducted their own studies.

Mueller *et al.* (1995) analysed data from 11 715 groundwater sites that were located in 23 study areas of the National Water-Quality Assessment Program (NAWQA) from 1970 to 1992. Water samples collected from wells (and a few springs) to monitor for compliance or to conduct research were selected after the elimination of those that were not considered to be representative. The median concentrations of nitrate-N were 0.2 mg/L in public water supplies, 1.3 mg/L in domestic wells and 2.4 mg/L in wells that were used for irrigation and stock water. The concentration in 1% of public water supplies exceeded the 10-mg/L standard compared with 9% of domestic supplies and 16% of samples from irrigation and stock water. Median levels of nitrate were higher in samples from agricultural land (3.4 mg/L) than those from urban land (1.8 mg/L) or forest land (0.1 mg/L). Highest levels were found in wells with a depth of less than or equal to 100 feet [30 m], in well drained soil and in areas where cropland was important. Among shallow wells, more than 20% exceeded the 10-mg/L guideline. Data on time trends were limited. Nevertheless, some areas showed a clear gradual increase in concentration of nitrate over the years (Georgia–Florida and Central Columbia Plateau). Nolan and Stoner (2000) analysed data from 2130 wells sampled in 20 study units of the NAWQA Program during 1992–95. Results were similar to those of Mueller *et al.* (1995).

In California, Kloos (1997) analysed data on concentrations of nitrate in 302 community wells in the Fresno-Clovis metropolitan area. Two annual test results were obtained in 1988–93. Nitrate was detected in samples from all wells. Six wells were closed because they had concentrations of nitrate-N that exceeded 10 mg/L and 86 (29%) had concentrations between 4.5 and 10 mg/L. Major sources of nitrate in groundwater in this area were sewage plant effluent, septic tank disposal systems and agricultural use of fertilizers.

Chowdhury *et al.* (2003) analysed data from 2435 domestic wells in Kalamazoo County, southwestern Michigan. The mean concentration of nitrate-N was 1.3 mg/L (range, 0–48.6 mg/L), and about 3% of the wells had concentrations above the 10-mg/L standard. The relative vulnerability of wells (based on the thickness of clay and partial clay layers and depth of the well) was associated with an increase in the concentration of nitrate. Agriculture was the largest land-use category of the county (about 40%).

Owens and Bonta (2004) conducted a study at the North Appalachian Experimental Watershed near Coshocton, Ohio. Concentrations of nitrate-N in groundwater were

assessed in four small watersheds over a 7-year period; they increased with degree of use of fertilizers (range, 13–25.5 mg/L) and decreased after 5 years of alternative management practice of grazing and growing hay with no use of fertilizers (range, 2.1–3.9 mg/L).

Kampbell *et al.* (2003) analysed data on water quality during drought conditions from 55 monitoring wells that surround Lake Texoma (border of Oklahoma and Texas). Median concentrations of nitrate-N were higher in samples from the agricultural zone (2.0 mg/L) and the area with septic tanks (1.4 mg/L), followed by those from land used for recreational purposes (0.6 mg/L) and oil production (0.1 mg/L). The maximal concentrations were 12.0 mg/L nitrate-N in the agricultural zone and 6.5 mg/L in the area with septic tanks. Concentrations of nitrate in groundwater were higher in sandy soils than in clay soils and increased during the drought season. The occurrence of nitrate in the agricultural area was explained by the application of fertilizers in vulnerable areas. An *et al.* (2005) extended this study over a 2-year period and found that residential areas had wider ranges of levels and slightly higher median levels of nitrate-N than agricultural areas (3.1 versus 2.5 mg/L).

Three studies were conducted in Texas. Hudak and Wachal (2001) evaluated concentrations of nitrate in 253 wells in the southeastern Gulf Coast aquifer and found that shallow wells and those located near cropland contained higher levels. Hudak (2003) evaluated groundwater supplies from 174 wells of five aquifers in the El Paso area. Median concentrations in aquifers ranged from 0.4 to 1.7 mg/L nitrate-N with maxima of 0.7–11.7 mg/L. The use of fertilizers was suspected to be the major source of nitrate in groundwater. Hudak and Sanmanee (2003) evaluated 110 wells in the Woodbine aquifer and found that the median concentration of nitrate-N was below the limit of detection (0.07 mg/L) with a maximum of 23 mg/L. The use of lawn fertilizers in urban areas and a combination of crop fertilizer and septic tank systems in agricultural areas were identified as possible sources of contamination.

Mitchell *et al.* (2003) studied the distribution of nitrate in the Abbotsford-Sumas aquifer in northwestern Washington State. A 22-month assessment of nitrate in groundwater was performed on a total of 504 samples from 26 domestic wells. The median concentration of nitrate-N ranged from 0 to 25.3 mg/L in shallow wells and from 0 to 13.5 mg/L in deep wells. The highest concentrations were linked to agricultural activity with a maximum of 32.4 mg/L. The impact of agricultural activity on a karst aquifer in southeastern West Virginia was assessed by Boyer and Pasquarell (1995). Two basins that had high levels of agricultural activity were compared with two others that were mostly forests. Water samples were taken weekly over several months. The median concentration of nitrate-N ranged from 2.8 to 3.5 mg/L in the agricultural areas and from 0.1 to 0.6 mg/L in the forest and areas with low levels of agriculture.

South America

Morales *et al.* (2000) studied the concentration of nitrate in groundwater in three water networks (one urban and two rural) in the Lake Maracaibo basin in Venezuela.

About 15 samples were taken at each site over several months. The mean concentrations of nitrate-N were 5.5 mg/L (range, 3.6–9.5 mg/L) in the urban network and 1.3 and 0.7 mg/L in the two rural areas. The sources of contamination of the urban network were not identified.

(ii) *Levels and sources of contamination of surface water*

The concentration of nitrate is usually lower in surface water than in groundwater, partly due to the uptake of nitrate by algae and other plants. Although the major sources of nitrate in surface waters are fertilizers and manure, in some areas, they are also contaminated by atmospheric sources (Graffy *et al.*, 1996). Several studies have recently been conducted worldwide and the results are presented in Table 1.5.

Africa

Ismail and Ramadan (1995) studied water samples from the Nile at several locations between Aswan City and Cairo. Levels of nitrate were very low (range, 0–0.2 mg/L nitrate-N). Koukal *et al.* (2004) analysed data on the quality of water from the Fez river in Morocco upstream from the Fez medina. The concentrations of nitrate-N ranged from undetectable to 2.5 mg/L.

Olajire and Imeokparia (2001) investigated the quality of water from six rivers in southwestern Nigeria (Osun State and Osogbo) in 1998. Mean concentrations of nitrate-N ranged from 4.6 to 8.9 mg/L. Intensive agricultural practices and inadequate disposal of domestic and municipal wastes were identified as the probable sources of contamination. Onyeike *et al.* (2002) studied levels of nitrate in streams located in four zones in Nigeria that had soils polluted from crude oil spillage and compared them with unpolluted waters. Concentrations of nitrate-N in the polluted areas ranged from 1.5 to 2.4 mg/L. Two control streams had concentrations of 0.6–0.8 mg/L.

Jonnalagadda and Mhere (2001) evaluated the quality of water from the Odzi River in the eastern highlands of Zimbabwe. River samples were collected at six sites over 9 months, and concentrations ranged from 0.4 to 1.0 mg/L nitrate-N.

Asia and Oceania

Cheung *et al.* (2003) studied the concentrations of nutrients in the Pearl River delta located in South China. Samples were collected at 16 sites along the estuary; concentrations of nitrate-N were typically inferior to 10 mg/L, except at one site where a concentration of 22 mg/L was measured. This high level was attributed to the influence of nearby cultivated areas.

Massoud *et al.* (2006) conducted an intense sampling programme to screen the water quality of water from the Abou Ali River in Lebanon. Three types of subcatchment were considered: mountainous forest, mountainous rural and urban. Samples were taken during three periods: the end of the dry season, and the peak and end of the wet season. Average concentrations of nitrate-N ranged from 1.8 to 3.4 mg/L in the forest area, from 1.8 to 4.7 mg/L in the rural area and from 2.9 to 4.4 mg/L in the urban area. There was little

Table 1.5. Levels of nitrate in surface water of different regions of the world

Country, region	Type of water	Population	Nitrate-N (mg/L)	Main activities or sources of pollution	Reference
Africa					
Egypt	River Nile between Aswan City and Cairo		0.0–0.2 (mean, 0.1)	Dam and industrial plants	Ismail & Ramadan (1995)
Morocco, region of Fez	River (main streams)	850 000	0–2.5	Extensive industrial development	Koukal <i>et al.</i> (2004)
Nigeria, southwestern	River Osum	557 000	4.6–8.9	Growing urbanization	Olajire & Imeokparia (2001)
Nigeria, Niger River delta	Streams		0.6–2.4	Heavily populated with surface oil pipelines	Onyeike <i>et al.</i> (2002)
Zimbabwe, eastern	River in plains and highlands		0.4–1.0	Some abandoned mines and mine dumps	Jonnalagadda & Mhere (2001)
Asia					
China, South	Pearl River delta	21.4 million	0–22	Industrial wastewater and domestic sewage	Cheung <i>et al.</i> (2003)
Lebanon, Tripoli area	Abou Ali River	350 000	1.8–4.7	Forest, rural and urban	Massoud <i>et al.</i> (2006)
Europe					
Germany, Saxony	Seven reservoirs and 22 tributaries	~2 million	0.28–6.0	Deforestation and reforestation	Ulrich <i>et al.</i> (2006)
Spain, South central (Castilla–La Mancha region)	Five rivers	~2 million	0.9–3.2 (pristine rivers); 2.7–8.7 (polluted rivers)	Mostly agricultural	Moreno <i>et al.</i> (2006)
United Kingdom, southern England	River Pang, tributary of the River Thames		5.0–8.4	Rural, sparsely populated	Neal <i>et al.</i> (2000a)

Table 1.5 (contd)

Country, region	Type of water	Population	Nitrate-N (mg/L)	Main activities or sources of pollution	Reference
United Kingdom, central England	Great Ouse River	1.6 million	5.4–15.7	Essentially rural, some important cities (Cambridge) and 500 sewage works	Neal <i>et al.</i> (2000b)
United Kingdom, southern central England	River Thames, upstream of London		4.2–12.9 (mean, 8.3)	Rural farming area	Neal <i>et al.</i> (2000c)
United Kingdom, Northeast England	River Wear		1.1–3.3 (mean, 2.1)	Arable farmland and former mining activity	Neal <i>et al.</i> (2000d)
United Kingdom, Aberdeenshire	Newmills burn		1.1–12.7 (mean, 6.1)	Pasture and farming activities	Petry <i>et al.</i> (2002)
North America					
USA, nationwide	20 national water-quality assessment units (different streams)		Agricultural land use, 1.1; agricultural/urban land use, 0.8; urban land use, 0.8; forest and rangeland, <0.2	Forest, agricultural and urban land	Mueller <i>et al.</i> (1995)
USA, Colorado, New Mexico	River Rio Grande		0.10–0.57 (median across 25 years)	Agricultural, residential and industrial uses	Passell <i>et al.</i> (2005)
Oceania					
Australia	River Tully		0.03–1.0 (mean, 0.1)	Cultivation of sugarcane and bananas	Mitchell <i>et al.</i> (2001)

difference in concentrations between the dry and wet seasons. The forested subcatchment was affected by nearby agricultural activity.

Mitchell *et al.* (2001) evaluated levels of nitrogen in the Tully River in North Queensland, Australia. Sugarcane and bananas were the main crops grown in the river catchments studied for the period 1989–2000. Concentrations of nitrate-N (plus nitrite) ranged from 0 to 1 mg/L (mean, 0.1 mg/L). Levels increased with agricultural activity, especially in the low-flow sectors.

Europe

Ulrich *et al.* (2006) conducted a study in seven acidified reservoirs that were affected by acid rain and 22 tributaries in the Erzgebirge region in southeastern Germany. The concentrations of nitrate-N in the reservoirs were monitored from 1993 to 2003 and ranged from 0.28 to 6.0 mg/L with a marked reduction in more recent years. When all study sites were considered, the authors estimated that the concentration of nitrate-N had decreased by an average of 41%. This decrease was found in 83% of the waters and was attributed to the possible effect of the regenerating vitality of forest vegetation and reforestation that could be associated with a reduction in deposition of atmospheric acidic sulfur.

Moreno *et al.* (2006) conducted a large study in Spain that compared relatively pristine and polluted rivers. The quality of water from five of the main rivers of Spain was assessed during 2001–03. Four main river ecotypes were studied: calcareous headwaters, siliceous rivers, plain rivers and large rivers. Polluted sites were selected in the Castilla–La Mancha River. In the unpolluted rivers, average concentrations of nitrate-N ranged from 0.9 to 3.2 mg/L. In comparison, polluted sites had average concentrations of 2.7–8.7 mg/L. The highest levels in relatively unpolluted rivers were found in calcareous headwaters. In polluted rivers, a strong relationship between the percentage of land used for agriculture and levels of nutrients was recorded.

Many studies have been conducted in the United Kingdom. Neal *et al.* (2000a,b,c,d) conducted a series of studies to evaluate the impact of agriculture on the quality of river water. The first (Neal *et al.*, 2000a) evaluated the River Pang, which is a tributary of the River Thames that drains a rural part of the Thames basin. Four sites were established along the River Pang and periodic sampling was carried out over a 2-year period. Concentrations of nitrate-N ranged from 5.0 to 8.4 mg/L and were higher during winter. This was attributed to increased surface run-off related to agricultural activities. The second study (Neal *et al.*, 2000b) was conducted over a 1-year period on the Great Ouse river that is located in central England. It flows through a highly agricultural area but also receives discharges from sewage works and industrial treatment plants. Concentrations of nitrate-N ranged from 5.4 to 15.7 mg/L. The higher concentration was associated with increased flow which is indicative of increased run-off from the agriculturally impacted soils. The third study (Neal *et al.*, 2000c) was carried out on the River Thames at a rural site downstream from Oxford, which has major agricultural activity. The data covered a 2-year period; concentrations of nitrate-N ranged from 4.2 to 12.9 mg/L with a mean of

8.3 mg/L and increased with higher flow that was related to run-off from agricultural land. The final study (Neal *et al.*, 2000d) was conducted over 1 year on the River Wear, which is located in northeastern England and has low agricultural input; the mean concentration of nitrate-N was 2.1 mg/L. Petry *et al.* (2002) studied the quality of water from the Newmills burn, Aberdeenshire, in agricultural parts of lowland Scotland. The mean concentrations of nitrate-N ranged from 4.28 to 7.08 mg/L with individual values in the range of 1.1–12.7 mg/L.

America

Mueller *et al.* (1995) analysed data on nutrients in more than 22 000 surface water samples in the USA. Data sets were compiled for each of the 20 NAWQA study units of the Geological Survey and were restricted to 1980–90. Samples were limited to one per month and several data sets were excluded because of potential bias (e.g. streamflows of less than 1 ft³/s [~ 28 dm³/s]). Unit land use was assessed to determine the dominant type of use that affected each of the selected sites. Median concentrations of nitrate-N ranged from about 1 mg/L (for agricultural use of the land) to <0.2 mg/L (for forest and rangeland). Large variations were observed within and between units. For instance, in the South Platte River Basin unit, median concentrations of nitrate-N were about 3 mg/L for agricultural land use. Generally, for non-point sources (such as agricultural activities), the concentration of nitrate increased with streamflow. The 10-mg/L drinking-water standard for nitrate-N was rarely exceeded in rivers and was less than 1% at agricultural sites. In forest/rangeland areas, the concentration of nitrate-N was generally less than 0.7 mg/L.

Passell *et al.* (2005) analysed 25-year temporal trends and spatial patterns for nutrients measured at six Geological Survey stations in the upper 600 km of the Rio Grande in Colorado and New Mexico. The monthly average concentration of nitrate-N (plus nitrite) ranged from 0.1 to 0.57 mg/L. The concentration of nitrate was stable in four sites and increased in two sites during this period. These two sites were characterized by predominant agriculture use of land.

(iii) *Levels of nitrate in drinking-water*

Few studies have been carried out that specifically evaluated the concentration of nitrates in drinking-water. Studies on the quality of water consumed by humans are presented in Table 1.6. Most studies were conducted in Europe and North America and some in Asian countries. Nitrite is usually integrated with the measurement of nitrate in these studies, and, therefore, most measurements refer to nitrate plus nitrite.

Few reports on public supplies were available. In China, a limited study reported that seven (64%) of 11 medium-sized cities (between 10 000 and 100 000 inhabitants) had water supplies with a concentration greater than 11.3 mg/L nitrate-N (Zhang *et al.*, 1996). Randomly selected public wells were also frequently found to be contaminated in an Indian region (28% above 11.3 mg/L; Manjappa *et al.*, 2003). In Saudi Arabia, a survey of representative samples of nationwide wells found that 13% had concentrations greater than 11.2 mg/L nitrate-N (Alaa el-Din *et al.*, 1994).

Table 1.6. Concentration of nitrate in drinking-water

Country, region	Location; type of water	Date; number of samples and source	Potentially exposed population	Nitrate-N (mg/L)	Reference
Asia					
China, Beijing, Hebei, Shanong, Tianjin	3 large cities, 11 towns and rural areas; mostly groundwater	1993–94; 69 locations, house wells and some waterworks	Millions	Major cities: 0–7.9; towns: 0–67.7 (7/11 >11.3); farmers' household wells: 0–67.7 (5/9 >23)	Zhang <i>et al.</i> (1996)
India, Karnataka State	61 public borewells selected at random	1999–2000; 3 samples per well	NR	0.02–69.6 (mean, 12.6; 28% >11.3; 16% >22.6)	Manjappa <i>et al.</i> (2003)
Saudi Arabia, nationwide	Groundwater, 1062 wells	1984–88; 4255 samples	NR	0–31.6 (13% >10.2; 1% >20.3)	Alaa el-Din <i>et al.</i> (1994)
Turkey, Ankara	Groundwater with potential use by military personnel	2002; 34 wells	NR	ND–14.7 (mean, 5.9)	Bakir <i>et al.</i> (2003)
Europe					
France	Cities with ≥5 000 inhabitants	1993–95; 2109 distribution units	73% of the French population	~6% >11.3 (max., 37.3)	Godet <i>et al.</i> (1998)
Greece, Thessaloniki province	Groundwater from 52 villages	2001; 52 samples of tap-water	NR	0.3–31.9 (mean, 5; 8% >11.3)	Fytianos & Christophoridis (2004)
Netherlands, Limburg region	Plateau of Schimmert; groundwater	1984–99; 16 natural springs	NR	9–45 (94% >11.3)	van Maanen <i>et al.</i> (2001)
Spain, Canary Islands	Island of Tenerife; 31 municipal areas	1999; 218 samples of tap-water	4.7 million tourists/year	0.1–12.9 (means; 2% >11.3)	Caballero Mesa <i>et al.</i> (2003)
Spain, Galicia region	31 springs	2000–01; 185 samples	NR	0–12.2 (mean, 1.9; 6% >11.3)	Salgado <i>et al.</i> (2003)
United Kingdom, West Midlands	Private water supplies in rural areas	1995–96; 1297 samples	NR	21% >11.3, mostly <22.7	Harrison <i>et al.</i> (2000)

Table 1.6 (contd)

Country, region	Location; type of water	Date; number of samples and source	Potentially exposed population	Nitrate-N (mg/L)	Reference
North America					
Canada, New Brunswick	Carleton County (three rural areas); groundwater	1984–85; 300 rural wells	NR	10–17%, 10–20; 1.1–1.5% >20	Ecobichon <i>et al.</i> (1985)
Canada, Québec Province	Portneuf (near Québec City), rural area	1995; 71 private wells	52 500	20% >3; 6% >10	Levallois <i>et al.</i> (1998)
Canada, Québec Province	Ile d'Orléans (near Québec City), rural area	1995; 87 private wells	NR	41% >3; 5% >10 (max., 16)	Chartrand <i>et al.</i> (1999)
Canada, Saskatchewan	Rural area; groundwater	2000; 3425 private wells	500 000	14% >11.3; 6% >22.6 (max., 216)	Thompson (2001)
USA, nationwide	Rural area; groundwater	1990; 2213 domestic wells	NR	2.4% >10 4.9 % >10	Spalding & Exner (1993)
USA, 18 states	Swine farms; groundwater	1991; 631 private wells	631 families	0.25–111.3 (mean, 4.0; 11.7% >10.2; 4.3% >22.6)	Bruning-Fann <i>et al.</i> (1994)
USA, Midwest	Nine states; domestic wells	1994; 5520 samples	5520 families	Mean, 8.4 (range, 0.01–266; 13.4% >10)	Center for Disease Control and Prevention/ National Center for Environmental Health (1998)
USA, Iowa	Statewide; groundwater	1988–89; 686 private rural wells	NR	<0.1–100 (mean, 6.2; 18.3% >10)	Kross <i>et al.</i> (1993)

Table 1.6 (contd)

Country, region	Location; type of water	Date; number of samples and source	Potentially exposed population	Nitrate-N (mg/L)	Reference
USA, New York State	Groundwater	1995–96; 419 private rural wells	NR	0–31.5 (16% >10)	Gelberg <i>et al.</i> (1999)
USA, Oregon	Umatilla and Morrow Counties; groundwater	1990–91; 80 private wells	219 people	25% >10 (median, 4.5)	Mitchell & Karding (1996)
USA, Pennsylvania	35 counties; groundwater	1989–92; 1583 private wells	NR	9.4% >10	Swistock <i>et al.</i> (1993)
USA, South Carolina	21 counties in Piedmont and Upper Coastal Plain; groundwater	2000–01; 70 private wells	NR	Average, 0.94 (29% >1)	Aelion & Conte (2004)

max., maximum; ND, not detected

NR, not reported

In France, a study of the quality of public water supplies reported that about 6% of distribution units that serve at least 5000 or more inhabitants had concentrations, at least once during 1993–95, greater than 11.3 mg/L nitrate-N (Godet *et al.*, 1998). A recent report of the European Environment Agency (2003) that used data from 28 countries concluded that pollution ‘hot spots’ occur in several European countries and that, in about seven of 17 countries, the guideline level of 5.65 mg/L nitrate-N is exceeded at more than a quarter of the sampling sites; the most affected area was the Republic of Moldova where about 35% of sampling sites exceeded the 11.3-mg/L nitrate-N guideline.

Most of the studies in North America concerned quality of private well-water. Contamination of wells situated in agricultural areas was frequent: 5–10% or more of the wells had concentrations above 10 mg/L nitrate-N, occasionally with very high maxima. For instance, in Saskatchewan (Canada), a report on 3425 private wells found that 14% had a concentration of nitrate-N (plus nitrite) above 11.3 mg/L with a maximum of 216 mg/L. The sample was not taken at random. Since this province offers free measurements of nitrate to private well owners, it was considered to be a good example of the quality of the groundwater at least for some high-risk areas (Thompson, 2001). In another study of 631 private wells on swine farms in 18 states in the USA, about 12% of wells were found to have a concentration of nitrate-N greater than 10.2 mg/L, with a maximum of 111 mg/L (Bruning-Fann *et al.*, 1994).

(b) *Nitrite*

The content of nitrite in groundwater or surface water is generally negligible compared with that of nitrate. However, under anaerobic conditions, nitrate may be converted to nitrite through microbial contamination (Health Canada, 1992).

Few studies have specifically evaluated the presence of nitrite in sources of water. Table 1.7 presents results of recent available studies.

Concentrations of nitrite-N rarely exceeded 1 mg/L. The highest level reported (7.9 mg/L) was found in a private well located on a swine farm in the USA (Bruning-Fann *et al.*, 1994).

The use of chloramines as a drinking-water disinfectant has been responsible for the presence of nitrite in some distribution networks that used this technology (Health Canada, 1996). Ammonia, which is used in the formation of chloramines, can be oxidized to nitrite. This process can increase levels of nitrite in distribution systems by 0.2–1.5 mg/L (WHO, 2004a).

1.5 Exposure

Exposure to nitrate and nitrite may occur by inhalation during the spreading of fertilizer. This route of exposure is beyond the scope of this monograph and is not reviewed here.

1.5.1 Food

Nitrate is a potential hazard because it can be reduced to nitrite *in vivo* and in food; nitrites can react with amines or other nitrosatable substances present in food to produce N-nitroso compounds (Shephard & Lutz, 1989).

(a) *Methodology*

Dietary exposure can be estimated by several methodologies. One method uses food consumption surveys in combination with levels of the chemical of interest in the food items, and results in so-called modelled dietary exposure that can be based on several scenarios. This method provides deterministic or probabilistic assessments. Food consumption can be derived from individual data or from per-capita estimates; the latter are the only available and comparable data at international levels (FAO-WHO, 2005). Levels of nitrates and nitrites can be determined in raw commodities or in food as consumed, in ‘total diet studies’ (see Section 1.4). Levels in food as consumed are closer to real intake than those in raw commodities (WHO, 1999, 2002b, 2004b).

Another method is based on the analysis of the total content of a meal or of a diet during 1 or several days of a limited number of subjects, and is known as the ‘duplicate diet method’. Results from such studies are available only for the Netherlands and the United Kingdom and are described below.

Table 1.7. Levels of nitrite in sources of water of different regions of the world

Country, region	Type of water/identification	Population	Nitrite-N (mg/L)	Main activities in the area or sources of pollution	Reference
Africa					
Morocco, Loukkos region	Groundwater	500 000	0–0.2	Mainly agricultural	Mourabit <i>et al.</i> (2002)
Niger, Niamey	Groundwater wells	700 000	0–0.03	Urban	Chippaux <i>et al.</i> (2002)
Senegal, Dakar	Dakar aquifer	2 million	0–1.43 (mean, 0.26)	Urban and wastes	Tandia <i>et al.</i> (2000)
Asia					
Saudi Arabia, nationwide	Groundwater wells; 4255 samples from 1062 wells		70%, not detected; 30%, 0.001–1.5	Septic tanks and agriculture	Alaa el-Din <i>et al.</i> (1994)
Europe					
Spain, Galicia region	Springs		0–0.27 (mean, 0.006)	Small towns and pilgrimage	Salgado <i>et al.</i> (2003)
Spain, Castilla–La Mancha region	Five rivers Pristine rivers Polluted rivers	~2 million	0.006–0.014 0.02–0.4	Mostly agricultural	Moreno <i>et al.</i> (2006)
United Kingdom, central England	Great Ouse river	1.6 million	0–0.34	Essentially rural, some large cities (Cambridge) and 500 sewage works	Neal <i>et al.</i> (2000b)
United Kingdom, West Midlands	1297 private water supplies	5.3 million	0–1.5 (1% >0.03)	Mainly rural	Harrison <i>et al.</i> (2000)
North America					
USA, 18 states	Groundwater, 631 private wells		Detected in 1.2% of wells (max., 7.9)	Swine farms	Bruning-Fann <i>et al.</i> (1994)
South America					
Venezuela, Maracaibo area	Groundwater	~2 millions	<0.003	Rural and urbanized areas	Morales <i>et al.</i> (2000)

max., maximum

(b) *Food consumption*

International food consumption data can be extracted from the Global Environmental Measuring Survey food diets. These data are based on the availability of food for each country that is included (production plus imports minus exports) and are collected by the Food and Agricultural Organization (FAO). The data are then grouped on the basis of statistical analyses; this results in the formation of clusters of the countries included according to their dietary information and pattern. These data thus provide average per-capita consumption for given countries or regions. Additional information can be found at www.who.org/foodsafety.

(c) *Dietary exposure assessment*

(i) *Nitrate*

The average dietary ingestion of nitrate by adults in the United Kingdom using the 1997 Total Diet Study was estimated to be 57 mg/consumer per day, with a 97.5th percentile for consumers only for each type of food of 105 mg per person per day (Food Standards Agency, 1998a). Dietary exposure for adult consumers of vegetables commonly eaten in the United Kingdom were estimated at 93 mg per day and 140 mg per day for the mean and the 97.5th percentile, respectively (Ysart *et al.*, 1999b). In a vegetarian duplicate diet over 7 days, the average dietary exposure was estimated to be 53 mg per person per day and up to 84 mg per person per day when taking into account contributions from water and beer (Clarke *et al.*, 2003).

In the Netherlands, the mean dietary exposure of adults estimated by the 'duplicate diet method' in 1994 was 80 mg per person per day (range, 1–322 mg per day). These results are higher than those obtained in 1984–85 (52 mg per day) in the same country and with a similar methodology (Vaessen & Schothorst, 1999).

When the average concentration of nitrate in food from the United Kingdom total diet studies were combined with the corresponding consumption from the 13 clustered diets (FAO WHO, 2005), total dietary exposure ranged from 58 to 218 mg per day. Such a scenario is intended to cover the variability in average dietary patterns around the world. [This range does not take into account variability of nitrate concentration between countries or interindividual variation in the specific food consumed, as well as seasonal or other variations within countries.]

(ii) *Nitrite*

Almost all exogenous exposure to nitrite occurs through food. The average dietary exposure of adults in the United Kingdom using the 1997 Total Diet Study was estimated to range between 0.74 and 1.3 mg per person per day, with a 97.5th percentile for consumers only of 2.2 mg per person per day (Food Standards Agency, 1998a).

In the Netherlands, the mean dietary exposure of adults estimated by the 'duplicate diet method' in 1994 was below 0.2 mg per person per day in the autumn and 0.6 mg per

person per day in the spring (range, 0.1–16 mg per person per day) (Vaessen & Schothorst, 1999).

When the average concentration of nitrate in vegetables, cereals, meats and fish from the United Kingdom Total Diet Study was combined with the corresponding consumption from the 13 clustered diets (FAO WHO, 2005), total dietary exposure ranged from 0.7 to 1.6 mg per day.

The relative contribution of cured meats to total dietary intake of nitrite has decreased substantially over the past 30 years (National Research Council, 1981; Cassens, 1997).

1.5.2 *Drinking-water*

Groundwater is an important source of drinking-water in many countries, and is the main source of drinking-water in Europe (European Chemical Industry Ecology and Toxicology Centre, 1988; European Environment Agency, 2003). In the USA, about 15% of the population has a private water source, mainly from groundwater wells (Environmental Protection Agency, 2002); 40% of water drawn for public supply is groundwater and the drinking-water of more than half of the population is groundwater (Nolan & Ruddy, 1996). In Canada, 30% of the population (almost 9 million people) rely on groundwater for domestic use; two-thirds of them live in rural areas (Environment Canada, 2004/2006). Groundwater, and especially that taken from private wells located in agricultural areas, is the source that is most affected by contamination above the present guideline value of 50 mg/L nitrate (10 mg/L nitrate-N) (WHO, 2003a, 2004a).

Infants constitute a special case. Breast-fed infants are exposed to very little nitrate or nitrite, but bottle-fed infants can be exposed to nitrate from the water used to prepare their formula. In particular, breast-fed infants of mothers who ingest water with a high content of nitrate (up to 100 mg/L nitrate-N) are not considered to be at risk for over-exposure (Greer *et al.*, 2005). In contrast, bottle-fed infants are at risk for over-exposure in areas where the levels of nitrate in drinking-water exceed 50 mg/L (WHO, 2003a).

1.5.3 *Estimated daily intake of nitrate and nitrite from diet and water based on different scenarios of exposure*

Tables 1.8 and 1.9 present the relative contribution of food and water to the total nitrate and nitrite intake of adults according to different scenarios of exposure and consumption. The calculations are based on the average content of nitrate and nitrite in food in the United Kingdom and the average and 95th percentile of food consumption in the 13 clusters of the Global Environmental Measuring Survey studies. The different scenarios are: (i) average consumption of food and water, (ii) high consumption of vegetables (for nitrate) or food that is rich in nitrite (for nitrite), (iii) average consumption of water contaminated with 50 mg/L and (iv) high consumption of water contaminated at this level.

Table 1.8. Estimated daily intake of nitrate in various scenarios

	Average water intake (1.4 L/day)		Average food consumption	
	Average food consumption	High-vegetable diet	Average water intake (1.4 L/day)	High water intake (2.7 L/day)
	mg/day (%) ^a	mg/day (%)	mg/day (%)	mg/day (%)
Diet	52–80 (89–93)	140–220 (96–97)	52–80 (45–56)	52–80 (33–43)
Water	6 (7–11)	6 (3–4)	70 (47–57)	135 (63–72)
Total	58–86 (100)	146–226 (100)	122–150 (100)	187–215 (100)

[The values do not take into account variability of nitrate concentration between countries and location, or interindividual variation in the specific food consumed, or seasonal or other variations within countries. All values were calculated by the Working Group; these calculations were based on average and 97th percentile food consumption in the United Kingdom and average and 95th percentile water intake in the USA.]

^a % of total daily intake

Table 1.9. Estimated daily intake of nitrite from food and water

	Average water intake (1.4 L/day)		Average food consumption	
	Average food consumption	High-nitrite food consumption	Average water intake (1.4 L/day)	High water intake (2.7 L/day)
	mg/day (%) ^a	mg/day (%)	mg/day (%)	mg/day (%)
Diet	0.74 (98)	2.2 (99)	0.74 (14)	0.74 (7.7)
Water	0.014 (2)	0.014 (1)	4.6 (86)	8.9 (92.3)
Total	0.754 (100)	2.214 (100)	5.34 (100)	9.65 (100)

[The values do not take into account variability of nitrite concentration between countries and location, or interindividual variation in the specific food consumed, or seasonal or other variations within countries. All values were calculated by the Working Group; these calculations were based on average and 97th percentile food consumption in the United Kingdom and average water intake in the USA. The high water intake is the 95th percentile of consumption in the USA.]

^a % of total daily intake

1.5.4 *Studies of exposure that used biomarkers*

Due to the important fluctuation in nitrate concentration in both urine and saliva, ‘spot samples’ of either fluid give poor estimates of past exposure to nitrate. Therefore, 24-hour collection of urine is recommended to evaluate total recent exposure to nitrate (Packer *et al.*, 1989). Four studies evaluated the impact of water contamination on total exposure by

assessing urinary excretion of nitrate. In three of the studies, 24-hour collection of urine was used (Table 1.10).

In East Anglia (United Kingdom), Chilvers *et al.* (1984) studied 404 adult users of well-water who completed a diet diary over a 48-hour period and provided a 24-hour urine specimen and a sample of their drinking-water. Mean daily dietary intake of nitrate was estimated by multiplying the food content reported in the diary with the nitrate content of food estimated from the literature. Ingested nitrate from water was estimated by multiplying the mean of the consumption of tap-water on the 2 days of data collection by the concentration of nitrate (plus nitrite) in the water sample. When data were stratified by nitrate intake from food, there was a constant relationship between nitrate intake from water and urinary excretion of nitrate (plus nitrite): the slope of the regression line was 0.6–0.8. The relative contribution of water to total intake (food plus water) of exogenous nitrate increased constantly with increasing concentration of nitrate in the water.

Møller *et al.* (1989) studied 294 adults in Denmark who used underground water from waterworks. Dietary and liquid intake of nitrate (plus nitrite) was estimated by means of a duplicate portion technique for 1 day followed by collection of urine overnight. A sample of tap-water was also taken from the home at each visit. Nitrate concentration in food and liquid was determined using a standard protocol. The concentration of nitrate (plus nitrite) in the water and urinary excretion of nitrate (plus nitrite) were correlated. The relative contribution of water to total intake of exogenous nitrate was estimated to be around 60% when the concentration in water was about 11 mg/L nitrate-N, and around 70% when the concentration in water was 20 mg/L nitrate-N.

van Maanen *et al.* (1994) studied 60 adult women in the Netherlands who used groundwater from public water supplies and private wells. Participants were requested to answer a questionnaire about their food and water consumption and provide a 24-hour urine sample. A sample of water was taken from each private well. Nitrate intake from food was estimated using data from Dutch laboratory studies of nitrate content of food. There was a clear association between urinary nitrate and concentration of nitrate in the water, despite an apparent plateau for the most highly exposed groups. [The number of subjects was small in these two groups (six and ten, respectively).] The relative contribution of water to the total intake of exogenous nitrate was correlated with the concentration of nitrate in the water and was estimated to be 45% when this was about 30 mg/L nitrate-N.

Levallois *et al.* (2000) studied 187 adults in the Province of Québec (Canada) who used private wells as a source of drinking-water. Each participant collected a 24-hour urinary sample and answered a 24-hour dietary and liquid questionnaire. A sample of tap-water was taken in each home and was analysed for nitrate and nitrite. Using the same procedure as Chilvers *et al.* (1984), intake of nitrate (plus nitrite) from food and water was estimated. Two groups were assembled: one with a low concentration of nitrate in the water (< 3 mg/L nitrate-N; geometric mean, 0.18 mg/L) and the other with a moderate concentration of nitrate (geometric mean, 7.1 mg/L nitrate-N; range, 3–28 mg/L). The

Table 1.10. Summary of studies of nitrate intake from water that used urinary nitrate as a marker of internal exposure

Country	Area	Subjects and type of water consumed	Mean concentration ^a in water (mg/L)	Mean intake ^a from water (mg/day)	Mean intake ^a from food (mg/day)	% from water	% from food	Urinary nitrate-N (mg/day)	Reference
United Kingdom	East Anglia	404 adult well-water users	0 (<i>n</i> = 170)	0	13.5	0	100	[19.4]	Chilvers <i>et al.</i> (1984)
			6 (<i>n</i> = 106)	7.45	15.6	32	68	[26.7]	
			15 (<i>n</i> = 93)	20.7	14.0	60	40	[31.6]	
			35 (<i>n</i> = 35)	44.7	18.3	71	29	[58.8]	
Denmark	West Himmerland (northern Jutland)	294 adults, aged 20–64 years, using 21 waterworks	0.07 (<i>n</i> = 115)	1.8**	8.7	17	83	8.2***	Møller <i>et al.</i> (1989)
			11 (<i>n</i> = 76)	13.8**	9.2	60	40	12.4***	
			19 (<i>n</i> = 103)	19.8**	12.6	61	39	16.4***	
Netherlands	Province of Limburg	60 adult women (44 with municipal supply and 16 using private wells)	0 (<i>n</i> = 21)	0	36.6	0	100	10.4	van Maanen <i>et al.</i> (1994)
			4.0 (<i>n</i> = 23)	5.7	37.5	13	87	14.5	
			5.0 (<i>n</i> = 6)	7.9	27.1	23	77	24.2	
			29.6 (<i>n</i> = 10)	24.7	31.4	45	55	26.2	
Canada	Province of Quebec	187 adults, aged 22–73 years, using well-water	0.18* (<i>n</i> = 100)	0.25*	23.0*	1	99	16.86*	Levallois <i>et al.</i> (2000)
			7.10* (<i>n</i> = 87)	9.81*	18.5*	35	65	23.33*	

* Geometric means

** Ingested liquid, including tea and soups

*** Overnight sample only

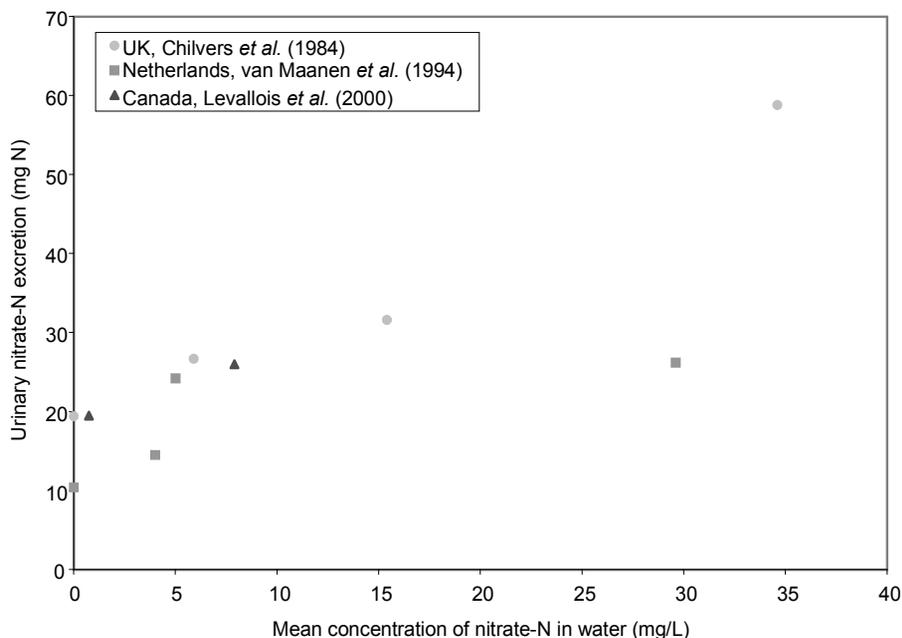
^a Values were converted to nitrate-N

impact of water on exposure to nitrate was clearly observed, with a 33% increase in urinary excretion in the most highly exposed group. The relative contribution of water to the total intake of exogenous nitrate in the moderately exposed group was estimated to be about 30%.

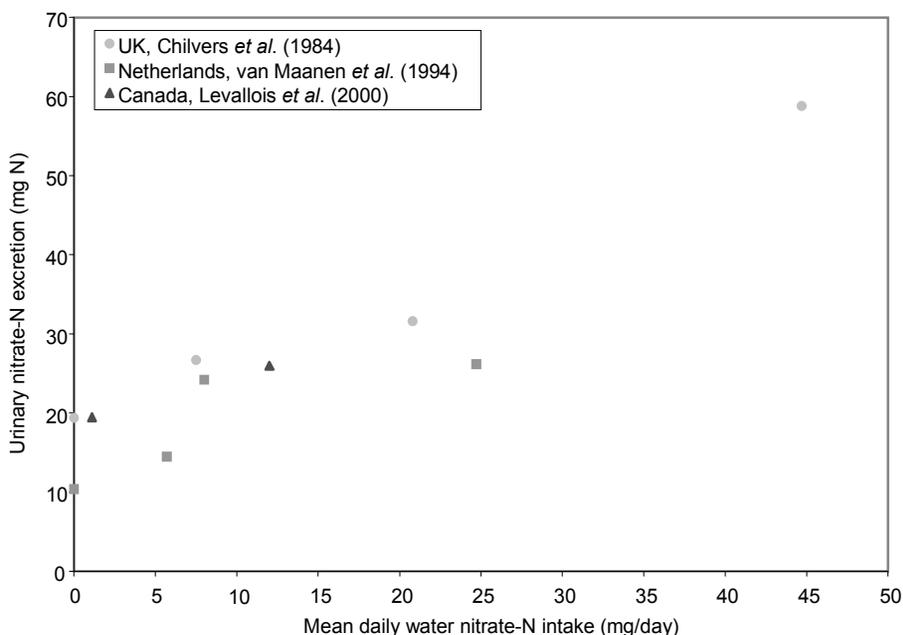
Because of differences in methodology between these studies and some limitations, especially with regard to the assessment of food intake, it is difficult to evaluate the relative importance of nitrate in water to total intake of nitrate. Nevertheless, it can be concluded that, when the concentration of nitrate is greater than the present standard (10 mg/L nitrate-N), nitrate in water becomes an important source of exposure to, and generally the principal source of intake of exogenous nitrate.

Figures 1.1 and 1.2 present the relationship found in these studies between 24-hour urinary excretion and concentration of nitrate in the water or daily intake of nitrate from water, respectively. From the available data, it can be concluded that urinary excretion increases steadily with increasing intake of nitrate from water.

Figure 1.1. Mean concentration of nitrate-N in water versus urinary nitrate-N excretion



Compiled by the Working Group

Figure 1.2. Mean daily water nitrate-N intake versus urinary nitrate-N excretion

Compiled by the Working Group

1.6 Regulations and guidelines

1.6.1 Food

(a) Acceptable daily intake

The Joint FAO/WHO Expert Committee on Food Additives (WHO, 2003b,c) has evaluated the health effects of nitrate and/or nitrite and confirmed the previous acceptable daily intake (ADI) of 0–3.7 mg/kg body weight (bw) per day for nitrate ion and has established an ADI of 0–0.06 mg/kg bw per day for nitrite ion (WHO, 1995). This ADI was also endorsed by the Scientific Committee on Food of the European Commission (1997). However, it was noted that these ADIs do not apply to infants under the age of 3 months. Bottle-fed infants under 3 months of age are most susceptible to methaemoglobinaemia following exposure to nitrate and/or nitrite in the drinking-water (WHO, 2003a).

(b) Permissible levels in food

At the international level, the *Codex alimentarius* elaborates guidelines for maximum permitted levels of residues in food, based on technological needs. In support of its work on food standards and codes of practice, the *Codex alimentarius* generates reputable texts

for the management of food safety and consumer protection based on the work of the best-informed individuals and organizations concerned with food and related fields. Countries have responded by introducing long-overdue food legislation and *Codex*-based standards and by establishing or strengthening food control agencies to monitor compliance with such regulations. The maximum permitted level for nitrate in the *Codex* draft General Standard for Food Additives is 37 mg/kg in cheese and cheese products, 3650 mg/kg in processed meat, poultry and game, 360 mg/kg in comminuted [powdered] meat poultry and game products and 220 mg/kg in semi-preserved fish and fish products. The maximum permitted level for nitrite is 17 mg/kg in cheese and cheese products, 420 mg/kg in processed meat, poultry and game (whole pieces and cuts) and 130 mg in comminuted processed meat and in processed fish (WHO, 2003c).

Permissible levels of nitrate (used as a source of nitrite) in meat and poultry products used to be in the 250–500 ppm [mg/kg] range, but in recent years the trend has been to reduce levels of nitrate (and combined nitrate and nitrite) to inhibit the formation of nitrosamines. In the European Union, it has been suggested that input levels of nitrate for heat-treated meat products could be reduced to 150 ppm and residual levels to 100 ppm. In Japan, permissible nitrate input for meat products is 70 ppm, measured as residual nitrite; in the European Union, up to 50 ppm sodium or potassium nitrate (as sodium nitrate) can be used in foie gras; and in the USA, nitrate cannot be used to cure bacon. The content of nitrate is limited to 200 mg/L in raw milk for cheese in Japan and to 50 ppm in cheese in the European Union (European Commission, 1995; Department of Agriculture, 1995; Ministry of Health and Welfare, 2000; Department of Agriculture, 2003; Food and Drug Administration, 2004; European Commission, 2005; Food Standards Australia New Zealand, 2006).

Permissible residual levels of nitrite (usually expressed as sodium nitrite) in meat and poultry products generally range from 50 to 200 ppm (Australia/New Zealand, European Union, Japan, USA). Permissible levels are typically 120–175 ppm for pork bacon (European Union, USA) and 70 ppm for whale bacon (Japan). Nitrite is also used to cure fish products; for example, in Japan, permissible residual levels are up to 50 ppm in fish ham and fish sausage and no more than 5 ppm in certain salted fish products; in the USA, levels up to 200 ppm are permitted in certain smoked fish (European Commission, 1995; Department of Agriculture, 1995; Ministry of Health and Welfare, 2000; Department of Agriculture, 2003; Food and Drug Administration, 2004; European Commission, 2005; Food Standards Australia New Zealand, 2006).

In some regions, additional limits are placed on baby foods. In the European Union, nitrate is limited to 200 ppm and, in the USA, no nitrate or nitrite can be added to food specifically produced for babies or young children (European Commission, 2004; Food and Drug Administration, 2004).

The addition of ascorbate as an antioxidant is also regulated in some countries. Regulations in the USA specify that, in addition to sodium nitrite or potassium nitrite, 550 ppm sodium ascorbate or sodium erythorbate should be added to prevent the formation of nitrosamines (Food Safety Inspection Service, 2006). The maximal levels

reported by the Directive 95/2/EC of the European Commission (1995) for most products, including pre-packed preparations of fresh minced meat, are 'quantum satis' [quantity required].

1.6.2 *Water*

The WHO (2004b) has established a drinking-water guideline value (short-term exposure) for nitrate and nitrite of 50 mg/L and 3 mg/L, respectively, to protect against methaemoglobinaemia in bottle-fed infants. For methaemoglobinaemia in infants (an acute effect), it was confirmed that the existing guideline value for nitrate of 50 mg/L is protective. For nitrite, human data reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) support the current short-term guideline value of 3 mg/L, based on the induction of methaemoglobinaemia in infants. Toxic doses of nitrite that are responsible for methaemoglobinaemia range from 0.4 to more than 200 mg/kg bw. By applying the lowest level of the range (0.4 mg/kg bw), a body weight of 5 kg for an infant and a consumption of drinking-water of 0.75 L, the guideline value of 3 mg/L (rounded figure) was derived (WHO, 2003a, 2004b). A provisional guideline value (long-term exposure) for nitrite was set at 0.2 mg/L.

WHO has proposed this guideline value for nitrite associated with chronic exposure based on the analysis by JECFA of animal data that showed nitrite-induced morphological changes in the adrenal glands, heart and lungs. Using the JECFA ADI of 0.06 mg/kg bw per day, assuming a 60-kg adult who ingests 2 L of drinking-water per day and allocating 10% of the ADI for drinking-water, this guideline value of 0.2 mg/L nitrite (rounded figure) has been calculated. However, owing to the uncertainty surrounding the relevance to humans of the adverse health effects observed in animals and the susceptibility of humans compared with that of animals, this guideline value should be considered as provisional (WHO, 2003a, 2004b). The occurrence of nitrite in distribution as a consequence of the use of chloramine is intermittent, and average exposures over time should not exceed the provisional guideline value. Because of the possibility of the simultaneous occurrence of nitrite and nitrate in drinking-water, the sum of the ratios of the concentrations of each to its guideline value should not exceed 1.0 (WHO, 2003a, 2004b).

The Australian Government (2004) drinking-water guideline has adopted the WHO guidelines for infants under 3 months of age. However, the guideline is up to 100 mg/L nitrate for adults and children over 3 months of age.

The Canadian drinking-water (health-based) guideline for nitrate is 45 mg/L. Where nitrate and nitrite are determined separately, concentrations of nitrite should not exceed 3.2 mg/L (Health Canada, 1992, 2006).

In the USA, the Environmental Protection Agency maximum contaminant level for nitrates (measured as nitrogen) is 10 mg/L (45 mg/L nitrate); that for nitrites (measured as nitrogen) is 1 mg/L. The limit for nitrate in drinking-water was established as a safeguard

against infantile acquired methaemoglobinaemia. (Environmental Protection Agency, 2003).

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2. Studies of Cancer in Humans

2.1 Introduction

Ingestion of nitrate and nitrite can result in the endogenous formation of *N*-nitroso compounds in the presence of nitrosatable precursors that are contained in meats, fish and some common drugs (see Sections 1 and 4). Nitrate is ingested from dietary sources and drinking-water. Vegetables are usually the primary source when levels of nitrate in the drinking-water are below 50 mg/L, which is the regulatory limit in many countries. Many vegetables contain vitamin C and other compounds such as polyphenols that inhibit endogenous nitrosation. Whereas nitrate from both vegetables and drinking-water is reduced in the body to nitrite, sources from vegetables probably result in less endogenous formation of *N*-nitroso compounds because of the presence of inhibitors of nitrosation (see Section 1). For these reasons, the Working Group evaluated ingested nitrate from dietary sources and drinking-water separately.

Epidemiological studies that assessed the relationship between nitrate in the drinking-water and cancer have been primarily ecological in design and focused on stomach cancer. Fewer case-control and cohort studies were available for other cancer sites. Ecological studies can provide important information on causal inference when exposure circumstances contrast greatly (between regions or population subgroups) and migration of populations is limited, particularly if there is almost homogenous exposure within each region and there are no serious potential confounders (see Cantor, 1997; IARC, 2004).

However, inference from ecological studies of exposure to waterborne nitrate is more difficult because of the complexity of and intra-individual variation in endogenous nitrosation (see Section 4). Specific subgroups of a population that have higher exposure to nitrosation precursors (from nitrate in water and amines and amides in the diet) and lower exposure to inhibitors of nitrosation (e.g. dietary antioxidants) are probably at highest risk. Ecological studies of nitrate in drinking-water, therefore, are not liable to be highly informative unless levels of exposure are high and exposure to waterborne nitrate and other factors that affect nitrosation are homogeneous across the population groups. Therefore, the Working Group gave much greater weight to case-control and cohort studies in their evaluation.

Most case-control and cohort studies of exposure to nitrate in the drinking-water have been conducted in areas where levels of nitrate in drinking-water supplies are elevated due to the use of nitrogen fertilizers. Some of the highest levels of nitrate are found in shallow wells and surface water supplies which contain high levels in the spring due to run-off of excess nitrogen (see Section 1.4.2). Public water supplies are monitored routinely, and historical data on levels of nitrate are available; however, exposure to levels greater than the maximum contaminant limit in the USA of 10 mg/L (as nitrate-N) (Environmental Protection Agency, 2003) or the WHO drinking-water guideline of

50 mg/L as nitrate (WHO, 2004) are rare. Private wells tend to contain higher levels of nitrate than public supply wells because they are unregulated, are frequently shallower and more poorly constructed and are often located in close proximity to sources of contamination by nitrogen (crop fields, animal feed lots, septic tank systems). Since historical data from monitoring are available, most studies have focused on populations who use public water supplies and have excluded populations who use private wells with potentially higher exposures.

Occupational groups that may be at risk of ingesting nitrate were also considered. Workers in the manufacture of nitrate-based fertilizer can have high exposures to dusts that contain nitrate ($> 10 \text{ mg/m}^3$) (see Section 1). One study (Al-Dabbagh *et al.*, 1986) among men whose jobs entailed exposure to different levels of nitrates in dust (maximum, 5 mg/m^3) demonstrated that, at the end of a workday, salivary nitrate levels from highly exposed workers were approximately twice those of workers who had no exposure to nitrate in dust; however, dietary intake of nitrate was not controlled and the range of salivary levels of nitrate among highly exposed workers overlapped with those of unexposed men in several regions of England. Furthermore, a twofold variation in nitrate in saliva in non-occupationally exposed groups has been observed even under controlled conditions (Weisenberg *et al.*, 1982; Bos *et al.*, 1988). The Working Group considered that the evidence for exposure to nitrate via ingestion was lacking in these studies and could not be quantified; these studies were therefore not reviewed.

A few case-control studies have investigated exposure to nitrate from tap-water and have measured levels of nitrate and/or nitrite at the current residence or at the residence at the time of diagnosis of disease. Levels of nitrate and/or nitrite may change over time; therefore, considerable misclassification of exposure may occur if current levels of exposure are used to estimate past exposure. Variation in levels of nitrate across the distribution system of a public water supply could also introduce misclassification of exposure. In addition, nitrate and/or nitrite in water may be correlated with other environmental exposures such as agricultural pesticides that may be potentially relevant to some cancers.

Dietary intake of nitrite occurs primarily from the consumption of cured meats and fish, bakery goods and cereal products. Nitrite is also found as a contaminant of drinking-water but only in unusual circumstances (see Section 1.5). Several epidemiological studies evaluated the risk for specific cancers among subjects who had a higher intake of nitrite and a lower intake of vitamin C; this dietary pattern is liable to result in an increase in the endogenous formation of *N*-nitroso compounds. Studies of this design were considered by the Working Group to be particularly informative in the evaluation of human carcinogenicity.

Studies that estimated intake of nitrite from all dietary sources were reviewed, but these that only evaluated consumption of cured meat and risk for cancer were not reviewed specifically since they do not represent complete dietary nitrite intake. This is because many, but not all, cured meats contain nitrite and because other foods can also be

important sources of nitrite. The Working Group also noted the results of some studies that estimated intake of nitrite and preformed *N*-nitroso compounds.

Several ecological studies in high- and low-risk areas for stomach and oesophageal cancers evaluated the potential for endogenous formation of *N*-nitroso compounds using the *N*-nitrosoproline (NPRO) test developed by Ohshima and Bartsch (1981). Some studies also measured urinary excretion of nitrate, levels of nitrate and nitrite in the saliva and excretion of other specific *N*-nitroso amino acids. Excretion of NPRO was generally higher in high-risk areas; however, not all of the differences were statistically significant (Bartsch *et al.*, 1992; van Maanen *et al.*, 1996). Urinary and salivary levels of nitrate reflect exposures to nitrate from both dietary sources and drinking-water; therefore, these studies were evaluated as a separate group. However, the Working Group did not give substantial weight to these studies in their evaluation because of the ecological study design and because recent excretion of nitrate or levels of nitrate in saliva may not reflect past exposures.

2.2 Stomach and oesophageal cancer

2.2.1 *Ingested nitrate*

(a) *Ecological studies* (Table 2.1)

Several ecological studies have considered the relationship between exposure to nitrate and the risk for stomach or oesophageal cancer; most of these used mortality as the end-point. The concentration of nitrate in the drinking-water was often used as indicator of exposure, and some studies assessed its correlation with the occurrence of disease or investigated the differences in mortality or incidence of disease across exposure levels. Alternatively, the concentrations of nitrate were compared in populations who were classified according to different levels of risk. In many instances, urinary excretion of nitrate or its concentration in other body fluids in groups of individuals were used as indicators of exposure rather than levels in drinking-water and food. The main features and results of these studies are summarized in Table 2.1.

(i) *Nitrate in the drinking-water*

Asia

Two studies addressed the association between exposure to nitrate and the occurrence of tumours of the stomach and oesophagus in China. An early study (Wang *et al.*, 1979) reported a positive significant correlation ($r = 0.23$) between concentrations of nitrate in the drinking-water (mean, 10.55 mg/L) and cancer of the oesophagus in Linxien, while a more recent study (Zhang *et al.*, 2003) reported a concentration of 19.6 mg/L nitrate-N in well water in the county of Cixian, an area where mortality from oesophageal cancer was very high. These studies reported nitrate levels higher than the level (8 mg/L) in Chichen county, a low-risk area for oesophageal cancer.

Table 2.1. Ecological studies of ingested nitrate and nitrite and the risk for stomach and oesophageal cancer

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Asia					
<i>China</i>					
Wang <i>et al.</i> (1979), 49 production brigades, Linxian County	Oesophagus (150)	Incidence, 1969–76	Nitrate in wells 1976–77, 4 seasons	Correlation (summer), $r = 0.233$; P -value < 0.05	Average concentration of nitrates, 10.56 mg/L
Zhang <i>et al.</i> (1984), 6 high-risk and 4 low-risk districts	Stomach (151)	Mortality ($\times 10^{-5}$): high risk (range), 31–113, low risk (range), 7–12	Nitrate and nitrite in gastric juice: 809 (high risk) and 554 (low risk) subjects Nitrate Nitrite	High- versus low-risk areas (mg/mL) 24.3 versus 30.9 ($P < 0.01$) 0.27 versus 0.10 ($P < 0.001$)	Concentrations are medians; subjects were patients with chronic stomach complaints.
Lu <i>et al.</i> (1986), Linxian (high-risk area) and Fanxian (low-risk area) counties	Oesophagus (150)	Mortality, 1978; rate $\times 10^{-5}$, men: Linxian, 151; Fanxian, 35	Nitrate in 24 h-urine, 1982, healthy subjects (148 high risk, 96 low risk) High-risk area Low-risk area	94 mg 48 mg P -value = 0.001	Nitrate levels are median in mg/person/day.

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Wu <i>et al.</i> (1993), 69 counties from 25 provinces	Stomach (151), oesophagus (150)	Mortality, 1975–75; cumulative rates, 0–64 years	Nitrate in 12-h urine, 1982, healthy subjects (about 30 per xiang^a, 2 xiangs per county) Stomach Oesophagus	Correlation coefficient $r = -0.14$ (NS) $r = -0.10$ (NS)	Nitrate was significantly associated (positive correlation) with tumours of nasopharynx and liver and leukaemia.
Lin <i>et al.</i> (2003), Lufeng County (low-risk area), Nan'ao County (high-risk area)	Oesophagus (150)	Mortality, age-adjusted rate ($\times 10^{-5}$); 10 (Lufeng); 110 (Nan'ao)	Nitrate in 12-h urine (nmol/g creatinine), 120 healthy persons per county High-risk area Low-risk area	7.1 3.1 P -value = 0.01	Daily intake of nitrate also higher in high-risk area; period to which mortality rates and urinary measurements refer not reported
Zhang <i>et al.</i> (2003), Cixian County (high-risk area), Chichen County (low-risk area)	Oesophagus (150)	Mortality, 1974–76 rate ($\times 10^{-5}$) men: Cixian, 147.7; Chichen, 8.3	Nitrite and nitrate in well water, 1993–96 (33 in Cixian, 31 in Chichen; mg/L nitrogen), 1996 Nitrate Nitrite	Cixian versus Chichen 19.6 versus 8.0 ($P < 0.01$) 0.01 versus 0.002 ($P < 0.01$)	Rates adjusted for Chinese population; similar results for years 1993–95

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
<i>Japan</i>					
Kamiyama <i>et al.</i> (1987), Akita (high-risk area), Iwate (low-risk area) Prefectures	Stomach (151)	Mortality, 1969–78; rate $\times 10^{-5}$, men: Akita, 33, Iwate, 101	Nitrate in 24-h urine, 104 healthy subjects, 1983 High-risk area Low-risk area	116 mg 140 mg <i>P</i> -value = 0.07	Nitrate levels are median in mg/person/day.
Tsugane <i>et al.</i> (1992), Yokote, Saku (high-risk areas), Ninohe, Ishikawa (low-risk areas) cities	Stomach (151)	Mortality, 1985–87; rate, men ($\times 10^{-5}$): high-risk, 49, 43; low-risk, 30, 17	Nitrate in 24-h urine, 134 adults, 40–49 years, 1989–90 High-risk area Low-risk area	<i>Mean nitrate (mg/day/person)</i> 120–166 167–203	Age-adjusted mortality (world population)

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Europe					
<i>Denmark</i>					
Jensen (1982), towns of Aalborg and Aarhus and other towns in northern Jutland	Stomach (151), oesophagus (150)	Incidence (1968–72) from the Danish Cancer Registry	Nitrate in drinking-water (mg/L) in 1976 <i>Stomach</i> Men Aalborg: 27.1 Aarhus: 0.2 Women Aalborg: 27.1 Aarhus: 0.2 <i>P</i> -value <i>Oesophagus</i> Men Aalborg: 27.1 Aarhus: 0.2 Women Aalborg: 27.1 Aarhus: 0.2 <i>P</i> -value	Incidence ($\times 10^{-5}$) 41.5 28.4 21.8 13.2 > 0.0001 5.6 4.1 2.0 2.4 NS	Incidence adjusted for age using European population; for stomach cancer, similar results for 5-year periods 1943–47 and 1963–67

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
<i>Eastern Europe</i>					
Zemla & Kolosza (1979), Zabrze, cities of Maciejov, Mikulczyce (low-nitrate and-nitrite districts) and Grzybowice, Pawlow (high-nitrate districts), Poland	Stomach (151)	Incidence, 1965–75	Mean level in drinking-water (mg/L) <i>Low (0.076–0.125)</i> Nitrate Nitrite <i>High (0.365–0.699)</i> Nitrate Nitrite	Incidence rates ($\times 10^{-5}$) <i>Men</i> <i>Women</i> 11.9 3.7 13.4 9.6 36.8 NR 52.6 4.8	Age-standardized rates, population of Zabrze; all rates but one significantly different from the city mean; no differences for women
Juhasz <i>et al.</i> (1980), 230 communities in Szabolcs-Szatmar county, Hungary	Stomach (151)	Incidence, 1975	Nitrate in drinking-water > 100 mg/L < 100 mg/L	No. of communities $\geq 20 \times 10^{-5}$/ Total No. of communities 127/205 12/25 Prevalence ratio, 1.29 (NS)	Prevalence ratio (proportion of communities with nitrate >100 mg/L divided by the proportion of communities with nitrate <100 mg/L); source of rates and period of nitrate measurement not stated
Zatonski <i>et al.</i> (1989), town of Opole (low risk), and Swierzow and Domaszowice rural areas (high risk), Poland	Stomach (151)	Mortality, 1980–84	Urinary nitrate (24 h) from 50 (low-risk town) and 47 (high-risk areas) subjects Low-risk areas High-risk areas	Nitrate excretion (median, mg/day) 76 106 $P = 0.0072$	Age-adjusted rates

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Sandor <i>et al.</i> (2001), 192 communities of the county of Baranya, Hungary	Stomach (151)	Mortality, 1984–93; SMR	Nitrate in drinking-water (mg/L), 1974–93 < 80 > 80	SMR (95% CI) 0.96 (0.79–1.13) 1.42 (1.25–1.58) Correlation: 0.59 (<i>P</i> = 0.072)	Expected deaths from national rates; correlation significant if nitrate log-transformed
Gulis <i>et al.</i> (2002), 60 villages in the district of Trnava, Slovakia	Stomach (151)	Incidence (1986–95) from the National Cancer Registry; SIR	Nitrate in drinking-water (mg/L), 1975–95 0–10 10–20 > 20	SIR (95% CI) 0.96 (0.71–1.30) 0.87 (0.69–1.10) 1.08 (0.87–1.35)	Expected cases calculated from the district rates
<i>Italy</i>					
Gilli <i>et al.</i> (1984), 1199 communities of the Piemonte region	Stomach (151)	Incidence (1976–79) from the Tumour Register of Piemonte and hospital releases; SIR	Nitrate in drinking-water (recent years) > 20 mg/L < 20 mg/L	No. of communities with SIR significantly high/Total No. of communities Relative Risk (95% CI) 10/155 1.0 (reference) 5/1059 13.7 (3.4–60.0)	Expected cases from regional rates (Piemonte)

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments		
Knight <i>et al.</i> (1990), Cagliari, Verona (low risk), Siena, Arezzo (high risk) cities	Stomach (151)	Mortality, 1976–80; age, 35–64 years	Salivary nitrate and nitrite (nmol/mL) from hospital visitors (187 low-risk areas, 144 high-risk areas)		Rates standardized to the world population; concentrations are geometric means.		
						<i>Nitrate</i>	
						Low-risk areas	88.3
						High-risk areas	65.5
							<i>P</i> = 0.094
						<i>Nitrite</i>	
Low-risk areas	101.4						
High-risk areas	82.4						
	<i>P</i> = 0.082						
Knight <i>et al.</i> (1992), urban areas of Florence (high-risk) and Cagliari (low-risk)	Stomach (151)	Mortality, 1980–92; age, 0–74 years	Urinary nitrate from healthy subjects (39 in low-risk areas, 40 in high-risk areas)		Rates standardized to the entire Italian population; concentrations are geometric means.		
						<i>Nitrate (mg/12 h)</i>	
						Cagliari (low-risk area)	33.7
						Florence (high-risk area)	37.1
							<i>P</i> = 0.55
						<i>Rate (× 10⁻⁵)</i>	
Men							
Cagliari	15.6						
Florence	27.2						

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
<i>Spain</i>					
Sanz Anquela <i>et al.</i> (1989), 9 areas (230 municipalities) of the province of Soria	Stomach (151)	Mortality and incidence, 1977–86	Nitrate in drinking-water (mg/L) (1986) Men Mortality Incidence	Correlation coefficient $r = 0.825 (P < 0.01)$ $r = 0.701 (P < 0.05)$	Age- and sex-adjusted, overall province as standard population; incidence from pathology reports
Morales-Suárez-Varela <i>et al.</i> (1995), 258 municipalities of the province of Valencia	Stomach (151)	Mortality, 1975–80	Nitrate in drinking-water (1968) Men < 25 mg/L 25–50 mg/L > 50 mg/L Women < 25 mg/L 25–50 mg/L > 50 mg/L	Rate ($\times 10^{-5}$) 21.3 16.7 28.9 16.5 13.2 19.0	Significant differences for level >50 mg/L compared with <25 mg/L only for the age group 55–75 years (both men and women)

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
<i>United Kingdom</i>					
Hill <i>et al.</i> (1973), Worksop and 9 neighbouring 'control' towns	Stomach (151)	Mortality, 1963–71; SMR	Nitrate in drinking-water: Worksop, 93 mg/L; control towns, 15 mg/L	SMR, 1.27 ($P < 0.05$); all SMRs but one NS	Expected deaths calculated from national rates
Davies (1980), Nottinghamshire: 5 mining towns (including Worksop) and 4 non-mining towns	Stomach (151)	Mortality, 1958–75; SMR	<i>Men</i> Worksop Mining towns Non-mining towns <i>Women</i> Worksop Mining towns Non-mining towns	SMR 0.97 (P -value, NS) 0.92 (P -value, NS) 0.91 ($P < 0.5$) 1.23 (P -value, NS) 1.04 (P -value, NS) 0.86 ($P < 0.5$)	Expected deaths from national rates; populations in 1961 and 1971 plus adjustment for social class and mining

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Fraser & Chilvers (1981), 32 rural districts of Anglia and Yorkshire	Stomach (151)	Mortality, 1969–73 and 1974–78; SMR	Nitrate in drinking-water	SMR (1974–78)	Expected deaths from national rates of population in 1971
			<i>Men</i>		
			Anglia		
			< 25 mg/L	0.90 ($P < 0.05$)	
			25–50 mg/L	1.07 ($P < 0.05$)	
			> 50 mg/L	1.29 ($P < 0.05$)	
			Yorkshire		
			< 25 mg/L	0.99 ($P < 0.05$)	
			25–50 mg/L	1.05 ($P < 0.05$)	
			> 50 mg/L	NR	
			<i>Women</i>		
			Anglia		
			< 25 mg/L	1.00 ($P < 0.05$)	
			25–50 mg/L	0.98 ($P < 0.05$)	
> 50 mg/L	1.05 ($P < 0.05$)				
Yorkshire					
< 25 mg/L	0.88 ($P < 0.05$)				
25–50 mg/L	1.40 ($P < 0.05$)				
> 50 mg/L	NR				
Beresford (1985), 229 urban areas of England, Scotland and Wales	Stomach (151)	Mortality, 1969–73; SMR	Nitrate in drinking-water (1971) as mg/L nitrogen	Regression coefficient (SMR for a each mg/L)	Expected deaths from national rates, population in 1971; results adjusted for social class.
Men			–1.19 ($P < 0.05$)		
Women			–2.02 ($P < 0.01$)		

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Forman <i>et al.</i> (1985), Wales and Northeast (high risk), Oxford and Southeast (low risk)	Stomach (151)	Mortality, 1981; SMR	Salivary nitrate and nitrite from selected subjects in each region (fasting samples) <i>Nitrate (nmol/mL)</i> Low risk High risk <i>P</i> -value <i>Nitrite (nmol/mL)</i> Low risk High risk <i>P</i> -value	SMR Low risk, 0.59–0.91 High risk, 1.12–1.62 169 92.5 < 0.05 80.8 44.7 < 0.05	Fasting samples; similar results for both sexes and social class
Barrett <i>et al.</i> (1998), 148 water supply zones in Yorkshire	Stomach (151), oesophagus (150)	Incidence (1975–94) from the Yorkshire Cancer Registry	Nitrate in drinking-water (average 1990–95) <i>Stomach</i> Quartile 1, 2.4 mg/L Quartile 2, 5.0 mg/L Quartile 3, 13.7 mg/L Quartile 4, 29.8 mg/L <i>Oesophagus</i> Quartile 1, 2.4 mg/L Quartile 2, 5.0 mg/L Quartile 3, 13.7 mg/L Quartile 4, 29.8 mg/L	Rate ratio (95% CI) 1.00 1.02 (0.98–1.07) 0.86 (0.82–0.90) 0.91 (0.87–0.95) 1.00 1.01 (0.93–1.09) 1.01 (0.94–1.09) 1.06 (0.96–1.14)	Rates adjusted by age, sex, socioeconomic status and population density

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
North America					
Geleperin <i>et al.</i> (1976), Illinois, USA	Oesophagus (150)	Mortality, 1959–66; whites	Nitrate in drinking-water <i>Men</i> < 10 mg/L 40–45 mg/L, spring only > 40 mg/L, constant <i>Women</i> < 10 mg/L 40–45 mg/L, spring only > 40 mg/L, constant	Rate ($\times 10^{-5}$) 43.0 40.3 38.4 6.0 4.2 15.3	All comparisons NS
Van Leeuwen <i>et al.</i> (1999), 40 ecodistricts of Ontario, Canada	Stomach (151)	Incidence (1987–91) from the Ontario Cancer Registry	Nitrate in drinking-water (mg/L), 1987–91	Regression coefficient for ln(nitrate), $b = -0.136$ ($-0.151, -$ 0.122)	Age-standardized rates; results shown are for men.
South and Central America					
Cuello <i>et al.</i> (1976), several municipalities of the department of Nariño, Colombia	Stomach (151)	Incidence, 1968–72; 261 incident cases and 269 matched controls	Nitrate in 173 water sources; nitrate and nitrite in urine and saliva of 282 subjects Low-risk area High-risk area	Nitrate (mg/L) 1.7–14.6 12.5–39.0 (test not given) No differences in salivary nitrite	Level of risk by the case:control ratio in each area compared with the whole department

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
Zaldívar & Wetterstrand (1978), 25 provinces of Chile	Stomach (151)	Mortality (mean of 1960, 1962 and 1964)	Nitrate in drinking-water (as mg/L nitrogen), 1953–75 Men Women	Correlation coefficient $r = 0.0335$ (NS) $r = 0.0486$ (NS)	Age-adjusted rates $\times 10^{-5}$
Armijo <i>et al.</i> (1981), Chillán, Linares (high risk), Antofagasta and Punta Arenas (low risk), Chile	Stomach (151)	Mortality	Nitrate in urine and saliva of 243 children aged 11–13 years (1977–80)	Antofagasta had significantly higher mean of urinary nitrate; no differences for nitrite in saliva	Actual values for concentration not provided, only graph and results of the test
Sierra <i>et al.</i> (1993), communities of Turrubares (high risk) and Hojanca (low risk), Costa Rica	Stomach (151)	Incidence (age-adjusted rate $\times 10^{-5}$): 66.1 (high risk), 26.8 (low risk)	Nitrate in urine of children Low-risk area ($n = 25$) High-risk area ($n = 26$)	Nitrate (mmol/12 h) 0.20 0.23 ($P = 0.75$)	Median: urinary nitrate excretion

Table 2.1 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure	Main results	Comments
International					
Hartman (1983), 12 countries (10 European, USA, Japan)	Stomach (151)	Mortality, 1974–75, adjusted rates ($\times 10^{-5}$)	Estimates of nitrate intake (mmol/day), 1970s	Correlation, $r = 0.88$	Correlation improved when nitrate-squared was used as a covariate.
Joossens <i>et al.</i> (1996), 24 countries	Stomach (151)	Mortality, (1984, 1986, 1987 or 1988), 45–74 years	Nitrate in 24h urine 1986–87, 3303 adults aged 20–59 years Men Women	Correlation $r = 0.63$ ($P = 0.001$) $r = 0.56$ ($P < 0.05$)	Age-adjusted rates, $\times 10^{-5}$, nitrate in mmol/day

CI, confidence interval; ICD, International Classification of Diseases; NR, not reported; NS, not significant; SIR, standardized incidence ratio; SMR, standardized mortality ratio

^a Formerly called communes

Europe

Denmark

In Denmark, Jensen (1982) compared the incidence of cancer at several sites in the towns of Aalborg and Aarhus, which were known to have high and low levels of nitrate in the water (27.1 and 0.2 mg/L, respectively). Significantly higher rates of stomach cancer were reported for the high-nitrate area (Aalborg), but no differences were seen for oesophageal cancer.

Eastern Europe

In Poland (Zemła & Kolosza, 1979), significantly higher incidence rates of stomach cancer among men were observed in districts of the town of Zabrze that had higher levels of nitrate in the drinking-water.

In Hungary, a positive but non-significant association between the incidence of stomach cancer and the level of nitrate in the water was reported for the county of Szabolcs-Szatmar (Juhász *et al.*, 1980), and a non-significant correlation between the level of nitrate in the water and mortality from stomach cancer was found in 192 communities of the county of Banya (Sandor *et al.*, 2001); however, in the latter study, there was a significantly increased standardized mortality ratio (SMR) of 1.42 for communities that had concentrations of nitrate greater than 80 mg/L.

Gulis *et al.* (2002) analysed the incidence of stomach cancer in 60 villages in the district of Trnava, Slovakia. The number of observed cases from 1986 to 1995 was retrieved from the national cancer registry and expected cases were calculated for each village using the age- and sex-specific rates for the whole district. No association was observed between incidence rates of stomach cancer and levels of nitrate in the drinking-water. The villages were grouped into three categories according to levels of nitrate (0–10 mg/L, 10–20 mg/L and > 20 mg/L); standardized incidence ratios (SIRs) for the three groups were close to unity and none was significant.

Italy and Spain

A strong positive association was also observed between the incidence of stomach cancer and the level of nitrate in the drinking-water in the Piemonte area, Italy (Gilli *et al.*, 1984).

The relationship between nitrate in the drinking-water and the risk for stomach cancer was also assessed in two studies in Spain. In the province of Soria (Sanz Anquela *et al.*, 1989), a high-risk area for stomach cancer, a significant positive correlation was observed with both mortality ($r = 0.83$) and incidence ($r = 0.70$), while no clear pattern was observed in the analysis of 258 municipalities of Valencia (Morales-Suárez-Varela *et al.*, 1995).

United Kingdom

Several studies compared indicators of the occurrence of stomach cancer with the concentration of nitrate in the drinking-water in defined geographical areas with a high degree of variability in exposure, ranging from 2.4 mg/L nitrate in some areas of Yorkshire to 93 mg/L in a town in Nottinghamshire. An early report showed a significant

increase in mortality from stomach cancer (standardized mortality ratio (SMR), 1.27; $P < 0.05$) in Worksop, a town that had high levels of nitrate (93 mg/L) in the drinking-water (Hill *et al.*, 1973), but not in control towns where levels of nitrate were lower (15 mg/L). However, a re-analysis of mortality in the same area (Davies, 1980) calculated expected deaths using SMRs standardized by social class and occupational group at the national level and also using the proportion of subjects in each community by social class and occupation in mining. No increase in mortality was found when mining activity and social class were taken into account. A trend towards higher SMRs for stomach cancer with increasing level of nitrate in the drinking-water was observed in rural districts of Anglia and Yorkshire (Fraser & Chilvers, 1981) (although most estimates were not significant) while a significant inverse relationship was estimated in the analysis of 229 urban areas of England, Wales and Scotland (Beresford, 1985). Barrett *et al.* (1998) conducted a study in Yorkshire, where cancer incidence was available from a regional cancer registry. Ecological assignment was carried out by mean levels of nitrate in tap-water (148 water supply zones, average levels of monthly measurements in 1990–95) and some other variables (socioeconomic status, population density), while individual variables (age and sex) were available from the cancer registry (1975–94) for subjects who were diagnosed with tumours of the stomach (15 554), oesophagus (5399) and brain (3441). For each tumour site, the expected number of cases for each water supply zone, sex and age group was calculated and Poisson regression was employed to model the potential effects of levels of nitrate on incidence rates. For stomach cancer, a significant reduction in risk was found for the third (relative risk, 0.86; 95% confidence interval [CI], 0.82–0.90) and fourth quartile (relative risk, 0.91; 95% CI, 0.87–0.95), while no association was observed for cancer of the oesophagus. [Levels of nitrate in this region were modest: only 0.3% (31/9333) of samples contained > 50 mg/L, and 16% of water supply zones had average levels > 25 mg/L].

North America

In the USA, Geleperin *et al.* (1976) did not find significant differences in mortality from cancer of the oesophagus with levels of nitrate in the drinking-water in Illinois.

Van Leeuwen *et al.* (1999) conducted an ecological study in 40 ecodistricts of Ontario, Canada. Levels of nitrate in groundwater were assessed for each district using regional data from the monitoring system for municipal water supplies, and sex- and age-standardized cancer rates for stomach and several other cancer sites were calculated from data from the population-based cancer registry (1987–91). Regression analysis weighted by population size was used to examine the correlation between levels of nitrate and incidence of cancer. A significant inverse association was reported between the incidence of stomach cancer and level of nitrate in the drinking-water among men.

South America

The risk for stomach cancer in relation to nitrate was investigated in Nariño, a department located in the South of Colombia (Cuello *et al.*, 1976); two areas were defined

within the department based upon the differential distribution of subjects in a case-control study. Concentrations of nitrate in wells and artesian waters from the high-risk area (13–39 mg/L) were much higher than those in the low-risk area (1.7–15 mg/L), although no formal tests were provided.

Following a previous report that showed a strong positive relationship between mortality from stomach cancer and the use of nitrate fertilizers (Zaldivar, 1977), a correlation study was undertaken in Chile that compared mortality from stomach cancer and levels of nitrate in the drinking-water in 25 provinces (Zaldivar & Wetterstrand, 1978); no association was found; non-significant correlation coefficients were 0.03 and 0.05 for men and women, respectively.

(ii) *Biomarkers of nitrate*

Asia

Two Japanese studies compared excretion of nitrate in 24-h urine samples from subjects selected in areas of low and high risk for mortality from stomach cancer. Kamiyama *et al.* (1987) observed lower levels of urinary nitrate in the prefecture of Akita, an area where mortality from stomach cancer was high, compared with the low-risk area of Iwate: median levels of excretion of nitrate were 116 and 140 mg per day per person, respectively ($P = 0.07$). No association was observed for urinary nitrate in the high-mortality areas of Yakote and Saku cities compared with the low-risk areas of Ninohe and Ishikawa cities (Tsugane *et al.*, 1992).

In China, Zhang *et al.* (1984) analysed levels of nitrate in the fasting gastric juice of subjects from six areas where mortality from stomach cancer was high and four where it was low and showed an inverse significant association. [However, this study was carried out among patients with chronic stomach complaints.] Three studies in China used excretion of nitrate in urine samples as an indicator of exposure. Wu *et al.* (1993) compared urinary nitrate in healthy subjects and mortality rates from 69 counties in 25 Chinese provinces and found inverse non-significant associations with mortality from both cancer of the stomach ($r = -0.14$) and cancer of the oesophagus ($r = -0.10$). Lu *et al.* (1986) had previously reported an excretion of 94 mg nitrate per person per day in Linxian County (an area that had the highest mortality from cancer of the oesophagus), which was significantly higher than the level of 48 mg measured in Fanxian county, an area that had much lower mortality. A very similar result was reported in a more recent study (Lin *et al.*, 2003): the level of urinary nitrate was 7.1 nmol/g creatinine in subjects from Nan'ao county, a high-risk area, which was more than twice that measured in Lufeng county (3.1 nmol/g), where mortality from cancer of the oesophagus was much lower.

Europe

In Poland, Zatonski *et al.* (1989) reported higher levels of urinary nitrate in subjects from rural areas where mortality from stomach cancer was high compared with residents in the town of Opole that had lower mortality from stomach cancer.

In Italy, lower levels of nitrate in the saliva were found in subjects from regions with high mortality from stomach cancer (Siena, Arezzo) compared with those from Cagliari and Verona, where mortality rates were lower, although the differences did not reach statistical significance (Knight *et al.*, 1990). In another Italian study, no differences were found in levels of urinary nitrate measured in children in Florence and Cagliari, areas with high and low mortality from stomach cancer, respectively (Knight *et al.*, 1992).

Forman *et al.* (1985) compared concentrations of nitrate in the saliva of subjects from areas where mortality from stomach cancer was high (Northeast and Wales) with those from subjects in low-risk areas (Southeast and Oxford) and found an inverse relationship, with significantly higher levels of nitrate in areas that had lower mortality from stomach cancer.

South and Central America

Armijo *et al.* (1981) compared levels of nitrate in the urine and saliva of children aged 11–13 years from four cities in Chile, two with high (Chillán, Linares) and two with low mortality rates (Antofagasta, Punta Arenas) for stomach cancer. Children in the two areas with lower mortality had the highest and the lowest mean levels of urinary nitrate; however, children from only one of the low-risk areas (Antofagasta) had significantly higher urinary excretion of nitrate in pairwise comparisons with the other three areas.

In a study in Costa Rica (Sierra *et al.*, 1993), children from two areas with high and low incidence rates of stomach cancer had similar levels of urinary excretion of nitrate.

International

In the multinational INTERSALT study (Joossens *et al.*, 1996), the mortality rates for stomach cancer were compared with excretion of nitrate in 24-h urine samples from 24 countries; significant correlations of 0.63 and 0.56 were observed for men and women, respectively. Although the source of data to estimate average intake of nitrate at the country level was not specified, a review by Hartman (1983) reported a correlation of 0.88 between the mortality rates from stomach cancer and the average intake of nitrate in 12 countries.

(b) Cohort studies (Table 2.2)

The Netherlands Cohort Study is a prospective study of diet, other lifestyle characteristics and the risk for cancer that was started in September 1986 and initially included 58 279 men and 62 573 women aged 55–69 years at recruitment (van Loon *et al.*, 1997, 1998). Usual diet was assessed by means of a 150-item semiquantitative food-frequency questionnaire. Intake of nitrates was estimated using data from the databank of contaminants in foods from the State Institute for Quality Control of Agricultural Products (RIKILT, Wageningen). Information on water intake was combined with data from all waterworks in the country to determine the concentration of nitrate in the drinking-water for each home address by postal code. A case-cohort approach was used for data analysis: a subcohort of 3500 subjects was randomly sampled and followed every

Table 2.2. Cohort studies of ingested nitrate and nitrite and the risk for stomach and oesophageal cancer

Reference, location, name of study	Organ site (ICD code)	Cohort description	Exposure assessment	Exposure categories	No. of cases/deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
van Loon <i>et al.</i> (1998), Netherlands, The Netherlands Cohort Study	Stomach (151)	Cohort of 58 279 men and 62 573 women, 55–69 years; prospective follow-up September 1986–December 1992 by regional cancer registries and national pathology register; case–cohort analysis of 282 incident cases (219 men, 63 women) and 3123 subcohort members (1525 men, 1598 women)	Self-administered 150-item food-frequency questionnaire; food composition and nitrate in drinking-water from databank on contaminants in food (nitrate) and a food composition database (nitrite)	Total nitrate, quintile (mean, mg/day)		Rate ratio	Age, sex, smoking, education, coffee, vitamin C, β -carotene, family history of stomach cancer, prevalence of stomach disorders, use of refrigerator or freezer	Similar results after exclusion of subjects with stomach disorders or those diagnosed during the first year
				I (59.8)	63	1.00 (reference)		
				II (84.7)	67	1.25 (0.84–1.86)		
				III (104.4)	42	0.74 (0.47–1.15)		
				IV (127.3)	54	0.92 (0.59–1.44)		
				V (179.8)	56	0.90 (0.53–1.55)		
						<i>P</i> trend = 0.30		
				Nitrate from drinking-water (mean, mg/day)				
				Q1 (0.02)	61	1.00 (reference)		
				Q2 (1.65)	54	0.93 (0.62–1.39)		
				Q3 (3.85)	53	0.87 (0.51–1.31)		
				Q4 (6.91)	57	0.83 (0.55–1.24)		
				Q5 (16.5)	57	0.88 (0.59–1.32)		
						<i>P</i> trend = 0.39		
				Nitrate from foods (mean, mg/day)				
				Q1 (55.8)	69	1.00 (reference)		
				Q2 (79.4)	61	1.02 (0.69–1.51)		
				Q3 (98.7)	45	0.71 (0.46–1.09)		
				Q4 (120.7)	49	0.80 (0.51–1.25)		
				Q5 (172.2)	58	0.80 (0.47–1.37)		
		<i>P</i> trend = 0.18						
Total nitrite, quintile (mean, mg/day)								
I (0.01)	47	1.00 (reference)						
II (0.04)	51	1.20 (0.78–1.86)						
III (0.09)	58	1.18 (0.77–1.82)						
IV (0.16)	46	0.88 (0.56–1.37)						
V (0.35)	80	1.44 (0.95–2.18)						
		<i>P</i> trend = 0.24						

Table 2.2. (contd)

Reference, location, name of study	Organ site (ICD code)	Cohort description	Exposure assessment	Exposure categories	No. of cases/deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Knekt <i>et al.</i> (1999), Finland, several regions	Stomach (151)	9985 subjects, part of a multiphasic screening examination (the Mobile Health Clinic of the Social Insurance Institution); follow-up 1967–90 through nationwide Finnish Cancer Registry; 68 incident cases of stomach cancer (43 men, 25 women)	Dietary history interview, referred to previous year	<i>Nitrate</i>	Not given	1.00 (reference)	Age, sex, municipality, smoking, energy intake	No association with NDMA for stomach cancer; mean daily intake: 77 mg nitrate (92% from vegetables); 5.3 mg nitrite (94% from cured meats and sausages)
				Q1	1.01 (0.56–1.84)			
				Q2	0.52 (0.25–1.08)			
				Q3	0.56 (0.27–1.18)			
				Q4	<i>P</i> trend = 0.09			
				<i>Nitrite</i>		1.00 (reference)		
				Q1	1.10 (0.58–2.11)			
				Q2	1.88 (1.01–3.49)			
Q3	0.71 (0.28–1.78)							
Q4	<i>P</i> trend = 0.90							

CI, confidence interval; ICD, International Classification of Diseases; NDMA, *N*-nitrosodimethylamine; Q, quartile

2 years for vital status and record linkage with all regional cancer registries. During a follow-up period of 6.3 years (up to December 1992), 347 cases of stomach cancer were identified. After exclusion of prevalent cases and those not microscopically confirmed or having incomplete dietary information, the analysis was carried out on 282 cases (219 men and 63 women) and 3123 subcohort members (1525 men and 1598 women). Total mean intake of nitrate in the subcohort was 111 mg per day (105 mg from food, 5.8 mg from drinking-water). No significant inverse associations were found for total nitrate intake, intake of nitrate from foods or intake of nitrate from water after adjustment for several factors including vitamin C intake. An analysis that excluded subjects with stomach disorders or those who were diagnosed during the first year did not modify these patterns substantially.

During 1966–72, a multiphasic screening examination was undertaken in several regions of Finland by the Mobile Health Clinic of the Social Insurance Institution. As part of the main study, data on food consumption were collected from 9985 individuals (5274 men and 4711 women) who had never had cancer (Knekt *et al.*, 1999). Dietary information was collected by means of an interview, and several food composition databases were used to calculate intake of nitrate. During a 24-year period (1967–90), 68 cases of cancer of the stomach were ascertained through the Finnish Cancer Registry. A non-significant inverse association was observed for nitrate intake: compared with the first quartile, relative risks for the second, third and fourth quartiles of dietary nitrate were 1.01, 0.52 and 0.56, respectively, none of which was significant. All of these estimates were adjusted for age, sex, municipality, tobacco smoking and energy intake.

(c) *Case-control studies* (Table 2.3)

Several case-control studies have analysed the relationship between the occurrence of tumours of the stomach or oesophagus and intake of nitrate from foods, but very few provided separate estimates for the intake of nitrate from drinking-water. Most of these studies were carried out in populations who had relatively low levels of nitrate in the drinking-water and the majority of ingested nitrate was provided by diet.

China

One case-control study from Taiwan, China (Yang *et al.*, 1998) was designed to assess the relationship between levels of nitrate in the drinking-water and mortality from stomach cancer. Information on the levels of calcium, magnesium and nitrate in the drinking-water were available from the Taiwan, China Water Supply Corporation for 252 of 361 municipalities. Cases were 6766 registered deaths from stomach cancer in residents from these municipalities from 1987 to 1991; age- and sex-matched controls were selected from among deaths from other causes excluding gastrointestinal disorders, cardiovascular diseases and some tumours. Levels of nitrate measured in 1970 in water in the municipality of residence was used as an indicator of exposure. No association was observed between mortality from stomach cancer and levels of nitrate in the drinking-water

Table 2.3. Case-control studies of ingested nitrate and nitrite and the risk for stomach and oesophageal cancers

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
China									
Yang <i>et al.</i> (1998), China (Province of Taiwan), 1987–91	Stomach (151)	6766 (4480 men, 2286 women) deaths from 1987 to 1991 obtained from the Bureau of Vital Status, residents in 252 municipalities of the Province of Taiwan	6766 selected among deaths from other causes, excluding those associated with gastrointestinal problems, cardiovascular and cerebrovascular diseases and tumours of the bladder, prostate, lung, oesophagus, head and neck	Levels of nitrate in each municipality's treated drinking-water supply for the year 1990, from the Taiwan Water Supply Corporation	Nitrate-N from drinking water, tertiles (mg/L) range (median) < 0.22 (0.04) 0.23–0.44 (0.37) ≥ 0.45 (0.67) P for trend	2109 2126 2531	1.00 0.95 (0.87–1.03) 1.02 (0.93–1.11) 0.44	Age, sex, year of death	Significant inverse association with magnesium and calcium in drinking-water; relative risks for nitrate (tertiles) adjusted for calcium and magnesium: 1.10 (1.00–1.20) and 1.14 (1.04–1.25)

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Europe									
Buiatti <i>et al.</i> (1990), 4 areas in northern and central Italy, 1985–87	Stomach (151)	1016 (640 men, 376 women) aged 75 years or under from all hospitals in the areas of study; 100% histologically confirmed; participation rate, 83%	1159 population controls from municipal lists of residents; stratified by age, sex according to distribution of cases; participation rate, 81%	Interviewer-administered structured questionnaire of 146 food items; Italian and British FCTs	Nitrate, quintiles (mean, mg/day)			Age, sex, area, urban/rural residence, migration from south, socioeconomic status, familial history of stomach cancer, body mass index, total energy intake	Interaction between antioxidant nutrients (vitamin C plus α -tocopherol) and nitrosating/nitrosable compounds (protein plus nitrite)
					1 (53)	NR	1.0 (reference)		
					2 (81)		0.9 (0.7–1.1)		
					3 (103)		0.9 (0.6–1.1)		
					4 (130)		0.7 (0.5–0.9)		
					5 (193)		0.9 (0.7–1.2)		
					<i>P</i> for trend		[0.68]		
					Nitrite, quintiles (mean, mg/day)				
					1 (2.1)	NR	1.0 (reference)		
					2 (2.8)		1.0 (0.8–1.4)		
					3 (3.4)		1.2 (0.9–1.7)		
					4 (4.1)		1.4 (1.0–2.0)		
					5 (5.9)		1.9 (1.3–2.7)		
					<i>P</i> for trend		[0.005]		

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Boeing <i>et al.</i> (1991), Southeast Bavaria and Hesse, Germany, 1985–87	Stomach (151)	143 (50% men, 50% women) aged 32–80 years from five main hospitals in the two areas; 100% histologically confirmed; participation rate, 85%	579 in total; 328 hospital controls plus 251 visitor controls selected from the same hospitals as the cases; participation rate, 90% for hospital controls; unknown for visitors	Interviewer-administered questionnaire of 74 food items; German FCT	Nitrate, quintiles (mg/day)	NR	1.00 (reference) 0.93 (0.53–1.64) 0.61 (0.32–1.19) 0.61 (0.30–1.27) 1.26 (0.59–2.70) [1.0]	Age, sex, hospital, vitamin C, carotene, calcium	Both control groups were pooled in this analysis; having well-water supply (supposed high nitrate contents) had a relative risk of 2.26 (1.19–4.28) compared with having central water supply.
González <i>et al.</i> (1994), five provinces in northern and central Spain, 1988–89	Stomach (151)	354 (235 men, 119 women) aged 31–88 years from 15 public hospital, residents in the study areas; 100% histologically confirmed; participation rate, 100%	354 admitted to the same hospitals as the cases; individually matched by age (± 3 years), sex, area of residence	Interviewer administered diet history, structured by meals; English and Spanish FCTs	Nitrate, quartiles (mg/day)	NR	1.00 0.80 (NR) 0.65 (NR) 0.45 (NR) 0.007	Age, sex, area of residence, total caloric intake	CIs not provided; in this study a significant increased risk was observed with higher intake of NDMA; significant interaction between nitrosamines and vitamin C.
					Nitrite, quartiles (mg/day)	NR	1.00 1.20 (NR) 1.09 (NR) 1.28 (NR) 0.38		

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Hansson <i>et al.</i> (1994), five counties in central and northern Sweden, 1989–92	Stomach (151)	338 (sex distribution not given) aged 40–79 years resident in the study areas; 100% histologically confirmed; participation rate, 74%	679 randomly sampled from a population register in the areas of study; stratified by age, sex according to the distribution of the cases; participation rate, 77%	Interviewer-administered FFQ of 45 items referred to adolescence and 20 years prior to interview; Swedish FCT	Quartiles (Q; 20 years prior to the interview) <i>Nitrate</i> Q4 versus Q1 <i>P</i> for trend <i>Nitrite</i> Q4 versus Q1	NR		Age, sex	Mean intake of nitrate (controls), 42 mg/day
							0.55 (0.38–0.80) 0.002		
La Vecchia <i>et al.</i> (1994, 1997), Milan area, Italy, 1985–92	Stomach (151)	723 (443 men, 280 women) aged 19–74 years from four largest teaching and general hospitals; 100% histologically confirmed; participation rate, 95%	2024 (1189 men, 835 women) admitted to the same hospitals (excluding neoplastic and digestive wards); participation rate, 95%	Interviewer-administered FFQ of 29 items; Italian FCT	Nitrate, quintiles (mg/day) 1 (< 63) 2 (63–80) 3 (81–96) 4 (97–116) 5 (> 116) <i>P</i> for trend Nitrite, quintiles (mg/day) 1 (< 1.91) 2 (1.92–2.41) 3 (2.42–2.94) 4 (2.95–3.64) 5 (> 3.64) <i>P</i> for trend Nitrite (mg/day) < 2.7 ≥ 2.7	228	1.00 (reference)	Age, sex, education, family history of stomach cancer, body mass index, energy intake	Combined effect of nitrite with methionine high/high versus low/low, 2.45 (1.9–3.2)
						156	0.64 (0.49–0.83)		
						117	0.50 (0.38–0.67)		
						117	0.52 (0.39–0.70)		
						105	0.43 (0.32–0.59)		
							< 0.001		
						123	1.00 (reference)		
						128	0.98 (0.72–1.33)		
						126	0.99 (0.72–1.36)		
						153	1.15 (0.84–1.59)		
193	1.35 (0.96–1.88)								
	< 0.05								
NR	1.00 (reference)								
	1.44 (1.2–1.7)								

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Pobel <i>et al.</i> (1995), Marseilles, France, 1985–88	Stomach (151)	92 (59 men, 33 women; mean age, 67 years) from 8 major centres for gastric surgery in Marseilles; 100% histologically confirmed; participation rate, 100%	128 hospital (74 men, 54 women) controls from two specialized centres for re-education for trauma or injuries; stratified by age, sex according to the distribution of the cases; participation rate, 100%	Interviewer-administered diet history, structured by meals; French FCT	Nitrate, tertiles	NR	1	Age, sex, occupation, total energy intake	Mean intake of nitrate (controls), 143 mg/day; no significant association with nitrate from different sources; a significant increased risk was observed with higher intake of NDMA.
					2		0.49 (0.24–1.01)		
					3		0.76 (0.38–1.50)		
						<i>P</i> for trend		0.96	
					Nitrite, tertiles	NR	1	1.0	
					2		0.83 (0.41–1.67)		
3	0.88 (0.44–1.79)								
	<i>P</i> for trend		0.49						

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
North America									
Risch <i>et al.</i> (1985), Newfoundland, Manitoba and Toronto, Canada, 1979–82	Stomach (151)	246 (163 men, 83 women) aged 35–79 years from the province tumour registries (Newfoundland, Manitoba) or pathology reports of 21 hospitals in Toronto; 100% histologically confirmed; participation rate, 44%	246 population controls from provincial electoral lists or municipal lists of residents; individually matched to cases by age (within 4 years), sex, province of residence; participation rate, 58%	Interviewer-administered diet history; FCT from the USDA and other sources	Nitrate, per 100 mg/day Nitrite, per 1 mg/day	NR NR	0.66 (0.54–0.81) 1.71 (1.24–2.37)	Age, sex, province of residence (matched analysis), total food consumption, ethnicity	No association with NDMA
Rademacher <i>et al.</i> (1992), Wisconsin, USA, 1982–85	Stomach (151)	1268 (758 men, 510 women) deaths from stomach cancer in the Wisconsin Bureau of Health Statistics	1268 randomly selected from all other deaths (excluded other gastrointestinal problems); matched for sex, year of birth, year of death	Recorded levels of nitrate in municipal water sources (1970 edition) and those measured in private wells (data not provided)	Level of nitrate-N (mg/L) > 0.5 versus ≤ 0.5 > 2.5 versus ≤ 25 > 5.0 versus ≤ 5 > 10.0 versus < 10 Private wells versus public	207 113 25 6	0.92 (0.75–1.12) 0.97 (0.74–1.35) 0.86 (0.69–1.08) 1.50 (0.12–18.25) 1.09 (0.82–1.47)	Age, sex (matched analysis)	Nitrate-N in private wells was significantly higher than that in public supplies.

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Rogers <i>et al.</i> (1995), three counties of Washington State, USA, 1983–87	Oesophagus (150)	645 upper aerodigestive tract cancers identified through the Cancer Surveillance System (SEER Program), of which 127 were oesophageal tumours (94 men, 33 women); participation rate, 67%	458 from random-digit dialling in the three counties; frequency-matched by sex, age (5-year interval)	Self-administered FFQ of 125 food items; food composition from report of the National Academy of Sciences	Nitrate, tertiles (mg/day)			Age, sex, pack-years of cigarettes, drink-years of alcohol, energy intake, vitamin C, body mass index, level of education	Interaction between nitrite and vitamin C (NS); slightly increased risk (NS) for intake of NDMA; high NDMA with low vitamin C had a significantly increased risk versus low NDMA and high vitamin C.
					1 (< 134)	33	1.00 (reference)		
					2 (134–226)	39	0.71 (0.38–1.33)		
					3 (> 226)	25	0.44 (0.24–0.93)		
					<i>P</i> for trend		0.078		
					<i>High vitamin C</i>				
					1 (< 134)	NR	1.00 (reference)		
					2 (134–226)		0.73 (NR)		
					3 (> 226)		0.44 (NR)		
					<i>Low vitamin C</i>				
					1 (< 134)	NR	1.63 (NR)		
					2 (134–226)		1.44 (NR)		
					3 (> 226)		1.27 (NR)		
					Nitrite, tertiles (mg/day)				
					1 (< 1.06)	26	1.00 (reference)		
2 (1.06–1.60)	28	1.17 (0.57–2.38)							
3 (> 1.60)	43	1.58 (0.73–3.44)							
<i>P</i> for trend		0.20							
<i>High vitamin C</i>									
1 (< 1.06)	NR	1.00 (reference)							
2 (1.06–1.60)		2.24 (NR)							
3 (> 1.60)		1.49 (NR)							
<i>Low vitamin C</i>									
1 (< 1.06)	NR	2.93 (NR)							
2 (1.06–1.60)		2.23 (NR)							
3 (> 1.60)		5.07 (<i>P</i> < 0.05)							

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Mayne <i>et al.</i> (2001); Engel <i>et al.</i> (2003), Connecticut, Washington state, New Jersey, USA, 1993–95	Stomach (151), cardia and non-cardia; oesophagus (150), SCC, ADC	Stomach: 255 cardia (217 men, 38 women), 352 non-cardia (244 men, 108 women); oesophagus: 282 ADC (235 men, 47 women), 206 SCC (166 men, 40 women) aged 30–79 years from three population-based tumour registries; 100% histologically confirmed; participation rate, 72% (overall)	687 from random-digit dialling and Health Care Finance Administration rosters: frequency-matched by age (5-year group), sex, geographic area; participation rate, 70%	Interviewer-administered FFQ of 104 items; FCT from the University of Minnesota and other databases	Nitrite, 75th percentile compared with 25th percentile	NR	<i>Oesophagus</i> SCC 1.13 (0.82–1.56) ADC 1.05 (0.80–1.38) <i>Stomach</i> Non-cardia 1.65 (1.26–2.15) Cardia 1.05 (0.79–1.40)	Age, sex, area (matching), race, proxy status, income, education, body mass index, cigarettes/day, years of consumption of beer, wine and liquor, sodium, energy intake	Interview with proxies for 30% of cases and 3.4% of controls

Table 2.3 (contd)

Reference, study location, study period	Organ site (ICD code)	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Mayne <i>et al.</i> (2001); Engel <i>et al.</i> (2003) (contd)		Stomach: 368 non-cardia	695 controls	Interviewer	Nitrite, quartiles	NR	1.0	Age, sex, area, race, respondent type, income, energy intake, smoking status, history of gastric ulcer	Levels (quartiles) of nitrite intake among controls by sex: men: 1.7–5.8, 5.9–7.5, 7.6–9.9, 10.0–39.2; women: 1.9–5.3, 5.4–6.9, 7.0–9.1, 9.2–31.2; increased risk of oesophageal ADC, SCC and stomach cardia cancer with low vitamin C intake (regardless of nitrite intake) compared with high vitamin C and low nitrite intakes
					1		1.5 (1.0–2.4)		
					2		1.8 (1.1–3.0)		
					3		2.5 (1.4–4.3)		
					4		1.7 (1.1–2.6)		
					2–4		1.0 (reference)		
					High vitamin C		2.10 (1.36–3.25)		
					Low nitrite		2.25 (1.46–3.47)		
					High nitrite		2.95 (1.90–4.59)		
					Low vitamin C				

ADC, adenocarcinoma; CI, confidence interval; DMA, dimethylamine; FCT, food composition table; FFQ, food-frequency questionnaire; ICD, International Classification of Diseases; NDMA, *N*-nitrosodimethylamine; NR, not reported; NS, not significant; SCC, squamous-cell carcinoma; USDA, US Department of Agriculture

However, a weak but significant association was seen after adjustment for levels of calcium and magnesium in the water.

Europe

A large case-control study was carried out in Italy (Buiatti *et al.*, 1990) based on 1016 cases and 1159 controls from two areas at high risk for stomach cancer (Florence-Siena and Forlì-Cremona-Imola) and two at low risk (Genoa and Cagliari). Dietary intake of nitrate (mg per/day) was derived from personal interviews using a structured questionnaire of 146 items and data on nitrate content in foods were obtained from Italian and British food composition tables. The association with risk for stomach cancer was assessed through the distribution of nitrate controls to define quintiles, taking the lowest as the reference category, and by means of a model that included age, sex, social class, familial history of stomach cancer and caloric intake. No association was found between dietary intake of nitrate and the risk for stomach cancer. Palli *et al.* (2001) updated the results of Buiatti *et al.* (1990) on the cases from the local cancer registry of Florence by expanding the series of controls and applying an updated Italian food composition table. Overall, this re-analysis included 382 cases and 561 controls. There was a significant reduction in risk for stomach cancer with increasing nitrate intake (P for trend = 0.01). [The authors stated that the effect of nitrate disappeared when adjustment was made for protein (associated with increased risk), and β -carotene and α -tocopherol (associated with protection against risk). However, adjusted estimates were not provided.]

Boeing *et al.* (1991) carried out a hospital-based case-control study in Southeastern Bavaria and Hesse (Germany). A total of 143 cases and 579 controls (patients and visitors) were interviewed using a standard questionnaire of 74 items coupled with a German food composition table to obtain dietary intake of nitrate. Information on the type of water supply for each residence was also collected. No association was observed between the risk for stomach cancer and quintiles of dietary intake of nitrate, adjusted for age, sex, hospital, vitamin C, carotene and calcium. Residence with a well-water supply was associated with an increased risk for stomach cancer (relative risk, 2.26; 95% CI, 1.19-4.28) compared with a central water supply. [The authors stated that private well-waters were supposed to have a higher content of nitrate, but information on levels of nitrate in water was not available for the areas under study.]

Another hospital-based case-control study that included 354 cases and 354 matched controls (González *et al.*, 1994) was carried out in five provinces of Spain (Barcelona, La Coruña, Lugo, Soria and Zaragoza). A diet history method administered personally by a trained interviewer was used to assess habitual diet during a typical week of the year before the diagnosis (cases) or interview (controls); Spanish and British food composition tables were used to estimate the intake of nutrients. In the matched analysis (age, sex, area), an inverse association between risk for stomach cancer and increasing quartiles of nitrate intake was observed with a significant trend (P for trend = 0.007) after also adjusting for total caloric intake. The effect was more evident when the analysis was restricted to cases with diffuse-type histology.

Hansson *et al.* (1994) reported the results of a case-control study in Sweden. A food frequency questionnaire was administered by an interviewer to 338 cases of stomach cancer and 679 population controls who were identified through the population register; information on usual diet during adolescence and the 20 years before the interview was collected and nitrate intake was calculated from a Swedish food composition table. The highest quartile of nitrate intake, adjusted for sex and age, was significantly associated with decreased risk for cancer of the stomach (odds ratio, 0.55; 95% CI, 0.38–0.80; *P* for trend = 0.002). However, no association was observed when dietary intake of nitrate was further adjusted for vitamin C and α -tocopherol. [A plausible interpretation of such results could be that the decrease in risk is probably due to the inhibitory effect on nitrosation of vitamin C and vitamin E.]

A hospital-based case-control study carried out in Italy included 723 cases and 2024 controls selected from the four largest teaching hospitals from the Greater Milan area (La Vecchia *et al.*, 1994, 1997). An interviewer administered a 29-item food-frequency questionnaire and an Italian food composition table was used to assess the usual dietary intake of nitrate. In a multivariate analysis that took into account potential dietary and non-dietary confounders, the highest quintile of nitrate consumption showed a significant reduction in the risk for stomach cancer (relative risk, 0.43; 95% CI, 0.32–0.59; based on 105 exposed cases; *P* for trend < 0.001) compared with the lowest quintile.

A hospital-based case-control study that included 92 cases and 128 controls was carried out in the area of Marseilles, France (Pobel *et al.*, 1995). A questionnaire on diet history was administered by an interviewer at the hospital and a French food composition table was used to estimate the intake of nitrates. No association was observed between intake of nitrate and the risk for stomach cancer overall or when different dietary sources were considered separately, while a significant increase in risk was reported for the dietary intake of preformed nitrosamines. [Potential limitations of this study are the small sample size and the fact that controls were selected in two specialized medical centres of re-education for trauma or injuries, which may not provide a good representation of the dietary habits of the study base.]

North America

The first case-control study on risk for stomach cancer that reported results for dietary intake of nitrates was a Canadian study by Risch *et al.* (1985). Overall 246 cases of stomach cancer were identified from the cancer registries of two provinces of Canada and from the pathology reports of hospitals in Toronto; individually matched controls were selected from provincial or municipal lists of residents. Usual diet was assessed by the diet history method administered by an interviewer and nutrient intakes were calculated using a food composition table from the USA and other sources. The matched analysis with further adjustment for ethnicity showed a significant reduction in risk for stomach cancer for each 100 mg nitrate ingested per day (odds ratio, 0.66; 95% CI, 0.54–0.81). However, the effect of nitrate totally disappeared when adjustment was made for intake of vitamin C from vegetables.

Rademacher *et al.* (1992) reported a case-control study based upon death certificates to assess the association of stomach cancer with nitrate in the drinking-water. Cases were 1268 deaths from stomach cancer from 1982 to 1985 in Wisconsin (USA) and matched controls were selected from among deaths from causes other than gastrointestinal problems. Levels of nitrate recorded in 1970 for municipal sources or measured in private wells were used as indicators of exposure at the residence of the subject at the time of death. Using several cut-off points to define exposure, no significant associations between nitrate in drinking-water and mortality from stomach cancer were observed. [The authors noted that the levels of nitrate in private wells were measured at the time of the study, which was about 20 years later than those recorded for public sources.]

Only one study has evaluated the potential association between dietary intake of nitrate and oesophageal cancer. Rogers *et al.* (1995) conducted a population-based case-control study in Washington State (USA) of 645 tumours of the upper aerodigestive tract, that included 125 oesophageal cancers, which were identified through the cancer surveillance system and 458 frequency-matched controls. A self-administered, 125-item food-frequency questionnaire and a food composition table from the National Academy of Sciences were used to estimate the usual intake of nitrate. High consumption (> 226 mg per day) was significantly associated with a decrease in the risk for cancer of the oesophagus compared with lowest intake (< 134 mg per day) after adjustment for potential non-dietary and dietary confounders (including vitamin C intake).

2.2.2 *Ingested nitrite*

(a) *Ecological studies*

Most ecological studies that addressed tumours of the stomach or oesophagus focused mainly on the intake of nitrates whereas nitrite was rarely assessed. The main features and results of ecological studies on the relationship between intake of nitrite and tumours of the stomach or oesophagus are summarized in Table 2.1.

China

In China, Zhang *et al.* (1984) analysed levels of nitrite in the fasting gastric juice of subjects from six areas where mortality from stomach cancer was high and from four areas with low mortality rates and showed a significant positive association for nitrite.

The only ecological study that assessed the association between intake of nitrite and oesophageal tumours was carried out in China. Zhang *et al.* (2003) observed a significantly higher level of nitrite in well-water in the county of Cixian (0.01 mg/L), an area where mortality from oesophageal cancer is very high, compared with the low-risk county of Chichen (0.002 mg/L).

Europe

In a study in Italy (Knight *et al.*, 1990), lower levels of nitrite in saliva were found in subjects from regions where mortality from stomach cancer was high (Siena, Arezzo)

compared with subjects from Cagliari and Verona, where rates were lower, although the differences did not reach statistical significance.

Only one study assessed measured levels of nitrite in the drinking-water in relation to stomach cancer. In Poland, Zemła and Kolosza (1979) reported a significantly higher incidence of stomach cancer among men in districts of the town of Zabrze that had higher levels of nitrite in the drinking-water.

In the United Kingdom, Forman *et al.* (1985) compared levels of nitrite in saliva in subjects from an area where mortality from stomach cancer was high (Northeast and Wales) with those from low-risk areas (Southeast and Oxford) and found an inverse relationship; levels were significantly higher in areas with lower mortality.

South America

The risk for stomach cancer in relation to ingested nitrite was investigated in the department of Nariño, Colombia (Cuello *et al.*, 1976). No differences in levels of salivary nitrite were seen between two areas of high or low risk. Armijo *et al.* (1981) carried out a study in children aged 11–13 years from four provinces in Chile, two of which had high and two of which had low mortality rates for stomach cancer. No differences were observed in the concentrations of nitrite in saliva gathered from these children.

(b) Cohort studies

Two cohort studies (described in detail in Section 2.2.1(b)) analysed the association between ingested nitrite and the risk for cancers of the stomach or oesophagus; their main findings are summarized in Table 2.2.

In the Netherlands Cohort Study (van Loon *et al.*, 1997, 1998), intake of nitrite was estimated using the database on food composition values from the Nutrition and Food Research Institute (TNO, Zeist). The association between stomach cancer and intake of nitrite, adjusted for age and sex, was not clear and did not show a clear dose–response relationship. The only significant estimate was for the fifth quintile of intake (age- and sex-adjusted relative risk, 1.49; 95% CI, 1.01–2.20), which became non-significant (relative risk, 1.44; 95% CI, 0.95–2.18) after simultaneous adjustment for vitamin C, β -carotene, level of education, tobacco smoking and use of a refrigerator or freezer. [The decreased risk after adjustment for vitamin C and β -carotene could reflect the potential inhibitory effect on nitrosation of such nutrients. The possible interaction between nitrite intake and vitamin C was not assessed; no data on dietary intake of nitrosamines were provided in this study.]

The Mobile Health Clinic of the Social Insurance Institution in Finland (Knekt *et al.*, 1999) collected data on food consumption by means of a diet history interview, and several food composition databases were used to calculate intake of nitrite. After a 24-year follow-up (1967–90), 68 cases of cancer of the stomach were identified. Dietary intake of nitrite was not associated with risk for stomach cancer after adjustment for age, sex, municipality, tobacco smoking and energy intake.

(c) *Case-control studies*

The findings of case-control studies on the risk for stomach cancer and oesophageal cancer in relation to ingested nitrites are summarized in Table 2.3. Most studies that addressed nitrite intake also considered nitrate and have thus been described in full in Section 2.2.1(c). Only the few studies that addressed exclusively intake of nitrite are described in detail here.

Europe

A large case-control study that included 1016 cases of stomach cancer and 1159 controls was carried out in Italy (Buiatti *et al.*, 1990). Nitrite intake (mg per day) was derived from personal interview using a structured questionnaire of 146 items and the application of food composition tables from Italian and English sources. The association with risk for stomach cancer was adjusted for age, sex, social class, familial history of stomach cancer, body mass index and caloric intake. There was a significant increase in risk with increasing levels (quintiles) of nitrite intake (P for trend = 0.005). Furthermore, an interaction was observed between antioxidants (vitamin C and α -tocopherol) and protein and nitrites: cases and controls were classified into three categories (low, medium, high) according to these two groups of compounds. Using the low/low category as the reference, the odds ratio for the high antioxidants/low protein and nitrite category was 0.4, while that for the low antioxidant/high protein and nitrite category was 2.1 (a fivefold difference in relative risk for the two extreme categories). Palli *et al.* (2001) updated the results of Buiatti *et al.* (1990) for the cases from Florence by expanding the series of controls and applying an updated Italian food composition table that included values for dimethylamine and *N*-nitrosodimethylamine (NDMA). Similarly to the previous study, a significant positive association with nitrite was observed (P for trend = 0.04); a positive association was also observed with estimated intakes of dimethylamine and NDMA.

Another Italian case-control study carried out in the four largest teaching hospitals of the Greater Milan area (La Vecchia *et al.*, 1994, 1997) assessed nitrite intake through a 29-item food-frequency questionnaire and an Italian food composition table. A significant trend for an increased risk for stomach cancer was observed with higher intake of nitrite (categorized in quartiles), although none of estimates was statistically significant (P for trend < 0.05); however, when two levels of nitrite intake were considered in relation to the median (≥ 2.7 versus < 2.7 mg per day), a significant increased risk was observed (odds ratio, 1.44; 95% CI, 1.2–1.7). In this study, methionine was associated with an increased risk for stomach cancer and the combined effect of both compounds was assessed; the odds ratio for the category with high intake of nitrite and methionine was 2.45 (95% CI, 1.9–3.2) compared with the category with lowest consumption for both compounds.

A hospital-based case-control study of stomach cancer in four provinces of Spain (González *et al.*, 1994) used a diet history method and Spanish and British food composition tables to estimate intake of nutrients. In the matched analysis of 354 pairs, a slightly increased risk for high intake of nitrite was observed, although the trend was not significant; the effect of nitrite was only evident for tumours of the intestinal type, while

no effect or inverse risk estimates were observed for diffuse-type tumours of the stomach. [In this study, there was a significant association with NDMA (results not provided) and an interaction with vitamin C: subjects who had a high intake of nitrosamines and a low intake of vitamin C had an odds ratio of 1.98 (95% CI, 1.3–3.1) compared with those who had a low vitamin C and low nitrosamine intake.]

Hansson *et al.* (1994) reported the results of a case–control study in Sweden of 338 cases of stomach cancer and 679 controls. Nitrite intake was calculated from a food-frequency questionnaire and the Swedish food composition table. The age- and sex-adjusted relative risk for the highest quartile of nitrite intake compared with the lowest quartile was 1.22 (95% CI, 0.82–1.81).

A hospital-based case–control study carried out in the area of Marseilles, France (Pobel *et al.*, 1995) used a diet history questionnaire combined with a French food composition table to estimate the intake of nitrites. No association was observed in relation to risk for stomach cancer overall or when different dietary sources of nitrite (from vegetables or other foods) were considered separately. [Potential limitations of this study are described in Section 2.2.1(c).]

North America

Risch *et al.* (1985) reported the results from a population-based case–control study in two provinces of Canada and the area of Toronto. Usual diet was assessed by the diet history method which was administered by an interviewer and nutrient intakes were calculated using a food composition table from the USA and other sources. The matched analysis with adjustment for ethnicity and total caloric intake showed a significant increase in risk for stomach cancer for every milligram of nitrite consumed per day (odds ratio, 1.71; 95% CI, 1.24–2.37). [No adjustment was made for β -carotene, although this nutrient was also found to be associated with a decreased risk for stomach cancer. The interaction between intake of nitrite and antioxidant micronutrients was not assessed.]

Two case–control studies from the USA addressed the association of nitrite intake with the risk for oesophageal cancer alone or with tumours at other sites. Rogers *et al.* (1995) conducted a population-based study in Washington State and used a 125-item food-frequency questionnaire and a food composition table from the National Academy of Sciences to estimate the usual intake of nitrite. A positive but non-significant association was observed. Although no statistically significant interactions were found, the data suggested that individuals with both low intake of vitamin C and high nitrite intake had a higher risk for oesophageal cancer (odds ratio, 5.07; $P < 0.05$). [In this study, the pattern of estimates for NDMA was very similar to that reported for nitrites yielding a positive trend (P for trend = 0.06), although estimates for tertiles of intake were not statistically significant. There was a suggestion of an interaction of NDMA with vitamin C: those with higher tertiles of NDMA and low vitamin C intake had a three-fold significantly increased risk of oesophageal cancer ($P < 0.05$).]

A more recent population-based case–control study in the USA (Mayne *et al.*, 2001) considered stomach and oesophageal tumours according to their localization or

histological type. Overall, 1095 cases were identified through population cancer registries; 458 frequency-matched controls were selected by random-digit dialling. Personal interviews were obtained using a 104-item food-frequency questionnaire and dietary data were linked to published databases on nitrite content. Separate results were provided for 352 non-cardia stomach cancers, 225 tumours located in the cardia, 206 squamous-cell type tumours of the oesophagus and 282 oesophageal adenocarcinomas. Adjusted for several potential confounders including total energy intake (but no nutrients), high intake of nitrite was significantly associated with an increased risk for non-cardia stomach cancer only. However, compared with persons with low nitrite and high vitamin C intakes, persons with low vitamin C intake had a significantly increased risk for cancers of the stomach (cardia and non-cardia) and oesophagus (adenocarcinomas and squamous-cell carcinomas). Intake of nitrite was further examined in cases of cardia stomach cancer from this study using categorization by quartiles according to the consumption of controls (Engel *et al.*, 2003). A significant increase in risk was found for all levels of intake compared with the lowest.

2.2.3 *Preneoplastic lesions*

Superficial gastritis, chronic atrophic gastritis, intestinal metaplasia and dysplasia have been postulated to be part of the sequential process that leads to stomach carcinoma, and are therefore considered to be precursor lesions. The roles of nitrate, nitrite and nitrosation have been investigated in relation to this process, mainly in some areas in Colombia that are at high-risk for stomach cancer. In the Nariño area, a cohort of subjects was recruited between 1973 and 1983, all of whom had a gastroscopy and biopsy at baseline. Cross-sectional analysis of the first biopsy was carried out for 1670 subjects (Correa *et al.*, 1990a). Taking normal mucosa as the reference category, positive versus negative level of nitrite in gastric juice (above or below the median) gave the following age-adjusted odds ratios: hyperplasia, 4.3; atrophy, 6.5; metaplasia, 8.3; and dysplasia, 16.8 (overall $P < 0.0001$); the corresponding values for nitrate (> 24 versus ≤ 24 mg/L) were 1.4, 1.8, 2.1 and 2.7 (P for trend < 0.0001). However, stomach nitrite failed to predict future changes in the same end-points (Correa *et al.*, 1990b). Within this cohort, a further analysis was carried out for 263 subjects, including 117 cases of stomach cancer and 146 controls [not specified] (Chen *et al.*, 1990). Odds ratios for the presence of nitrite in gastric juice and the risk for precursor lesions compared with subjects with normal mucosa, adjusted for age, sex, pH, sodium/creatinine ratio and addition of salt, were: superficial gastritis, 2.99 (95% CI, 0.88–10.17); chronic atrophic gastritis, 3.49 (95% CI, 0.84–14.40); intestinal metaplasia, 4.39 (95% CI, 1.36–14.16); and dysplasia, 24.72 (95% CI, 1.97–309.64). In the same region, Cuello *et al.* (1979) characterized the histopathology of stomach biopsies from 123 patients in a village of the department of Nariño, in the Andean region of Colombia. Measurable levels of nitrite in gastric juice were detected in patients who had chronic atrophic gastritis but not in those who had superficial gastritis or normal mucosa; the level was significantly elevated (4.2 mg/L;

$P < 0.01$) only among those who had intestinal metaplasia with dysplasia. There were no differences in the level of nitrate.

During 1989–90, a gastroscopic screening survey was carried out in a rural area of the province of Shandong (China), which has one of the highest rates of stomach cancer (You *et al.*, 1996). Endoscopic biopsies, fasting gastric juice and overnight urine were collected; data were available for 583 persons (312 men and 271 women). The level of nitrite in gastric juice was above the detection level for 7% of subjects with superficial/chronic atrophic gastritis versus 17% of those with intestinal metaplasia ($P < 0.01$). Furthermore, among those with detectable values, the geometric mean gastric content of nitrite was 0.8 ng/mL for subjects with intestinal metaplasia compared with 0.4 ng/mL for those with superficial/chronic atrophic gastritis ($P = 0.06$). This pattern was similar for subjects whose gastric juice had a pH > 2.4 , but not for the subgroup that had a lower pH. On the contrary, the level of nitrate in urine was higher among subjects who had superficial/chronic atrophic gastritis (281 ng/mL) than among those who had intestinal metaplasia (242 ng/mL), but the differences were non-significant.

2.3 Tumours of the brain

Epidemiological studies of brain cancer are challenging because of problems with the specificity of diagnosis and the difficulties of patients to recall past exposures while experiencing the effects of cerebral lesions, surgery or treatment. Numerous epidemiological studies have investigated the potential role of nitrate and nitrite in relation to the occurrence of central nervous system (mainly brain) tumours in adults and children. These were mostly case–control studies that estimated nitrate and nitrite consumption from either residential tap-water or other dietary sources and are summarized in Table 2.4.

In the studies that considered dietary exposure, participants, their mothers (childhood brain tumour studies) or proxy respondents (for deceased or disabled patients in some studies of adult brain tumours) were requested to recall consumption of selected foods; this information was then linked to data on the content of nitrate and nitrite in food. In each of the studies reviewed below, approximately 50 foods (range, 42–100) were included; in many, the authors stated that foods with a high content of nitrate, nitrite or *N*-nitroso compounds were intentionally well represented.

The studies that considered exposure from tap-water used past measures of nitrate in public utilities and/or measured nitrate and/or nitrite in newly collected water samples from the current home or the residence at the time of diagnosis. With the exception of one study (Ward *et al.*, 2005), water testing relied on semiquantitative test strips.

As outlined in Section 2.1, studies were not reviewed if they only considered less direct potential indicators of exposure to nitrate and/or nitrite. However, the potential association between the consumption of cured meat and the occurrence of brain tumours has been studied extensively. Many studies suggested an association between brain tumours in children and consumption of cured meat by the mother during pregnancy and/or

Table 2.4. Case-control studies of nitrate and nitrite in the drinking-water and from dietary sources and the risk for adult and childhood central nervous system tumours

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Adult								
<i>Australia</i>								
Giles <i>et al.</i> (1994), Melbourne and other cities of Victoria, Australia, 1987–91	Glioma primary	409 cases (243 men, 166 women) from registry/14 hospitals, aged 20–70 years; 100% histologically confirmed; response rate, 66%; 409 controls (243 men, 166 women) randomly selected from electoral roll; matched by sex, age; history of stroke, epilepsy, prior brain tumour excluded; response rate, 45%	Mailed questionnaire (59 foods), followed by in-person interview, linked to published tables of food content; proxy for 54.3% of male cases, 3.3% of male controls, 50.6% of female cases and no female controls	Nitrate in the diet			Alcohol consumption, tobacco smoking	Period for which diet was ascertained was not specified; among men, most food groups and nutrients were above null, and no adjustment was made for total energy intake [in this particular study, the Working Group believed this adjustment should have been made because of misclassification, not because energy intake is a potential risk factor].
				<i>Men</i>				
				T1	74	1.0		
				T2	95	1.42 (0.89–2.26)		
				T3	74	1.13 (0.68–1.86)		
				<i>Women</i>				
				T1	73	1.0		
				T2	48	0.48 (0.25–0.91)		
				T3	45	0.53 (0.28–0.96)		
				Nitrite in the diet				
				<i>Men</i>				
				T1	60	1.0		
T2	90	1.58 (0.96–2.61)						
T3	93	1.58 (0.96–2.58)						
<i>Women</i>								
T1	61	1.0						
T2	47	0.77 (0.43–1.37)						
T3	58	0.98 (0.55–1.72)						

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments			
<i>Europe</i>											
Boeing <i>et al.</i> (1993); Steindorf <i>et al.</i> (1994), Rhein–Neckar–Odenwald area, Germany, 1987–88	Brain, primary	226 cases (99 men, 127 women; 115 gliomas, 81 meningiomas, 30 acoustic neuromas) from 2 neurosurgery clinics, aged 25–75 years; 100% histologically confirmed; response rate, 97.8%; 418 controls (185 men, 233 women) from compulsory regional residential register; frequency-matched by sex, age; response rate, 72%	Diet: in-person questionnaire (42 foods, previous 5 years) linked to published tables of food content; proxy for 12% of cases, 3% of controls Water (test): semi-quantitative test strip estimation of tap-water at current home; available for 60.1% of cases and 90.4% of controls who had lived in the region > 1 year	Nitrate in the diet				Age, sex, alcohol consumption, tobacco smoking	Diet: odds ratios include 53 (23.5%) cases and no controls who did not live in the study region for at least 1 year since 1970 [approximately previous 17 years]. Water test: case water samples were sent to hospital, whereas control water samples were tested in home immediately after sample collection		
				<i>Glioma</i>		T1	29			1.0 (reference)	
				T2	34	1.0 (0.6–1.8)					
						T3	30	0.9 (0.5–1.5)			Crude
						<i>P</i> for trend		0.64			
				Nitrate in water (test)		Not detected	60	1.0 (reference)			
						10 mg/L	12	[0.5 (0.2–0.9)]			
		25 mg/L	28	[0.7 (0.4–1.2)]							
		50–100 mg/L	4	[0.3 (0.1–0.7)]							

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments	
Boeing <i>et al.</i> (1993); Steindorf <i>et al.</i> (1994) (contd)			Water (records): residential history linked to measures in 1971–87 from 69 water providers, plus other data for 2% with private well/spring; attempted for subjects living in region >1 year since 1970, 76.6% of cases and 100% of controls; data available for 168 cases and 406 controls (97% of each attempted)	Nitrate in water (records) (mean nitrate mg/L)				Age, sex (diet, tobacco smoking, bottled water considered)	Water records: mean nitrate levels were very similar for cases and controls during each year (1970–87); consideration of tumour type or latency period did not alter conclusions.
				Q1 (0–2.0)	45	1.0 (reference)			
				Q2 (> 2.0–11.3)	38	0.99 (0.60–1.63)			
				Q3 (> 11.3–25.2)	44	1.12 (0.69–1.83)			
				Q4 (> 25.2)	41	1.00 (0.61–1.64)			
				Nitrite in the diet <i>Glioma</i>			Age, sex, alcohol consumption, tobacco smoking		
				T1	26	1.0 (reference)			
T2	31	1.1 (0.6–1.9)							
	T3	36	1.1 (0.6–2.0)						
	<i>P</i> for trend		0.62						
<i>Israel</i>									
Kaplan <i>et al.</i> (1997), Tel Hashomer/central Israel, 1987–91	Brain tumour, primary malignant or benign	139 cases (mostly glioma and meningioma; 63 men, 76 women) from one medical centre, aged 18–75 years; 100% histologically confirmed; response rate, 77%; 278 controls (126 men, 152 women); matched by age, sex, ethnic origin (one friend, one orthopaedic patient per case); response rate not specified	Questionnaire (100 foods) for the period 10 years before diagnosis, linked to published tables of food content; proxy for 30% of each group	Nitrate or nitrite in the diet	NR	No association [no odds ratios reported]	Total energy intake, matching variables	Analyses repeated by tumour type, and for the two control groups separately in subanalyses	

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
<i>USA</i>								
Blowers <i>et al.</i> (1997), Los Angeles County, CA, USA, 1986–88	Glioma	94 women from tumour registry (county resident), alive and well enough to provide an interview, aged 25–74 years; 100% histologically confirmed; response rate, 67%; 94 female county residents; matched on neighbourhood of residence (at case diagnosis), age, race (black/white); response rate, 72%	In-person questionnaire (43 foods, 90% estimated ascertainment of nitrate and nitrite); no proxies used; complete nutrient data available for 91 (97%) case–control pairs	Nitrate in the diet	NR		Total food intake, body mass index	Only surviving female cases were included and may lead to survivor bias; ethnicity, religion, education, vitamin intake, diabetes and allergies, asthma and eczema considered but not included in the final models.
				Q1		1.0		
				Q2		1.3 (0.5–3.3)		
				Q3		1.1 (0.4–2.7)		
				Q4		0.7 (0.2–1.8)		
				<i>P</i> for trend		0.46		
				Nitrite in the diet	NR			
				Q1		1.0		
				Q2		0.6 (0.2–1.3)		
				Q3		0.4 (0.1–1.0)		
				Q4		1.4 (0.6–3.5)		
				<i>P</i> for trend		0.55		
				Nitrite from cured meat	NR			
Q1		1.0						
Q2		0.9 (0.3–2.4)						
Q3		1.3 (0.6–3.0)						
Q4		2.1 (1.0–4.6)						
<i>P</i> for trend		0.07						

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments	
Lee <i>et al.</i> (1997), San Francisco Bay area, CA, USA, 1991–94	Glioma	494 cases (282 men, 212 women) from one cancer centre rapid-ascertainment system, aged > 20 years; 100% histologically confirmed; response rate, 82%; 462 controls (254 men, 208 women) from random-digit dialling; frequency-matched by age, sex, race/ethnicity (white, black, Asian, Hispanic, other)	Mailed questionnaire (79 foods, usual consumption in previous year) followed by in-person interview, linked to database of nutrients; 46% proxies for cases; available for 434 (94%) cases and 439 (99%) controls	Nitrite with vitamin C				Age, education, income	Analyses repeated to exclude proxy respondents: difference in nitrate intake became non-significant for women; increased risk in men with high nitrite and low vitamin C intake was not attenuated and remained significant; nitrite and vitamin C each dichotomized at the median.
				<i>Men</i>					
				Low nitrite, high vit C	36	1.0 (reference)			
				Low nitrite, low vit C	75	1.3 (0.7–2.3)			
				High nitrite, high vit C	58	1.0 (0.6–1.8)			
				High nitrite, low vit C	71	2.1 (1.1–3.8)			
				<i>Women</i>					
				Low nitrite, high vit C	24	1.0 (reference)			
				Low nitrite, low vit C	52	1.2 (0.6–2.4)			
				High nitrite, high vit C	69	1.6 (0.9–3.0)			
				High nitrite, low vit C	36	1.5 (0.7–3.1)			
				Nitrate in the diet					
				<i>Men</i>					
				Cases		726.3 (26.8)			
				Controls		775.8 (26.3)			
				<i>P</i> -value		NS			
				<i>Women</i>					
Cases		52.1 (22.8)							
Controls		660.9 (41.2)							
<i>P</i> -value		≤ 0.05							
Nitrite in the diet									
<i>Men</i>									
Cases		6.5 (0.3)							
Controls		6.0 (0.2)							
<i>P</i> -value		NS							
<i>Women</i>									
Cases		4.9 (0.2)							
Controls		4.6 (0.2)							
<i>P</i> -value		NS							

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Schwartzbaum <i>et al.</i> (1999), Ohio, USA, 1993–96	Glioma	40 cases (19 men, 21 women) from 1 hospital network at time of first surgery for possible brain tumour; median age, 52.5 years; all white; 100% histologically confirmed; response rate, 95%; 48 controls (22 men, 26 women) from same hospital network (orthopaedic or gynecological surgery patients with no malignant neoplasm, diabetes, pregnancy, obesity, gastro intestinal disease, coronary heart disease or stroke); matched by age, sex; all white; response rate, 92%	Nitrite: diet questionnaires (prior year) translated to nutrients via software and published tables; no proxies Vitamins C and E: pre-surgery serum analysed; available for 23 (57.5%) cases	Nitrite from cured meat (mg/day)			Protein, fat, total calories, cured meat, body mass index	Nitrite intake from cured meats: median, 0.6 mg/day in cases; 0.3 mg/day in controls; exclusion criteria for controls probably resulted in a group with a different diet than population controls; odds ratios for nitrite intake above the median from individual types of cured meats at null or below. *low = < median values, high = ≥ median values
				Q1 (< 0.16)	9	1.0 (reference)		
				Q2 (0.16–0.39)	7	2.0 (NR)		
				Q3 (0.40–0.91)	14	2.2 (NR)		
				Q4 (≥ 0.92)	10	2.1 (NR)		
				<i>P</i> for trend		0.36		
				Nitrite-serum vitamin C				
				Low nitrite* low vit C	8	1.0 (reference)		
				Low nitrite high vit C	1	0.1 (0.0–1.2)		
				High nitrite, high vit C	2	0.7 (0.1–9.4)		
High nitrite, low vit C	12	1.6 (0.3–8.0)						

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments	
Chen <i>et al.</i> (2002); Ward <i>et al.</i> (2005), eastern Nebraska, USA, 1988–93	Glioma, primary	251 cases (139 men, 112 women) from cancer registry/11 hospitals in region, aged ≥ 21 years; all white; 100% histologically confirmed; response rate, 89% [among eligible]; 498 controls (283 men, 215 women) from a randomly selected control group (same base population) from a study of non-Hodgkin lymphoma (random-digit dialling, supplemented by Medicare records for age >65 years); frequency-matched on age, sex, vital status; all white; response rate, 71%	Diet: telephone questionnaire (48 food items), linked to published tables; available for 236 (94%) cases and 449 (90%) controls; proxies for 76% cases, 60% controls Public water: residence histories linked to nitrate water-quality monitoring data from 236 public water systems, 1947–85; available for 130 (52%) cases and 319 (64%) controls; ≥70% person-years after 1964 (when water data more complete); lag of 3–8 years between the exposure period and case diagnosis.	Nitrate in the diet				Energy, age, sex, farming, education, respondent, family history of central nervous system tumour Age, sex, respondent, education, farming Birth year, sex, respondent, education, farming Birth year, sex, respondent, education, farming	Subanalyses separate by respondent type, histological type (no meaningful differences); low statistical power to evaluate risk at public water system nitrate levels above 5–10 mg/L nitrate-N; only about 15% of cases and controls believed to be exposed to 10 mg/L nitrate-N in public water supplies >8 years. No interaction observed between vitamin C intake and dietary nitrite.
				Q1	59	1.0 (reference)			
				Q2	81	1.2 (0.7–1.9)			
				Q3	43	0.7 (0.4–1.2)			
				Q4	53	0.7 (0.4–1.2)			
				<i>P</i> for trend		0.1			
				Nitrate in water, public system, 1965–84 (Mean mg/L nitrate-N)					
				Q1 (< 2.38)	24	1.0 (reference)			
				Q2 (2.38–2.57)	39	1.4 (0.7–2.7)			
				Q3 (2.58–4.32)	33	1.2 (0.6–2.3)			
				Q4 (> 4.32)	34	1.3 (0.7–2.6)			
				Nitrate with vitamin C					
				Q1/high vit C	8	1.0 (reference)			
				Q2/high vit C	22	2.4 (0.8–6.9)			
				Q3/high vit C	13	2.0 (0.7–6.0)			
				Q4/high vit C	13	2.1 (0.7–6.4)			
Q1/low vit C	15	2.3 (0.8–6.9)							
Q2/low vit C	16	1.6 (0.5–4.8)							
Q3/low vit C	17	1.7 (0.6–4.7)							
Q4/low vit C	17	2.0 (0.7–5.8)							
Years of nitrate ≥ 5 mg/L nitrate-N									
0	22	1.0 (reference)							
1–4	62	1.3 (0.7–2.5)							
5–9	20	1.8 (0.8–4.1)							
≥ 10	26	1.1 (0.5–2.2)							

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Chen <i>et al.</i> (2002); Ward <i>et al.</i> (2005) (contd)			Private water: tap-water samples from well used in 1985, laboratory analysed; available for 47 cases and 43 controls (75% and 60%, respectively, of those who had used a private well in 1985)	Years of nitrate				
				≥ 10 mg/L nitrate-N				
				0	85	1.0 (reference)	Energy, age, sex, farming, education, respondent, family history of central nervous system tumour	
				1–8	25	1.1 (0.6–2.1)		
				≥ 9	20	1.1 (0.6–2.0)		
				Nitrate in water, private well				
				< 10 mg/L nitrate-N	40	1.0 (reference)		
				≥ 10 mg/L nitrate-N	7	1.2 (0.4–4.1)		
				Nitrite in the diet			Birth year, sex, respondent, education, farming, β-carotene, fibre, calories	Chen <i>et al.</i> (2002)
				Q1	66	1.0 (reference)		
				Q2	66	1.0 (0.6–1.7)		
				Q3	57	0.9 (0.5–1.5)		
				Q4	47	0.8 (0.5–1.3)		
				<i>P</i> for trend		0.3		
				Animal sources (mg/day)			Birth year, sex, respondent, education, farming, β-carotene, fibre, calories	Ward <i>et al.</i> (2005)
				Q1 (< 0.29)	29	1.0 (reference)		
				Q2 (0.29–< 0.46)	25	0.8 (0.4–1.7)		
				Q3 (0.46–0.63)	31	1.3 (0.6–2.7)		
				Q4 (≥ 0.63)	36	1.3 (0.6–2.9)		
				Plant sources (mg/day)				
				Q1 (< 0.31)	34	1.0 (reference)		
				Q2 (0.31–< 0.43)	29	1.0 (0.5–2.1)		
				Q3 (0.43–0.59)	28	3.2 (1.2–8.3)		
				Q4 (≥ 0.59)	30	2.8 (1.0–8.2)		
				Preformed dietary intake + endogenous nitrite from water nitrate (mg/day)				
				< 1.4	21	1.0 (reference)		
				1.4–< 2.1	43	1.8 (0.9–3.6)		
				2.1–< 3.3	28	1.1 (0.5–2.3)		
				≥ 3.3	28	1.1 (0.5–2.5)		

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Childhood								
<i>Europe</i>								
Cordier <i>et al.</i> (1994), Paris/Ile de France region, France, 1985–87	Intra-cranial tumour	75 cases (34 boys, 41 girls) from 13 hospitals in the region, aged 0–15 years, residents of Ile de France; 84% histologically confirmed; response rate, 69%; 113 controls (63 boys, 50 girls) from sample of residences in region, supplemented by telephone books; frequency-matched by birth year; response rate, 72%	Questionnaire (maternal diet during pregnancy) linked to French surveys and published tables of food content	Nitrate in the diet			Age, sex, maternal age, maternal education	<i>N</i> -Nitroso compounds not considered; vitamin C non-significantly protective
				Q1	NR	1.0 (reference)		
				Q2	NR	1.5 (0.5–4.5)		
				Q3	NR	0.5 (0.1–1.7)		
				Q4	NR	1.5 (0.5–4.6)		
				<i>P</i> for trend		NS		
				Nitrite in the diet				
				Q1	NR	1.0 (reference)		
				Q2	NR	0.9 (0.3–2.7)		
				Q3	NR	0.6 (0.2–2.3)		
Q4	NR	0.4 (0.1–0.4)						
<i>P</i> for trend		NS						

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments	
<i>North America</i>									
Bunin <i>et al.</i> (1993, 1994), Canada/USA, 1986–89	Brain tumours, PNET (1993), astrocytic glioma (1994)	321 cases with no previous malignancy from 33 paediatric oncology groups, aged <6 years; 166 (100 boys, 66 girls) PNET; 89% histologically confirmed; response rate, [77%]; 155 (81 boys, 74 girls) astrocytic glioma; 78% histologically confirmed; response rate, [74%]; 321 controls from random-digit dialing; matched on telephone area code + next 5 digits, birthdate (1 year), race (black/other); 166 PNET controls (90 boys, 76 girls); response rate, [73%]; 155 astrocytic glioma controls (85 boys, 70 girls); response rate, [74%]	Structured telephone interview (53 foods during pregnancy, 60–75% estimated ascertainment of nitrate and nitrite), linked to databases of portion size and food content	Nitrate in the diet			None (child diet, family income, maternal tobacco smoking considered)	Inverse PNET–nitrate association attenuated and became non-significant (<i>p</i> for trend = 0.19) after adjustment for other food components (vitamins A, C and E, folate, nitrite and nitrosamine) and vitamin/mineral supplements [only crude risk estimates provided in full].	
				<i>PNET</i>					
				Q1	NR				1.0
				Q2	NR				1.18 (0.64–2.19)
				Q3	NR				0.61 (0.34–1.09)
				Q4	NR				0.44 (0.23–0.84)
				<i>P</i> for trend					0.002
				<i>Astrocytic glioma</i>					
				Q1	40				1.0
				Q2	32				0.7 (0.3–1.4)
				Q3	38				0.9 (0.4–1.8)
				Q4	34				0.7 (0.3–1.4)
				<i>P</i> for trend					0.43
				Nitrite in the diet					None (child diet, family income, maternal tobacco smoking considered)
				<i>PNET</i>					
				Q1	NR				
Q2	NR	1.58 (0.79–3.16)							
Q3	NR	1.04 (0.57–1.89)							
Q4	NR	1.11 (0.58–2.10)							
<i>P</i> for trend		0.80							
<i>Astrocytic glioma</i>									
Q1	40	1.0							
Q2	31	0.8 (0.4–1.6)							
Q3	31	0.8 (0.4–1.5)							
Q4	42	1.3 (0.7–2.6)							
<i>P</i> for trend		0.54							
		Family income							

North America

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Preston-Martin <i>et al.</i> (1996); Pogoda & Preston-Martin (2001a,b), California (Los Angeles and San Francisco areas) and Washington (Seattle area) states, USA, 1984–91	Brain, cranial nerves, cranial meninges, primary	540 cases (298 boys, 242 girls) from 3 tumour registries, aged < 20 years, living in the study region at diagnosis in a home with a telephone; % histological confirmation not specified; response rate, [71%]; 801 controls (448 boys, 353 girls) from residents of the same counties and identified by random-digit dialling; frequency-matched by age, sex; response rate, [74%]	In-person questionnaire (47 foods, maternal intake during pregnancy), linked to published tables/software (1996 analyses) or year-specific nitrite levels from 26 food surveys of sodium nitrite levels (2001 analyses)	Nitrite in the diet			Age, sex, birth year, geographic area	No interaction with socioeconomic status (<i>p</i> for trend statistically significant for low and high socioeconomic groups); race and socioeconomic status considered but not included in final models.
				Q1	NR	1.0 (reference)		
				Q4	NR	1.1 (0.79–1.50)		
				[Other quartiles not reported]				
				Nitrite in vegetables only				
				Q1	NR	1.0 (reference)		
				Q4	NR	0.98 (0.71–1.3)		
				[Other quartiles not reported]				
				Nitrite in cured meat only (mg/day)				
				Q1 (<0.02)	122	1.0 (reference)		
				Q2 (0.02–0.29)	121	1.1 (0.78–1.5)		
				Q3 (0.30–1.28)	116	1.1 (0.78–1.5)		
				Q4 (> 1.28)	155	1.9 (1.3–2.6)		
<i>P</i> for trend		0.003						
Nitrite with prenatal vitamins supplements								
Low nitrite/vit +	195	1.0 (reference)	Low nitrite = < 0.3 mg/day and high nitrite = ≥ 0.3 mg/day from cured meats					
High nitrite/vit +	219	1.3 (1.0–1.7)						
<i>P</i> for trend		0.002						
Low nitrite/vit –	46	1.5 (0.93–2.3)						
High nitrite/vit –	47	2.2 (1.4–3.6)						
<i>P</i> for trend		0.007						

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Preston-Martin <i>et al.</i> (1996); Pogoda & Preston-Martin (2001a,b) (contd)				Nitrite in cured meat only (mg/day)*				Re-analysis of Preston-Martin <i>et al.</i> (1986) *Time-specific nitrite estimates from literature review Interaction with vitamin use or intake not addressed
				None	102	1.0 (reference)		
				0.01–0.49	293	1.1 (0.8–1.5)		
				0.50–0.99	68	1.9 (1.2–2.9)		
				1.00–1.99	28	1.3 (0.8–2.3)		
				2.00–2.99	12	1.8 (0.8–4.1)		
				≥ 3.00	11	3.0 (1.2–7.9)		
				Per mg/day		β = 0.22 (standard error, 0.08)		
				P-value		0.008		

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
<i>International</i>								
Mueller <i>et al.</i> (2004), regions near Winnipeg, Canada; Paris, France; Valencia, Spain; and Los Angeles, San Francisco and Seattle, USA; and countries not collecting water (Australia, Israel, Italy), also included in full study; 1976–94	Brain, cranial nerves, cranial meninges, primary malignant	1218 cases in full study from cancer incidence registries in each area, aged < 20 years; > 90.2% histologically confirmed; response rate in each country, 71–88%; 2223 controls in full study from electoral roll, telephone directory, census, population register, health service rosters, or random-digit dialling; response rate in each country, 60–87%	Semi-quantitative test strip estimation of tap-water (collected in 1990s in Canada, France, Spain, USA) from the residence at the time of interview; available for 283 cases and 537 controls (28% of all subjects); including 185 cases and 341 controls, representing 86% subjects from the participating countries who still lived in pregnancy home; including 131 cases and 241 controls with no bottled water use in the home during pregnancy; test strip: minimum 10 mg/L nitrate, 1 mg/L nitrite	Pregnancy home <i>Nitrate in water (mg/L)</i> All None detected 10–< 25 25–< 50 ≥ 50 No bottled water None detected 10–< 25 25–< 50 ≥ 50 Astroglial tumours None detected 10–< 25 25–< 50 ≥ 50 PNET None detected ≥ 10 Other tumours None detected 10–< 25 25–< 50 ≥ 50	128 31 12 14 NR NR NR NR NR NR NR NR NR NR NR NR	1.0 (reference) 0.5 (0.3–0.9) 0.5 (0.2–1.1) 1.0 (0.4–2.2) 1.0 (reference) 0.9 (0.5–1.8) 0.7 (0.2–2.1) 1.5 (0.6–3.8) 1.0 (reference) 0.7 (0.3–1.3) 0.5 (0.1–1.5) 1.9 (0.7–5.7) 1.0 (reference) 0.2 (0.1–0.8) 1.0 (reference) 0.4 (0.2–0.99) 0.6 (0.2–1.6) 0.7 (0.2–2.1)	Study centre, age, sex, year of diagnosis	Among control pregnancy homes, nitrate and nitrite were very prevalent considering that most were on public water supplies at the time of conception: 39.6% any nitrate; 8.2% nitrate ≥ 50 mg/L; 10.0% any nitrite; 2.4% nitrite ≥ 5 mg/L. Results for pregnancy home only presented because these subjects were targeted (others who provided water samples may not be representative; among controls, participation of those not targeted varied according to the presence of nitrite).

Table 2.4. (contd)

Reference, study location, period	Tumour type	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjustment for potential confounders	Comments
Mueller <i>et al.</i> (2004) (contd)				Nitrite in water (mg/L)				Results for nitrate not altered materially when individuals with nitrite detected excluded (nitrite interferes with nitrate measurement using these test strips)
				None detected	160	1.0 (reference)		
				1-< 5	19	1.7 (0.8-3.7)		
				≥ 5	6	2.1 (0.6-7.4)		
				<i>No bottled water</i>				
				None detected	NR	1.0 (reference)		
				1-< 5	NR	2.1 (0.8-6.0)		
				≥ 5	NR	5.2 (1.2-23.3)		
				<i>Astroglial tumours</i>			Center, age, sex, year of diagnosis	
				None detected	NR	1.0		
				1-< 5	NR	4.3 (1.4-12.6)		
				≥ 5	NR	5.7 (1.2-27.2)		
				<i>PNET</i>				
				None detected	NR	1.0		
				1-< 5	NR	0.9 (0.1-11.7)		
				≥ 5	NR	1.3 (0.1-30.9)		
				<i>Other tumours</i>				
				None detected	NR	1.0		
				1-< 5	NR	1.2 (0.3-4.2)		
				≥ 5	NR	0.7 (0.1-7.7)		

CI, confidence interval; NR, not reported; NS, not significant; PNET, primitive neuroectodermal tumours; Q, quartile; T, tertile; vit C, vitamin C; vit-, without vitamin supplement; vit+, with vitamin supplement

by the child (astrocytoma and astrocytic glioma: Preston-Martin *et al.*, 1982; Kuijten *et al.*, 1990; Bunin *et al.*, 1994; primitive neuroectodermal tumours: Bunin *et al.*, 1993; brain tumours: McCredie *et al.*, 1994a,b; Sarasua & Savitz, 1994; Preston-Martin *et al.*, 1996; Schymura *et al.*, 1996; Pogoda & Preston-Martin, 2001a,b) but a few other studies did not confirm this (Howe *et al.*, 1989; Cordier *et al.*, 1994; Lubin *et al.*, 2000; medulloblastoma: Bunin *et al.*, 2005). Studies in adults have yielded mixed results. Some suggested an increased risk for brain tumours (meningiomas in women: Preston-Martin *et al.*, 1982; astrocytoma versus population-based controls: Ahlbom *et al.*, 1986; cohort study of glioma: Mills *et al.*, 1989; meningioma in men: Preston-Martin *et al.*, 1989; Preston-Martin & Mack, 1991; glioma and meningioma: Boeing *et al.*, 1993; glioma: Giles *et al.*, 1994; gliomas in women: Blowers *et al.*, 1997; glioma: Lee *et al.*, 1997; Schwartzbaum *et al.*, 1999) whereas others did not (meningiomas in men: Preston-Martin *et al.*, 1983; astrocytoma versus clinical controls: Ahlbom *et al.*, 1986; hospital controls: Burch *et al.*, 1987; cohort study of meningioma: Mills *et al.*, 1989; glioma in men: Preston-Martin *et al.*, 1989; Preston-Martin & Mack, 1991; glioblastoma: Hochberg *et al.*, 1990; brain tumours: Kaplan *et al.*, 1997; glioma: Chen *et al.*, 2002). Meta-analyses of some of the studies cited above suggested an association between consumption of cured meat and the occurrence of brain tumours in children (Huncharek & Kupelnick, 2004) and adults (Huncharek *et al.*, 2003), but Murphy *et al.* (1998) observed that trends in the incidence of brain tumour and consumption of cured meat in the two age groups do not support such an association [all three analyses, especially the latter, had limitations that limit inference]. Some studies observed a possible interaction between consumption of cured meat and intake of vitamins (e.g. vitamin C), fruit or vegetables (i.e. the greatest risk is for those who have high consumption of cured meat and a low intake of antioxidants) (Bunin, 1994; Preston-Martin *et al.*, 1996; Blowers *et al.*, 1997).

2.3.1 Nitrate

(a) Ecological studies

Barrett *et al.* (1998) conducted a study (described in detail in Section 2.2) in the Yorkshire region of England, where only 31 (0.3%) of all water samples in public supplies contained > 50 mg nitrate/L. The residence at diagnosis of 3441 (of 3812) cases of brain cancer was linked to a water supply zone ($n = 148$) and its corresponding measurements of nitrate. Relative to the quartile of the population that had the lowest mean concentration of nitrate in water, individuals in all other quartiles (higher levels of nitrate) had a very modest but statistically significant increase in risk for brain cancer (relative risk, 1.14; 95% CI, 1.04–1.26 for the second quartile; 1.13; 95% CI, 1.03–1.25 for the third quartile; and 1.18; 95% CI, 1.08–1.30 for the fourth quartile, adjusted for socioeconomic status and population density).

Van Leeuwen *et al.* (1999) conducted an ecological study (described in detail in Section 2.2) of 40 'ecodistricts' in Ontario, Canada, where levels of nitrate in water ranged from 0 to 91 mg/L nitrate-N. A model that included levels of atrazine as an a-

priori variable and all other potentially confounding factors that were selected according to *p*-value was used to estimate incidence ratios for brain cancer [it was not specified which confounders were retained nor whether any non-significant factors that affected the coefficient for nitrate were appropriately included.] The resulting incidence ratios were non-significantly increased in both men ($P = 0.072$) and women ($P = 0.101$) in relation to increasing levels of nitrate [coefficients not presented].

(b) *Case-control studies in adults*

Giles *et al.* (1994) conducted a case-control study of primary glioma and diet among adult residents in five major cities in Victoria, Australia, including Melbourne. Cases, aged 20–70 years, were identified at 14 hospitals that had neurological services and were checked against the cancer registry of the region. Controls with no history of stroke, epilepsy or brain tumour were randomly selected from the electoral roll of Victoria that covers 97% of the population. One control was matched to each case by sex and age and subjects with no dietary data were excluded, which resulted in 409 case-control pairs. Odds ratios (tertiles of intake), stratified by gender, were adjusted for alcohol consumption and tobacco smoking. In men, odds ratios for nitrate were modestly and non-significantly above null, while in women, nitrate was associated with a significantly decreased risk for glioma. No dose-response effects were observed in either sex. [Among men, risk estimates for most foods and nutrients, including vitamin C, were above null, which suggests that cases and/or their proxies possibly reported greater food intake in general than controls, although odds ratios were not adjusted for total energy intake. In this particular study, the Working Group believed that this adjustment should have been made because of misclassification, not because energy intake is a potential risk factor.]

Boeing *et al.* (1993) conducted a case-control study of primary incident brain tumours in adult residents of the Rhein-Neckar-Odenwald area of Germany. Cases aged 25–75 years at diagnosis were identified from two neurosurgery clinics that treated 95% of brain tumour patients in the region (115 gliomas, 80 meningiomas, 30 acoustic neuromas); 418 controls were identified by the residential register for the region and were frequency-matched by sex and age. Results were presented for glioma only; odds ratios for dietary intake of nitrate in tertiles were close to null after adjusting for age, sex, alcohol consumption and tobacco smoking. [Steindorf *et al.* (1994) indicated that all controls but only 76.6% of cases had lived in the study region for at least 1 year since 1970 (in the approximately 17 years preceding diagnosis).]

In the same study, Boeing *et al.* (1993) used semiquantitative nitrate test strips (10, 25, 50, 100, 250 and 500 mg/L nitrate) to estimate the levels of nitrate in drinking-water samples from the current residence for a subset of subjects: cases who sent a water sample to the hospital and controls for whom the interviewer conducted the testing during the in-home interview. Proportionally fewer cases than controls provided a water sample (an estimated 60% of cases and 90% of controls, based on Steindorf *et al.* (1994)). [The water-related results are difficult to interpret because only 76.6% of cases but all controls (Steindorf *et al.*, 1994) had lived in the study region for at least 1 year since 1970 (in the

approximately 17 years preceding diagnosis); markedly fewer cases than controls provided water samples; a delay in water testing that applied only to cases raises the possibility that the estimates for cases may be systematically biased in relation to those of controls. It is unknown whether the 'current' residence was the residence at diagnosis.]

Steindorf *et al.* (1994), using data from the study by Boeing *et al.* (1993), focused on the 173 cases and 418 controls who had lived in the study region for at least 1 year since 1970. Historical information on residence was linked to data from water-quality monitoring that gave mean nitrate concentrations for each year in 1971–87 obtained from 69 public authorities and water-treatment plants to create indicators of exposure to nitrate in water for 168 (97.1%) cases and 406 (97.1%) controls. (Only 2% of subjects used private wells/springs and supplementary data were attempted for these individuals.) Nitrate values ranged from 0 to 97.8 mg/L and were very similar for cases (15.98 mg/L; standard deviation [SD], 15.61) and controls (16.16 mg/L; SD, 15.72), even when stratified by year of exposure. Odds ratios for estimated exposure to nitrate in quartiles yielded risk estimates for brain tumours at or very close to null, adjusted for age and sex (diet, use of bottled water and tobacco smoking were also considered). The authors indicated that the results were unchanged in subanalyses that considered latency and tumour type. The results of nitrate test strips obtained in Boeing *et al.* (1993) were presented for the subset of subjects (residents of the region) only; nitrate was associated with a decreased risk for brain tumours. [For the test strip results, most of the limitations noted for Boeing *et al.* (1993) apply. These do not apply to the results of water record linkage which suggested that these estimates are valid; when stratified by case status, there was good correlation ($r = 0.62$ in cases and $r = 0.59$ in controls) between the results of water record linkage and another non-gold-standard measure, the test strips. Although this also suggests that the test strips categorized subjects accurately according to nitrate levels within their respective case group (good precision), these results did not provide any information about possible non-differential or differential bias of the test strip measures.]

Kaplan *et al.* (1997) conducted a case–control study of primary adult brain tumours in central Israel. One hundred and thirty-nine cases, aged 18–75 years, were identified from one medical centre that served the region. Two controls (one friend and one orthopaedic patient from the region) of the same sex, age (within 5 years) and ethnic origin were located for each case. Analyses used nutrient densities (nutrient divided by total daily calories) or adjusted for total energy consumption, and were repeated by tumour type (glioma or meningioma) using only one control group at a time. The authors stated that total dietary intake of nitrate was not associated with the occurrence of brain tumours [the number of exposed cases and controls and the risk estimates were not provided].

Blowers *et al.* (1997) conducted a case–control study of gliomas in women and examined their diet. Cases, aged 25–74 years, were identified from a population-based tumour registry in Los Angeles County, CA, USA, and were restricted to 94 glioma patients who were alive and sufficiently well to undergo an interview. For each case, one female control who lived in the same neighbourhood as the case at the time of diagnosis

was selected and matched by age and race (black/white). There was no association between case status and dietary intake of nitrate in quartiles, adjusted for total food intake and body mass index (several other potential confounding factors were also considered). [Exclusion of deceased and very ill cases of glioma somewhat limits the interpretation of the results because surviving cases may not represent incident cases; therefore, selection bias may be possible and results cannot be generalized (such as for stage of tumour or treatment).]

Lee *et al.* (1997) conducted a case-control study of glioma in adults in the San Francisco Bay Area of California, USA. Four hundred and thirty-four cases were identified from a rapid case-ascertainment system at one cancer centre; 439 controls were selected using random-digit dialling and were frequency-matched by age, sex and race/ethnicity (white, black, Hispanic, Asian, other). All comparisons were stratified by sex and adjusted for age, education and family income. Mean weekly intake of nitrate was lower for cases than for controls in both men (non-significant) and women (significant). There was a statistically significant increased risk for glioma in men with high nitrate intake and low vitamin C intake (odds ratio, 2.1; 95% CI, 1.1–3.8; 71 exposed cases).

Chen *et al.* (2002) conducted a case-control study of diet and glioma in adults in eastern Nebraska, USA. Two hundred and fifty-one cases, aged ≥ 21 years, were identified through a cancer registry and 11 hospitals. Controls were mainly selected from among controls of a case-control study of non-Hodgkin lymphoma (that used random-digit dialling and Medicaid records in the same population) and were supplemented by others who were identified by random-digit dialling and death certificates; a total of 498 controls were included and were frequency-matched to cases by age, sex and vital status. Dietary nitrate calculated as nutrient intake in residual quartiles was adjusted for age, sex, respondent type, education, family history of central nervous system tumour and farming and was not significantly associated with the occurrence of glioma.

Ward *et al.* (2005) extended the study by Chen *et al.* (2002) of adult glioma and dietary nitrate by expanding intake of nitrate to drinking-water. This was largely assessed by linking residential histories to data from water quality monitoring for 236 public water sources from 1947 to 1984 (although most analyses presented included only records after 1964, when monitoring became more frequent); the study was restricted to the 130 (52%) cases and 319 (64%) controls who had $> 70\%$ person-years of use of public water after 1964 (and up to 1984, which resulted in a 3–8-year lag period before diagnosis). Mean levels assessed by this method ranged from 0 to 12 mg/L nitrate-N. Odds ratios for each quartile above the first quartile were slightly above null, but were non-significant and showed no dose-response pattern. Relative to low levels of nitrate in water and high vitamin C intake, all strata of exposure were above null but were non-significant. The number of years of use of a public water system that contained ≥ 10 mg/L nitrate-N was not associated with risk for glioma nor when 5 mg/L nitrate-N was used as the cut-off point, although the second to first quartile was non-significantly increased. In addition to these analyses, at the time of the interview, 75% of the cases and 60% of 72 controls who had used a private well in 1985 (47 cases, 43 controls) provided a tap-water sample that

was analysed for nitrate. Levels of nitrate ranged from < 0.5 to 67 mg/L nitrate-N. There was no association between this measure (< 10 mg/L nitrate-N versus \geq 10 mg/L nitrate-N) and case status. The majority of participants relied largely on public water supplies, and only about 15% of cases and controls were believed to have been exposed to 10 mg/L nitrate-N from public water sources for more than 8 years.

(c) *Case-control studies in children*

Cordier *et al.* (1994) conducted a case-control study of brain tumours in children < 15 years of age in the Paris/Ile de France region of France. Seventy-five cases were identified from 13 hospitals and 113 controls were selected from children who lived in the region through the census agency and telephone books. Relative to the first quartile of nitrate intake during pregnancy, the risk for brain tumours in the offspring in the higher quartiles (adjusted for sex, the age of both the mother and child and maternal education in years) ranged from 0.5 to 1.5, with no dose-response pattern. [Cases and controls differed in several demographic characteristics, including the length of time that mothers had to recall diet during pregnancy, but adjustment was made for several factors as noted above. Unexpectedly, a greater proportion of cases were female than male, but case ascertainment appeared to be complete.]

Lubin *et al.* (2000) conducted a case-control study of children (aged 0–18 years) with brain tumours in relation to diet of both the children and the mothers (during the index pregnancy) in Israel. Three hundred cases, drawn from the Jewish population only, were identified from all neurosurgical departments in Israel and 574 controls were selected from the population registry and were individually matched to cases by sex, birth year and either hospital of birth (in Israel) or country of birth (outside Israel). Based on tertiles of intake, there was no association between the occurrence of brain tumours and intake of nitrate; odds ratios for the upper tertiles were close to null and non-significant relative to the first tertile of intake for both the mother during pregnancy and for the child. [It was not specified whether controls were also required to be Jewish, although intake of nitrate, such as that from pork products, may differ between Jewish and non-Jewish individuals. However, cases and controls were very similar with regard to the ethnic background of the mother as categorized as Asian/African, European/American and Israeli.]

A case-control study of selected types of brain tumour, specifically primitive neuroectodermal tumours (Bunin *et al.*, 1993) and astrocytic gliomas (Bunin *et al.*, 1994), in children aged < 6 years was conducted on cases identified from 33 paediatric oncology groups in the USA and Canada. One control per case (166 controls matched to primitive neuroectodermal tumours, 155 controls matched to astrocytic gliomas) was identified by random-digit dialling and matched to the case on telephone area code plus the first five digits, date of birth (within 1 year) and race (black/non-black). Increased intake of nitrate was associated in a dose-response fashion with a statistically significantly (P for trend = 0.002) reduced risk for primitive neuroectodermal tumours in the offspring (Table 2.4). However, the inverse association between primitive neuroectodermal tumours and nitrate was attenuated and became non-significant (P for trend = 0.19) after

adjustment for other food components (vitamins A, C and E, folate, nitrite and nitrosamines) and vitamin/mineral supplements. More than 20 other factors relating to the child, the mother and the child's diet were considered, including family income, duration of nausea during pregnancy that interfered with normal eating, duration of breast-feeding and maternal smoking; of these, it appeared that the child's diet of cured meat further attenuated the association reported (fourth versus first quartile of nitrate intake during pregnancy attenuated to 0.81). Dietary nitrate was not associated with risk for astrocytic glioma in the offspring, and all odds ratios were at or below null after adjustment for family income (and consideration of other factors such as maternal smoking).

Mueller *et al.* (2004) conducted a case-control study of childhood brain tumours and intake of nitrate from drinking-water based on data from four countries: the USA (Mueller *et al.*, 2001), Canada, France and Spain. These data were collected as part of a larger international case-control study that also included subjects from Australia, Israel and Italy, but water samples were not collected in these countries. In-person interviews were conducted with the biological mother and, if she was still residing where she had lived during the index pregnancy, a water sample was sought (and obtained from 86% of these women; 185 cases and 341 controls), although water samples were provided by 283 cases and 537 controls. [The Working Group believed that the risk estimates for residences during pregnancy were more valid in terms of exposure assessment because the estimates for post-pregnancy residences may be misclassified due to mothers potentially having changed residence after pregnancy.] Interviewers estimated nitrate using a semiquantitative Merckoquant test strip (10, 25, 50, 100, 250 or 500 mg/L). With adjustment for study region, age, sex and year of diagnosis, there was no increased risk for childhood brain tumours associated with nitrate in tap-water at the residence of pregnancy; however, there was a non-significantly increased risk associated with the highest category of exposure to nitrate after excluding women who used bottled water during pregnancy (odds ratio, 1.5; 95% CI, 0.6–3.8). [Mothers who drank any bottled water were excluded from subanalyses to test reliance on well-water.]

2.3.2 Nitrite

(a) Dietary intake of nitrite

(i) Case-control studies in adults

Giles *et al.* (1994) conducted a case-control study (described in detail in Section 2.3.1) of adult glioma that linked data on food intake from interviews to published tables of nitrite content. A modest borderline significant increase in risk for glioma was observed among men in relation to estimated dietary intake of nitrite, adjusted for alcohol consumption and tobacco smoking, but with no dose-response relationship. Corresponding odds ratios were close to null among women. [Interpretation of the possible association in men is limited, as noted in Section 2.3.1.]

Boeing *et al.* (1993) conducted a case-control study (described in detail in Section 2.3.1) of adult brain tumours in Germany. The risk for glioma associated with tertiles of dietary nitrite intake was close to null. Nitrite was rarely detected in drinking-water from the current homes of subjects using semiquantitative nitrite test strips. [However this does not reflect the nitrite burden from water in previous places of residence.] The risk estimate for glioma was close to null, while that for meningioma was markedly but non-significantly increased. [Study limitations are noted in Section 2.3.1.]

Kaplan *et al.* (1997) conducted a case-control study (described in detail in Section 2.3.1) of adult brain tumours in Israel. Consumption of approximately 100 foods in the period 10 years before diagnosis was assessed and linked to published tables. The authors stated that dietary intake of nitrite was not associated with the occurrence of brain tumours. [However, neither the number of exposed cases and controls nor risk estimates were provided.]

Blowers *et al.* (1997) conducted a case-control study (described in detail in Section 2.3.1) of glioma in women in southern California, USA. With regard to total nitrite intake and glioma, there was no pattern of association in the risk estimates (P for trend = 0.55), but glioma risk increased with increasing quartiles of nitrite intake from cured meats (P for trend = 0.07). [Study limitations are noted in Section 2.3.1.]

Lee *et al.* (1997) conducted a case-control study (described in detail in Section 2.3.1) of glioma in northern California, USA. All comparisons were stratified by sex and adjusted for age, education and family income. In both men and women, the mean weekly intake of nitrite of glioma cases was slightly [non-significantly] higher than that of controls. In men, the risk for glioma only increased among those with a nitrite intake above the median and a vitamin C intake below the median. This comparison was not significant [interaction p -value not provided.] [For other comments, see Section 2.3.1.] Also, non-participating controls who were asked about vitamin intake reported a higher vitamin intake than participating controls. [It is unknown whether participating and non-participating controls differed with respect to nitrite intake overall or differentially by category of vitamin C intake.]

Schwartzbaum *et al.* (1999) conducted a hospital-based case-control study of glioma and diet in Ohio, USA, among forty cases who underwent their first (exploratory) brain tumour surgery (all histologically confirmed) and 48 age- and sex-matched hospital controls who underwent orthopaedic or gynaecological surgery. Diet was ascertained through a questionnaire and was converted into nitrite intake from cured meats specifically from software and published tables. Just before surgery, 23 (53.5%) cases and 27 (56%) controls provided serum for which results (levels of three antioxidants) were available; these were used to examine the interaction between nitrite and vitamin C/ α -tocopherol/ γ -tocopherol. Cases had a higher dietary intake of nitrite from cured meat than controls; however, after adjustment for total energy intake, glioma was not associated with an intake of nitrite greater than the median from any of several specific types of cured meat (all odds ratios at or below null). Interaction odds ratios, adjusted for energy, suggested that the risk for glioma was highest among individuals who had an intake of

nitrite from cured meats above the median and levels of intake of vitamin C or α -tocopherol below the median; interaction *p*-values were not provided. [The exclusion criteria for controls that included several diseases related to diet (listed in Table 2.4) probably resulted in a control group that had a different diet (and prevalence of cigarette smoking) from population controls.]

Chen *et al.* (2002) conducted a case–control study (described in detail in Section 2.3.1) of adult glioma in relation to diet in Nebraska, USA. A questionnaire (48 foods) and published literature were used to assess total nitrite intake from the diet, which was not associated with the risk for glioma; there was no interaction between intake of nitrite and that of vitamin C.

Ward *et al.* (2005) (study described in detail in Section 2.3.1) extended the study by Chen *et al.* (2002) to consider the source of the dietary nitrite (animal versus plant) and to estimate the intake of endogenously formed nitrite from drinking-water (5% of the estimated nitrate intake from drinking-water). For most subjects, nitrate intake from water was estimated by linking histories of residence to data from public water-quality monitoring (mainly for 1965–85 for 130 (52%) cases and 319 (64%) controls). For a small subset of subjects who used private wells in 1985 and who provided a water sample, nitrite was estimated by laboratory analysis. As in the larger study, odds ratios for quartiles of total dietary intake of nitrite were close to null, and there was no interaction with intake of vitamin C (Chen *et al.*, 2002). Odds ratios for nitrite from animal sources were also close to null and not statistically significant, but those for the upper two quartiles of nitrite from plant sources were markedly increased and statistically significant. When total nitrite intake from all dietary sources was combined with the estimated intake of nitrite from drinking-water, odds ratios were again close to null, with the exception of the second (versus the first) quartile (odds ratio, 1.8; 95% CI, 0.9–3.6). There was no interaction between this combined (diet and water) nitrite variable and vitamin C, nor between nitrite from water and either vitamin C or smoking status. [See comments in Section 2.3.1.]

(ii) *Case–control studies in children*

Cordier *et al.* (1994) conducted a case–control study (described in detail in Section 2.3.1) of childhood brain tumours in France. Maternal intake of selected foods during pregnancy was ascertained by structured interview and linked to a national survey and published tables to estimate total intake of nitrite. Odds ratios for brain tumours in the offspring were estimated by quartiles of nitrite intake, and a non-significant inverse dose–response relationship was observed [for possible limitations, see Section 2.3.1].

Lubin *et al.* (2000) conducted a case–control study (described in detail in Section 2.3.1) of childhood brain tumours and consumption of nitrite in childhood and during the index pregnancy in Israel. There was no association between brain tumours and intake of nitrite; odds ratios for the upper tertiles were close to null relative to the first tertile for intake by both the mother during pregnancy and the child. [See Section 2.3.1 for possible study limitations.]

Bunin *et al.* (1993, 1994) conducted a case-control study (described in detail in Section 2.3.1) of primitive neuroectodermal tumours and astrocytic gliomas in children < 6 years of age and their possible association with maternal dietary levels of nitrite in Canada and the USA. Intake of nitrite during pregnancy was not associated with the risk for either primitive neuroectodermal tumours or astrocytic gliomas in the offspring. Risk estimates for the latter were adjusted by family income, and numerous other potentially confounding factors were also considered (see Section 2.3.1).

Preston-Martin *et al.* (1996) conducted a case-control study of childhood brain tumours and diet in the states of California and Washington, USA. Five hundred and forty cases were identified from three tumour registries, and 801 controls, who were identified by random-digit dialling, were frequency-matched to cases by age and sex. A questionnaire (47 foods) was administered to biological mothers to identify the frequency and amounts of intake during the index pregnancy; these data were subsequently linked to published food tables and other software to determine levels of intake of nitrite. Odds ratios were adjusted for age, sex, birth year and geographical area; race and socioeconomic status were also considered but had no confounding effect. Risk for childhood brain tumour was not associated with total dietary nitrite or total intake of nitrite from vegetables. The highest quartile of nitrite intake from cured meats, however, was associated with a significantly increased risk for childhood brain tumours (odds ratio, 1.9; 95% CI, 1.3–2.6; 155 exposed cases), and yielded a significant *p* value for trend (*P* = 0.003); risk estimates for the second and third quartiles were close to null. The *p* value for trend was also significant among subjects with low and high socioeconomic status. When the authors considered the interaction between nitrite from cured meat and the use of prenatal vitamin supplements, no significant effect was observed, but the risk for brain tumours was increased in those with a high (above median) intake of nitrite from cured meats and no prenatal use of vitamins relative to those with an intake below the median plus prenatal use of vitamins (odds ratio, 2.2; 95% CI, 1.4–3.6; 47 exposed cases).

Pogoda and Preston-Martin (2001a) extended this work to refine the levels of nitrite linked to the earlier (Preston-Martin *et al.*, 1996) data on diet using 26 food surveys with data on sodium nitrite to consider levels of nitrite in specific cured meats according to the year of consumption (index pregnancy). This updated study also allowed greater flexibility in the modelling (splines), with adjustment for the same confounding factors as before. Results were consistent with the previous study, and were more pronounced. The authors also estimated the increase in risk associated with 1 mg per day increase in nitrite consumption (from cured meat) during pregnancy, which was statistically significant (*P* = 0.008).

(b) *Intake of nitrite from water*

Mueller *et al.* (2004) conducted a case-control study (described in detail in Section 2.3.1) of childhood brain tumours and nitrite from drinking-water in Canada, France, Spain and the USA. Interviewers used semiquantitative Merckoquant test strips to estimate nitrite levels in tap-water samples from the current residence, which were sought

if this was the same residence as that of the index pregnancy. A sample was obtained from 86% of these women (185 cases and 341 controls), although water samples were provided by 283 cases and 537 controls. Among the latter, there was no increased risk for brain tumours in children in relation to the presence of nitrite in tap-water. [Subjects who no longer lived at the same residence as that at the time of pregnancy but who provided water samples may not be representative; among controls, participation of those who no longer lived at the pregnancy home varied according to the presence of nitrite.] When water samples were collected from homes in which the index pregnancy occurred, increasing levels of nitrite in water were associated with an increased risk for brain tumours in the offspring. This association was stronger (and statistically significant for the highest level of nitrite) when analyses were further restricted to women who did not drink any bottled water in the home during pregnancy. The increased risk was present only with respect to astroglial tumours; risk estimates for both primitive neuroectodermal tumours and other tumour types were close to null. [Among the pregnancy homes of controls, most of whom used public water supplies at the time of conception, nitrate and nitrite were very prevalent: 39.6% any nitrate, 8.2% nitrate \geq 50 mg/L, 10.0% any nitrite and 2.4% nitrite \geq 5 mg/L. Because levels of nitrite were usually low in public water supplies, it is unknown whether this reflects measurement error and, if so, whether it could differ by case status; however, it is unlikely to differ by histological tumour type, although the association was confined to astroglial tumours. Also, it should be noted that the levels of nitrite in tap-water may correlate with other substances in the water, such as pesticides and trichloromethanes.]

2.4 Cancer of the urinary tract

2.4.1 *Ingested nitrate*

(a) *Ecological studies* (Table 2.5)

Ecological studies in Europe have evaluated concentrations of nitrate in the drinking-water in relation to mortality from or incidence rates of cancer of the urinary tract (urinary bladder, lower urinary tract (comprised largely of bladder cancers) and kidney). Generally, these studies obtained concentrations of nitrate in water for a specific time period from public water supply databases and computed rate ratios, mortality rates or standardized incidence ratios (SIR) or compared incidence rates for regions grouped according to their average concentrations of nitrate.

In northern Jutland, Denmark, Jensen (1982) computed incidence rates (age standardized to the European standard population) of cancers of the urinary tract for Aalborg, a town that had high levels of nitrate in the water, and Aarhus, a town that had low levels. Levels of nitrate in municipal water averaged 27.1 mg/L in Aalborg in 1976 and had been detected since the 1930s, i.e. for at least 50 years; average levels in Aarhus were 0.2 mg/L in 1976 (described in detail in Section 2.2). No significant differences in incidence rates (per 100 000 persons) were observed between the two towns.

Table 2.5. Ecological studies of ingested nitrate and tumours of the urinary tract and genital tumours

Reference, location	Organ site (ICD code)	End-point	Exposure assessment	Exposure categories	No. of cases/deaths	Main results	Adjustment for potential confounders	Comments	
Jensen (1982), northern Jutland, Denmark	Urinary system, male genital organs, uterus, other female genital organs, breast	Incidence of urinary system cancers, 1968–72	Annual average concentrations of nitrate for the municipality of Aalborg (27.1 mg/L) and Aarhus (0.2 mg/L) in 1976	Mean nitrate (mg/L) Aalborg Aarhus Aalborg Aarhus Aalborg Aarhus Aalborg Aarhus		Incidence rate per 100 000	Rates age-standardized to the European standard population		
						Men			Women
						<i>Urinary tract</i>			
						43.7			15.1
						47.3			19.1
						<i>Male genital organs</i>			
						49.9			
						47.8			
<i>Uterus</i>									
	70.5								
	49.0								
<i>Other female genital organs</i>									
	27.5								
	24.7								
<i>Breast</i>									
1.2	75.8								
0.4	76.5								
Morales-Suárez-Varela <i>et al.</i> (1993, 1995), Valencia, Spain; 261 (1993) and 258 (1995) municipalities	Bladder (188), prostate (185)	Mortality, 1975–88	Concentrations of nitrate in public drinking-water, 1975–80 (1993) and 1968 (1995)	Mean nitrate (>50 mg/L) versus all other concentrations for municipalities		Rate ratio (95% CI) <i>Bladder</i> Men, 1.31 (0.64–2.71) Women, 2.1 (0.42–10.39) All, 1.38 (0.68–2.8)	Rate ratio based on mortality rates directly age-standardized to the 1981 Spanish population	Number of deaths not provided	

Table 2.5 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure assessment	Exposure categories	No. of cases/deaths	Main results	Adjustment for potential confounders	Comments	
Morales-Suárez-Varela <i>et al.</i> (1993, 1995) (contd)				Concentrations of nitrate in municipality (mg/L)	Mortality rates [per 100 000 persons/year]		Age-adjusted mortality rates (direct method)		
					<i>Men</i>	<i>Bladder</i>			
					0–25	569			9.9
					25–50	25			7.32
					> 50	14			8.41
					<i>Women</i>				
					0–25	112			2.03
					25–50	0			–
					> 50	0			–
					<i>Men</i>	<i>Prostate</i>			
0–25	925	16.9							
25–50	58	16.9							
> 50	40	24.04							
Van Leeuwen <i>et al.</i> (1999), Ontario, Canada	Bladder	Incidence of bladder cancer, 1987–91	Nitrate in drinking-water, 1987–91; 40 'ecodistricts' of Ontario Cancer Registry, 1987–91	Mean concentrations of nitrate in the ecodistrict		No correlation was observed in women or men	Age-standardized		

Table 2.5 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure assessment	Exposure categories	No. of cases/deaths	Main results	Adjustment for potential confounders	Comments
Gulis <i>et al.</i> (2002), Trnavas District, Slovakia	Bladder (188), kidney (189)	Cancer incidence from regional cancer registry, 1986–95	Concentrations of nitrate in 60 municipal supplies, 1975–95	Average level of nitrate (mg/L)		SIR (95% CI)		SIR based on age- (10-year), calendar year- and sex-specific incidence rates for the district
				0–10	30	Bladder		
				10.1–20	53	<i>Men</i>		
				> 20	43	1.18 (0.82–1.68)	1.09 (0.83–1.43)	
						0.97 (0.71–1.30)	<i>P</i> for trend = 0.25	
						<i>Women</i>		
				0–10	13	1.32 (0.76–2.28)	0.81 (0.49–1.34)	
				10.1–20	15	0.82 (0.48–1.38)	<i>P</i> for trend = 0.13	
				> 20	14	Kidney		
						<i>Men</i>		
				0–10	14	0.99 (0.58–1.67)	1.06 (0.74–1.53)	
				10.1–20	29	1.11 (0.75–1.62)	<i>P</i> for trend = 0.53	
> 20	26	<i>Women</i>						
0–10	4	0.50 (0.19–1.32)	1.07 (0.66–1.75)					
10.1–20	16	0.96 (0.55–1.64)	<i>P</i> for trend = 0.15					
> 20	13							

Table 2.5 (contd)

Reference, location	Organ site (ICD code)	End-point	Exposure assessment	Exposure categories	No. of cases/deaths	Main results	Adjustment for potential confounders	Comments	
Volkmer <i>et al.</i> (2005), Bocholt, Germany	Urinary tract, testis, penis, prostate	Incidence of genitourinary tract cancers derived from medical facilities and practices, 1985–97	Concentrations of nitrate from two waterworks, 1957–85	Nitrate (60 mg/L versus 10 mg/L)	527	SIR (95% CI)	Age standardization based on the German population in 1993		
						<i>Transitional-cell carcinoma of the urinary tract</i>			
					NR	Men			2.26 (1.34–3.79)
					NR	Women			1.52 (0.78–2.96)
					210	Total			1.98 (1.10–3.54)
						<i>Renal-cell carcinoma</i>			
					NR	Men			0.61 (0.28–1.33)
					NR	Women			2.96 (0.66–13.18)
					57	Total			0.87 (0.34–2.22)
					42	Testis			0.43 (0.21–0.90)
9	Penis	0.66 (0.14–2.88)							
209	Prostate	1.06 (0.76–1.48)							

CI, confidence interval; ICD, International Classification of Diseases; NR, not reported; SIR, standardized incidence ratio

Morales-Suarez-Varela *et al.* (1993) studied cancer of the urinary bladder in Valencia, Spain, a region that has the highest reported levels of nitrate in the drinking-water in Europe. Mean concentrations of nitrate for 261 municipalities in the region were derived for the period 1975–80 from data obtained from the Department of Public Health, Hygiene and Environment. Mortality rates for urinary bladder cancer (International Classification of Diseases (ICD)-9 188) for the period 1975–88 were calculated from national death records and standardized directly for age and sex to the 1981 Spanish population. Compared to other regions, mortality rates in municipalities where water contained levels of > 50 mg/L nitrate were 38% higher overall, 31% higher among men and twice as high among women; however, these excesses were not statistically significant. [The Working Group noted that this study does not report the actual number of deaths from bladder cancer and the confidence intervals indicate imprecise rate ratios.] A subsequent report from Morales-Suárez-Varela *et al.* (1995) examined mortality rates for cancer of the urinary bladder (described in detail in Section 2.2). As in the previous study, deaths from cancer were obtained from national records (ICD-9 188 for bladder cancer) for 1975–88. Data from 268 municipalities were grouped into three categories of estimated concentrations of nitrate in the drinking-water (0–25, 25–50 and > 50 mg/L). Mortality rates for bladder cancer (directly age-standardized [standard population unspecified]) were similar across exposure categories for men: 9.9, 7.32 and 8.32 [per 100 000 person–years], respectively. In women, the rate was 2.03 [per 100 000 person–years] in the lowest exposure category and could not be computed for the other levels because there were no deaths.

An ecological study in Ontario, Canada (described in detail in Section 2.2) examined the incidence of urinary bladder cancer in relation to average concentrations of nitrate in 40 ‘ecodistricts’ (Van Leeuwen *et al.*, 1999). The authors indicated that no correlation was detected among men or women ($P > 0.25$), when levels of atrazine, alcohol consumption, tobacco smoking, level of education, income and occupational exposures derived from a provincial health survey were taken into account. [No correlations or risk estimates were provided.]

A study in the agricultural district of Trnava, Slovakia (Gulis *et al.*, 2002), computed incidence rates for urinary bladder cancer in 60 villages that used municipal water systems (described in detail in Section 2.2). Villages were grouped into three categories based on the average concentrations of nitrate in their water supplies for the period 1975–95 (0–10, 10.1–20 and ≥ 20 mg/L nitrate). Using data from the regional cancer registry for the period 1986–95, sex-specific SIRs were computed for cancers of the urinary bladder (ICD-0 188) and kidney (ICD-0 189) with incidence rates for the entire district as the standard, stratified by age (10 years) and calendar year. SIRs did not increase with exposure category for either bladder or kidney cancer (SIRs for bladder cancer, 1.22, 1.18 and 1.32; SIRs for kidney cancer, 0.81, 1.05 and 1.05, respectively).

An ecological analysis was performed in a community of Bocholt, Germany, that was served by two waterworks over a period of 28 years (Volkmer *et al.*, 2005). Concentrations of nitrate were distinct for the two waterworks: between 1957 and 1985,

one contained 60 mg/L nitrate and the other 10 mg/L, but both contained 10 mg/L nitrate from 1986 to 1997. Newly diagnosed cancers of the urinary tract among inhabitants of Bocholt were identified from medical departments (urology, pathology, oncology, radio-oncology) and outpatient urology practices between July 1985 and June 1997. Incidence rates in the two exposure groups were standardized to the German population for 1993. Rate ratios were higher in the area with higher levels of nitrate in 1957–85 for transitional carcinomas of the urinary tract (1.98; 95% CI, 1.10–3.54; overall rate ratio in men, 2.26; 95% CI, 1.34–3.79; overall rate ratio in women, 1.52; 95% CI, 0.78–2.96). For renal carcinomas, rate ratios were not increased overall among the high-nitrate group (0.87; 95% CI, 0.34–2.22); however, the rate ratio was above one among women (2.96; 95% CI, 0.66–13.18) and below one among men (0.61; 95% CI, 0.28–1.33), although, neither was statistically significant. [The investigators determined tobacco smoking histories from the medical records of the cases and these were not statistically significantly different between the two exposure groups; 15.9% in the high-exposure group and 20.6% in the low-exposure group had smoked > 20 pack-years; however, smoking could not be adjusted for in the analysis and the possibility of confounding remains. Further, the level of completion of case ascertainment is uncertain.]

(b) *Cohort study* (Table 2.6)

The US Iowa Women's Health Study (Weyer *et al.*, 2001) evaluated nitrate in both the diet and drinking-water in relation to the incidence of cancers of the urinary bladder and kidney. This was a prospective study of 21 977 women who were 55–69 years of age in 1986 (response rate, 43%) and who used the same water supply for more than 10 years (87% used the same water supply for more than 20 years). The baseline postal questionnaire included basic demographic information, cigarette smoking history and a 126-item food-frequency questionnaire (the 1984 version of the Nurses' Health Study) which asked about usual diet in the previous year. A follow-up postal questionnaire in 1989 asked about the type of water system at the current residence and duration of use. Concentrations of nitrate in municipal water systems were analysed by the University of Iowa's Hygienic Laboratory in 1955–64, 1976–82 and 1983–88. Only communities that used a 'single source' (surface source or specific aquifer for 90% of their water) were included; users of private wells formed a separate stratum. Cancers diagnosed through to 31 December 1998 were determined by linkage to the Iowa State Cancer Registry. After adjustment for age and total energy intake, relative risks for kidney cancer were non-significantly elevated in the top three quartiles of dietary intake of nitrate (> 11.6 mg nitrate-N); however, there was no clear evidence of a trend in risk across quartiles. For urinary bladder cancer, relative risks were slightly and non-significantly elevated in the top three quartiles of dietary nitrate intake, but again there was no consistent increase with increasing exposure. Using average levels of nitrate in water (all years), relative risks were

Table 2.6. Cohort study of ingested nitrate and tumours of the urinary tract and genital tumours

Reference, location, name of study	Description of the cohort	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments	
Weyer <i>et al.</i> (2001), Iowa, USA, Iowa Women's Study	Cohort of 21 977 Iowa women, 55–69 years of age in 1986, who had a valid driver's licence and no previous history of cancer and who had used the same water supply for > 10 years (87% > 20 years), linked to the Iowa Cancer Registry 1986–98; response rate, 43%	Mailed baseline questionnaire in 1986 included tobacco smoking and other factors and a 126-item food-frequency questionnaire (adapted from the 1984 version of the Nurses' Health Study); a follow-up questionnaire in 1989 included type of water system of current residence and duration of use.	Bladder, kidney, breast, ovary, uterine corpus	Diet nitrate (mg nitrate-N)				Age, total energy	Investigators did not examine interaction with vitamin C; for use of private wells, odds ratios were 1.31 (95% CI, 0.48–3.55) for bladder cancer and 1.07 (95% CI, 0.45–2.57) for kidney cancer.
				< 11.6	9	Bladder	1.00 (reference)		
				11.6–18.0	17		1.88 (0.84–4.24)		
				18.1–27.2	13		1.46 (0.62–3.47)		
				> 27.2	14		1.57 (0.66–3.75)		
						Kidney			
				< 11.6	12		1.00 (reference)		
				11.6–18.0	15		1.32 (0.62–2.83)		
				18.1–27.2	14		1.32 (0.60–2.89)		
				> 27.2	14		1.37 (0.61–3.06)		
						Breast			
				< 11.6	253		1.00 (reference)		
				11.6–18.0	252		0.98 (0.83–1.17)		
				18.1–27.2	265		1.04 (0.87–1.24)		
> 27.2	254		0.99 (0.83–1.19)						
		Ovary							
< 11.6	24		1.00 (reference)						
11.6–18.0	28		1.12 (0.65–1.94)						
18.1–27.2	28		1.10 (0.63–1.92)						
> 27.2	22		0.85 (0.47–1.55)						
		Uterine corpus							
< 11.6	71		1.00 (reference)						
11.6–18.0	41		0.60 (0.41–0.88)						
18.1–27.2	51		0.78 (0.54–1.12)						
> 27.2	61		0.97 (0.68–1.39)						

Table 2.6 (contd)

Reference, location, name of study	Description of the cohort	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Weyer <i>et al.</i> (2001) (contd)		Nitrate concentrations were derived from municipal water supplies over the periods 1955–64, 1976–82, 1983–88; average nitrate level computed over the 33-year time frame for communities using a ‘single source’ for 90% of their water and average level for 1955–64 exclusively; users of private wells formed a separate stratum		Mean nitrate in water (mg/L nitrate-N) 1955–88				Age, education, tobacco smoking (never/former/current and pack–years), physical activity, body mass index, waist-to-hip ratio, total energy, intakes of vitamin C, vitamin E, and fruits and vegetables (for water nitrate), dietary nitrate, water source (ground/surface)
				< 0.36	7	Bladder	1.00 (reference)	
				0.36–1.00	14		1.69 (0.66–4.30)	
				1.01–2.46	8		1.10 (0.38–3.20)	
				> 2.46	18		2.83 (1.11–7.19)	
				< 0.36	9	Kidney	1.00 (reference)	
				0.36–1.00	1		1.34 (0.55–3.25)	
				1.01–2.46	13		1.38 (0.56–3.41)	
				> 2.46	12		1.20 (0.46–3.16)	
				< 0.36	208	Breast	1.00 (reference)	
				0.36–1.00	209		1.03 (0.84–1.26)	
				1.01–2.46	185		0.97 (0.78–1.20)	
				> 2.46	208		1.03 (0.83–1.28)	
				< 0.36	13	Ovary	1.00 (reference)	
0.36–1.00	19		1.52 (0.73–3.17)					
1.01–2.46	24		1.81 (0.88–3.74)					
> 2.46	26		1.84 (0.88–3.84)					
< 0.36	44	Uterine corpus	1.00 (reference)					
0.36–1.00	44		0.86 (0.55–1.35)					
1.01–2.46	48		0.86 (0.55–1.36)					
> 2.46	32		0.55 (0.33–0.92)					

CI, confidence interval

higher among those in the top quartile (> 2.46 mg/L nitrate-N) versus the lowest quartile (< 0.36 mg/L nitrate-N) for bladder cancer (relative risk, 2.83; 95% CI, 1.11–7.19); for kidney cancer, the relative risk was 1.20 (95% CI, 0.46–3.16) [the mean concentration for the top quartile was 5.59 mg/L nitrate-N]. Use of private wells was more weakly associated with bladder cancer (relative risk, 1.31; 95% CI, 0.48–3.55) and appeared to be unrelated to kidney cancer. Results were similar for an additional analysis that was restricted to 1955–64 and to women who had lived in the same residence for > 20 years. Relative risks for nitrate in water and use of private wells were adjusted for multiple potentially confounding factors such as age, education, tobacco smoking, pack-years of smoking, physical activity, body mass index, waist-to-hip ratio, total energy, vitamin C, vitamin E and fruit and vegetable intake, dietary intake of nitrate and type of water source (ground-versus surface). [Although the study did not ascertain sources of drinking-water outside the home, only 33% of women worked away from home; the study did not account for the amount of water consumed on an individual level. There was no assessment of potential effect modification, i.e. by vitamin C intake.]

(c) *Case-control studies* (Table 2.7)

One case-control study examined ingested nitrate in relation to cancer of the urinary bladder (Ward *et al.*, 2003). Assessment of exposure to nitrate included evaluation of dietary intake and concentrations in the drinking-water. One small study of bladder cancer that analysed urinary concentrations of nitrate after diagnosis was not reviewed because the cancer potentially could have affected the levels of nitrate.

A large population-based case-control study of bladder cancer was conducted in Iowa, USA, and investigated intake of nitrate in both the diet and drinking-water (Ward *et al.*, 2003). Cases included 1452 histologically confirmed bladder cancers that were newly diagnosed between 1986 and 1989, who were between the ages of 40 and 85 years and had no history of malignancy (except for basal-cell or squamous-cell carcinoma of the skin). These were identified by the Iowa State Cancer Registry and controls were selected from driver's licence records (for those aged < 65 years) and US Health Care Finance Administration records (for those aged 65 years and older). Reported response rates were 85% for cases and 82% for controls. Subjects were mailed a questionnaire that covered demographic information, history of cigarette smoking, occupation and lifetime residences and sources of drinking-water, together with a 55-item food-frequency questionnaire that asked about usual adult diet. Dietary levels of nitrate were based on the published literature and were analysed in quartiles. There was no evidence of an association between dietary intake of nitrate among men (odds ratio, 0.9; 95% CI, 0.7–1.1 for ≥ 119 versus < 59 mg nitrate per day) or women (odds ratio, 0.8; 95% CI, 0.5–1.3 for ≥ 127 versus < 62 mg nitrate per day). Estimates of exposure to nitrate from drinking-water were based on residential concentrations of nitrate. Questions included the amount of tap-water consumed in and outside the home, but the latter accounted for only 10% of consumption. Samples taken from the distribution system in a given year were averaged and linked to an individual by year and town. When no data were available for that year and

Table 2.7. Case-control studies of ingested nitrate and nitrite and tumours of the urinary tract and genital tumours

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Barbone <i>et al.</i> (1993), Birmingham, AL, USA, 1985–88	Endo-metrial	168 women with endometrial cancer, 1985–1988, identified at the University of Alabama Hospital and a large private gynaecology–oncology practice in Birmingham, AL	334 women with an intact uterus attending the University optometry clinic; matched on age, race	Nurses' Health Study FFQ (116-item) administered in person with the assistance of the interviewer 103 cases and 236 controls	Dietary nitrate (calorie adjusted mg) T 1 (< 94.0) T 2 (94.0–<150.5) T 3 (≥ 150.5) <i>P</i> for trend	48 29 26	1.0 (reference) 0.7 (0.4–1.2) 0.4 (0.2–0.8) 0.0057	Age, race, years of schooling, total calories, use of unopposed estrogens, obesity, shape of obesity, smoking, age at menarche, number of pregnancies, diabetes, hypertension	

Table 2.7 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Wilkens <i>et al.</i> (1996), Oahu, Hawaii, USA, 1979–86	Lower urinary tract cancer (90% urinary bladder, 7% renal pelvis, 3% ureter)	261 cases (195 men, 66 women) of Caucasian or Japanese ancestry; 1977–86, hospitals covering 95.5% of eligible cases in the Hawaii Tumor Registry	522 population controls (390 men, 132 women); matched on sex, age (± 5 years), ethnic group; identified from Health Surveillance Program (annual survey of 2% of the population)	29-item dietary history of diet in a usual week one year prior to the reference date	Dietary nitrites ($\mu\text{g}/\text{week}$)	NR		Age, cigarette smoking status, pack/years, employment in a high-risk occupation (both men and women), consumption of dark green vegetables (men), total vitamin C intake (women)	The mean intake of nitrate ($\mu\text{g}/\text{week}$) was reported as 1.95 in male cases, 1.84 in male controls, 1.14 in female cases and 1.32 in female controls [the Working Group questioned that the unit was more probably mg/week]. The odds ratios for nitrosamine in Japanese were 1.9 (95% CI, 0.9–4.3) and 3.0 (95% CI, 1.4–6.4) for tertiles 2 and 3 compared with tertile 1 in men and 1.0 (95% CI, 0.3–3.3) and 1.9 (95% CI, 0.6–5.8), respectively, in women.
					<i>Men</i>				
					Japanese				
					T 1				
					T 2				
					T 3				
					<i>P</i> for trend				
					Caucasian				
					T 1				
					T 2				
					T 3				
					<i>P</i> for trend				
					<i>Women</i>				
					Japanese				
T 1									
T 2									
T 3									
<i>P</i> for trend									
Caucasian									
T 1									
T 2									
T 3									
<i>P</i> for trend									

Table 2.7 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Møller (1997), Denmark, 1986–88	Testicular cancer	514 men born between 1916 and 1970 identified by the Danish Cancer Registry; response rate, 88%	720 men selected randomly from the Danish population; matched on year of birth; response rate, 69%	Telephone interviews	Born in high nitrate area			Year of birth	
					Total	31	1.27 (0.99–1.64)		
					Non-seminoma	30	1.28 (0.91–1.80)		
					Seminoma	32	1.27 (0.93–1.74)		
					Lived in high nitrate area for the larger part of childhood				
					Total	32	1.40 (1.09–1.81)		
					Non-seminoma	33	1.49 (1.06–2.08)		
					Seminoma	33	1.36 (1.00–1.86)		
					High nitrate in childhood in the country				
					Total	18	1.10 (0.79–1.53)		
					Non-seminoma	17	1.04 (0.66–1.62)		
					Seminoma	19	1.14 (0.76–1.72)		
					High nitrate in childhood not in the country				
					Total	15	1.51 (1.03–2.20)		
					Non-seminoma	16	1.74 (1.07–2.82)		
					Seminoma	14	1.37 (0.86–2.20)		

Table 2.7 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments	
Ward <i>et al.</i> (2003), Iowa, USA, 1986–89	Urinary bladder	1452 cases (1135 men, 317 women) newly diagnosed and histologically confirmed, aged 40–85 years, identified from the Iowa State Health Registry; response rate, 85%	2434 population-based controls (1601 men, 833 women) from driver's licence records or US Health Care Finance Administration; frequency-matched on age, sex at a different ratio in 1986–87 and 1988–89; response rate, 82%	Mailed questionnaire covering basic demographics, smoking, occupation, residential and water source history, well depth and a 55-item FFQ on usual adult diet.	Dietary nitrate (mg/day)	NR		Age, cigarette smoking, education, duration of chlorinated surface-water use, study period	Water analysis restricted to those who used Iowa public water systems with known nitrate level for $\geq 70\%$ of their person-years starting in 1960. No evidence of a positive trend by years of use of a public water supply ≥ 10 mg/L nitrate-N. No positive association between use of private wells and risk.	
							<i>Men</i>			1.0 (reference)
							> 59			0.8 (0.7–1.1)
							59–< 84			0.9 (0.7–1.2)
							84–< 119			0.9 (0.7–1.1)
							≥ 119			1.0 (reference)
							<i>Women</i>			1.2 (0.8–1.9)
							< 62			0.9 (0.5–1.4)
							62–< 90			0.8 (0.5–1.3)
							90–< 127			
							≥ 127			
							Dietary nitrite (mg/day)			
							<i>Men</i>			1.0 (reference)
< 0.81	1.1 (0.9–1.4)									
0.81–< 1.06	1.2 (0.9–1.5)									
1.06–< 1.39	1.2 (0.9–1.6)									
≥ 1.39										
<i>Women</i>	1.0 (reference)									
< 0.58	1.0 (0.6–1.5)									
0.58–< 0.75	0.8 (0.5–1.3)									
0.75–> 0.98	1.0 (0.7–1.6)									
> 0.98										

Table 2.7 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Ward <i>et al.</i> (2003) (contd)				Residential concentrations of nitrate in water 1960 onwards; average concentrations and number of years of public water supply with ≥ 10 mg/L examined	Nitrate in water (mg/L nitrate-N) <i>Men</i> < 0.6 0.6–< 1.40 1.40–< 3.09 ≥ 3.09 <i>Women</i> < 0.67 0.67–< 1.18 1.18–< 2.48 ≥ 2.48 <i>High vitamin C</i> Low nitrate High nitrate <i>Low vitamin C</i> Low nitrate High nitrate	171 171 164 116 57 44 38 47 169 134 171 158	1.0 (reference) 0.9 (0.6–1.2) 0.8 (0.6–1.1) 0.5 (0.4–0.8) 1.0 (reference) 0.7 (0.4–1.2) 0.6 (0.3–1.1) 0.8 (0.4–1.3) 1.0 (reference) 0.7 (0.5–1.0) 0.8 (0.6–1.1) 0.7 (0.5–0.9)	Age, gender, smoking, duration, chlorinated surface water, education, study period	Nitrate (mg/L nitrate-N): low (< 1.25) high (≥ 1.25)

CI, confidence interval; FFQ, food-frequency questionnaire; T, tertile

the sources did not vary more than 10%, a weighted average of annual averages in adjacent years was used (with greater weights closer to the year without data); missing values were assigned when no data were available within 10 years. The analyses were restricted to persons who used Iowa public water systems with known levels of nitrate for 70% or more of their person-years starting in 1960, and individual levels of nitrate were assigned based on the average levels after 1960. The risk for bladder cancer was unrelated to quartiles of nitrate-N (mg/L) among women and, if anything, lower among men. This risk was statistically significant for the highest exposure quartile (odds ratio, 0.5; 95% CI, 0.4–0.8 for ≥ 3.09 versus < 0.6 mg/L nitrate-N) and similar after accounting for number of years of use of water that contained levels of ≥ 10 mg/L nitrate-N after 1934. Estimates were adjusted for age, education, cigarette smoking, years of use of chlorinated surface water and study period. Further, there were no apparent interactions between exposure to nitrate from water and vitamin C intake, ever having had a bladder or kidney infection or smoking status. The authors also stated that duration of use of private wells and duration of use of wells less than 50 feet [15.2 m] deep were weakly inversely related to the risk for bladder cancer among men and unrelated to the risk among women [the point estimates were not provided].

2.4.2 *Ingested nitrite*

Two case-control studies of dietary intake of nitrite and cancer of the urinary bladder or lower urinary tract have been conducted, but no cohort studies. Case-control studies that used urinary concentrations of nitrite after diagnosis were excluded (for reasons provided in Section 2.4.1). The reviewed case-control studies of nitrite and urinary tract tumours are summarized in Table 2.7.

A population-based case-control study (Wilkens *et al.*, 1996) from Oahu, Hawaii, USA, evaluated dietary intake of nitrites in relation to cancers of the lower urinary tract. The study included 195 men and 66 women of Caucasian or Japanese ancestry who were newly diagnosed with a lower urinary tract cancer between 1977 and 1986 at the seven largest civilian hospitals on the Island of Oahu (covering an estimated 95.5% of eligible cases based on the state cancer registry). Response rates were 89% for controls and 73% for living cases. Ninety per cent of the cases had tumours of the urinary bladder, 7% of the cancer of renal pelvis and 3% cancer of the ureter. Controls were selected from the Health Surveillance Program of the Hawaii State Department of Health (a survey of a 2% random sample of the population with participation rates of over 95%) and comprised 390 men and 132 women who had no history of a lower urinary tract tumour and who were matched to each case on sex, age (± 5 years) and ethnic group. A dietary history was obtained by personal interview that included a 29-item questionnaire that was designed to determine foods that contain nitrite and nitrosamines consumed in a usual week in the year before the reference date. Estimates of nitrite intake were obtained from published US Department of Agriculture tables. Among men of Japanese ancestry, there was a twofold risk for urinary tract cancers in the highest tertile of nitrite intake versus the

lowest tertile (odds ratio, 2.0; 95% CI, 1.0–4.0; P for trend = 0.05). The inverse was observed among Caucasian men (highest versus lowest tertile odds ratio, 0.6; 95% CI, 0.3–1.2), but not women. A statistically significant trend in risk ($P = 0.01$) was observed for dietary intake of nitrosamines among Japanese men (highest versus lowest tertile odds ratio, 3.0; 95% CI, 1.4–6.4). An elevated risk for nitrosamines was not statistically significant among Japanese women (highest versus lowest tertile odds ratio, 1.9; 95% CI, 0.6–5.8). Estimated intake of nitrosamines was unrelated to cancer of the urinary tract among Caucasian men or women. [The interaction between nitrite and vitamin C or other inhibitors of nitrosation was not evaluated.]

The population-based case–control study of bladder cancer from Iowa, USA (Ward *et al.*, 2003; see Section 2.4.1 (c)), also estimated dietary intake of nitrite. When quartiles of intake were analysed, no significant association with bladder cancer was observed among men or women (odds ratio for men, 1.2; 95% CI, 0.9–1.6 for ≥ 1.39 versus < 0.81 mg nitrite per day; odds ratio for women, 1.0; 95% CI, 0.7–1.6 for > 0.98 versus < 0.58 mg nitrite per day).

2.5 Genital and breast cancer

2.5.1 *Ingested nitrate*

(a) *Ecological studies*

Ecological studies of exposure to nitrate in Europe have evaluated regional concentrations of nitrate in the drinking-water in relation to mortality from or incidence rates of testicular, penile, prostatic and breast cancer. These are described in greater detail in Section 2.4 and are summarized in Table 2.5.

In northern Jutland, Denmark, incidence rates (age-standardized to the European standard population) of cancers of the male genital organs, uterus and other female genital organs were computed for Aalborg, a town that had high levels of nitrate in the water, and Aarhus, a town that had low levels. Incidence rates (per 100 000 persons) for cancers of the uterus (cervix and corpus combined) were 44% higher in Aalborg, but no significant differences were observed in the rates of other cancers (Jensen, 1982).

A study in Valencia, Spain (Morales-Suárez-Varela *et al.*, 1995), examined mortality rates (directly age-standardized [standard population unspecified]) for prostatic cancer (ICD-9 185) which were similar across exposure categories: 16.9, 16.9 and 24.04 [per 100 000 person–years].

(b) *Cohort study*

The US Iowa Women's Health Study (Weyer *et al.*, 2001) examined nitrate in both the diet and drinking-water in relation to the incidence of breast, ovarian and corpus uterine cancers (summarized in Table 2.6). No association was apparent between cancers of the breast, uterine corpus or ovary and dietary levels of nitrate. When average levels of nitrate in water (all years) were used, relative risks were increased non-significantly for

ovarian cancer (relative risk, 1.84; 95% CI, 0.88–3.84), reduced for cancer of the uterine corpus (relative risk, 0.55; 95% CI, 0.33–0.92) and close to unity for breast cancer (relative risk, 1.03; 95% CI, 0.83–1.28). Use of private wells was more weakly related to ovarian cancer (relative risk, 1.55; 95% CI, 0.77–3.13) and appeared to be unrelated to cancers of the breast or uterine corpus. Relative risks for nitrate in water and use of private wells were adjusted for multiple potentially confounding factors. [There was no assessment of potential effect modification, i.e. by vitamin C intake.]

(c) *Case-control studies*

(i) *Endometrial cancer*

A hospital/clinic-based case-control study (Barbone *et al.*, 1993; see Table 2.7) of endometrial cancer that was conducted in Birmingham, Alabama, USA, examined dietary intake of nitrate and included 103 women who were newly diagnosed with histologically confirmed endometrial cancer from June 1985 to December 1988 and were identified from the University of Alabama Hospital and a large private gynaecology-oncology practice in Birmingham. Controls were 236 women with an intact uterus matched on age and race who had attended the University optometry clinic. Information on reproductive history, medical history, anthropometric measurements and other factors was elicited through an in-person interview. Cases and controls also completed an interviewer-administered food-frequency questionnaire (the Nurses' Health Study 116-item questionnaire). Response rates were 93% for cases overall of whom 61% completed the nutritional assessment and 77% for controls overall of whom 71% completed the nutritional assessment. Dietary intake of nitrate was inversely related to the risk for endometrial cancer (odds ratio, 0.4; 95% CI, 0.2–0.8 in the highest versus lowest tertile of nitrate). These results were adjusted for age, race, years of schooling, total calories, use of unopposed estrogens, obesity, shape of obesity, tobacco smoking, age at menarche, age at menopause, number of pregnancies, diabetes and hypertension and changed only slightly with further adjustment for potassium (odds ratio, 0.5; 95% CI, 0.2–0.9).

(ii) *Testicular cancer*

Exposure to nitrate in relation to the incidence of testicular cancer was examined in a population-based case-control study in Denmark (Møller, 1997; see Table 2.7). Cases were 514 men (239 non-seminoma, 262 seminoma) who were born between 1916 and 1970 and were diagnosed with testicular cancer from 1986 to 1988. Controls were 720 men randomly sampled from the Danish population and frequency-matched on year of birth. Participants were interviewed by telephone. Of the cases approached, 88% participated, which represents 74% of the total incident cases in Denmark. Among controls, 69% of those approached took part in the study. The study assessed being born or living during childhood in areas known to have high groundwater levels of nitrate from manure and agricultural fertilizers. These regions included Aarhus, Viborg and Nordjylland counties. In 1994, 22% of the waterworks in these areas contained more than 25 mg/L nitrate compared with 6% for the rest of the country. Odds ratios were slightly

increased among men born in the area that had high levels of nitrate (odds ratio, 1.27; 95% CI, 0.99–1.64); risk estimates were similar for both non-seminoma and seminoma tumours. The association was stronger for men who had lived in the area that had high levels of nitrate for the larger part of their childhood (odds ratio, 1.40; 95% CI, 1.09–1.81); again, the magnitude of the risk estimates were comparable for both histological types of tumour. The association appeared to be limited to men who had lived in the area that had high levels of nitrate during childhood but who did not live in the country (odds ratio for men who did not live in the country, 1.51; 95% CI, 1.03–2.20; odds ratio for men who lived in the country, 1.10; 95% CI, 0.79–1.53). [In light of the heterogeneity in nitrate in the exposure groups, and lack of consistency between the results for urban versus rural residence, the results argue against the association being related to nitrates.]

2.5.2 Nitrite

No cohort or case-control studies of have examined genital or breast cancers in relation to ingested nitrites.

2.6 Leukaemia and lymphoma

2.6.1 Ingested nitrate

(a) Ecological studies

Two ecological studies of levels of nitrate in the drinking-water and non-Hodgkin lymphoma were conducted in North America. Weisenburger *et al.* (1987) reported a twofold higher incidence of non-Hodgkin lymphoma among 25 counties in Nebraska where 20% or more of the private wells contained levels of nitrate-N greater than 10 mg/L compared with 25 counties where fewer than 10% of wells contained this level. A Canadian study (Van Leeuwen *et al.*, 1999) (described in detail in Section 2.2) in 40 districts of Ontario evaluated the incidence of non-Hodgkin lymphoma in relation to levels of nitrate (weighted means of the levels in public and private wells). Incidence rates for non-Hodgkin lymphoma were not associated with the level of nitrate (range, 0.05–7.79 mg/L). The study also evaluated levels of atrazine, a crop herbicide that was weakly associated with higher rates of non-Hodgkin lymphoma among men but not among women.

Three ecological studies in Europe that evaluated levels of nitrate in drinking-water from public water supplies and the incidence of non-Hodgkin lymphoma had mixed results. A study in the agricultural district of Trnava, Slovakia (Gulis *et al.*, 2002) (described in detail in Section 2.2) found a significant positive trend ($P=0.021$) in incidence rates for non-Hodgkin lymphoma among men and women with increasing levels of nitrate in public water supplies (categories of nitrate: 0–<2.3, 2.3–4.5, >4.5 mg/L nitrate-N). Levels of nitrate were averaged over 20 years (1975–95) and incidence was calculated for the years 1986–95. An ecological study in the United

Kingdom (Law *et al.*, 1999) evaluated levels of nitrate in 1990–95 (mean of monthly averages in water supply zones with homogeneous levels of nitrate and a population of < 50 000) in relation to the incidence of non-Hodgkin lymphoma in 1984–93 adjusted for population density. Overall, incidence was not related to levels of nitrate; however, incidence in 1984–88 (but not 1989–93) was positively associated with the average level of nitrate. [The authors interpreted the inconsistent association in the two time periods as not supportive of the hypothesis that levels of nitrate in the drinking-water are associated with non-Hodgkin lymphoma.] An ecological study in Sardinia, Italy (Cocco *et al.*, 2003), compared incidence rates of non-Hodgkin lymphoma in 1974–93 with monitoring data on levels of nitrate in 1993 for 153 communes (median, 0.74 mg/L). There was no trend in the incidence of non-Hodgkin lymphoma among men or women by category of nitrate, although incidence rate ratios were significantly elevated for some categories of nitrate among men. Fifteen of the 153 communes had data on nitrate in 1971–84 when levels were higher (median, 1.83 mg/L); these higher levels of nitrate (≥ 5.7 versus < 1.81 mg/L) were not associated with incidence rates among men or women either.

One ecological study in Southwest England, United Kingdom (Foster *et al.*, 1997), evaluated incidence (1984–88) of leukaemia subtypes and myelodysplasias in relation to levels of nitrate in public water supplies (46 water supply zones). The specific diagnostic groups that were evaluated included acute myeloblastic leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemia, myeloproliferative disorders and myelodysplasias. No significant differences in SIRs were observed for any of the leukaemias, myeloproliferative disorders or myelodysplasias in relation to levels (1984–88) of nitrate in public water supplies (range, 0–18.6 mg/L; weighted mean, < 10 mg/L; measurements in three of the 46 water supply zones exceeded 10 mg/L).

One study in 65 counties in China (Wu *et al.* 1993) (described in detail in Section 2.2) evaluated urinary levels of nitrate, ‘nitrosation potential’ (decrease in urinary NPRO after feeding ascorbic acid) measured by the NPRO test and excretion of *N*-nitroso amino acids among men aged 35–64 years in relation to mortality rates (ages, 0–64 years in 1973–75) for leukaemia. Mortality rates were significantly positively correlated with urinary excretion of nitrate, nitrosation potential and the sum of the *N*-nitroso amino acids.

(b) Cohort and case-control studies

A cohort study of women in Iowa (Weyer *et al.*, 2001) (described in detail in Section 2.4.1) evaluated levels of nitrate in drinking-water from public supplies and dietary intake of nitrate. The water source at the current home was assessed in 1989 and cancer incidence was evaluated from the period of enrolment in 1986 through to 1998 (Table 2.8). The average level of nitrate was calculated from public monitoring data in 1955–88 for women who had used the same water supply for 10 or more years (396 towns/cities). Risk was also evaluated among women who used private wells in 1989, although no measurements of nitrate were available. In models adjusted for confounding factors that included intake of fruit and vegetables, intake of vitamins C and E, body mass index, education, physical activity and tobacco smoking, no significant

Table 2.8. Cohort study of nitrate in the drinking-water and nitrate and leukaemia and lymphoma

Reference, study location, study period	Organ site (ICD code)	Cohort description	Exposure assessment	Exposure categories	No. of cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Weyer <i>et al.</i> (2001), Iowa, USA; 1986–98	Leukaemia	Cohort of 21 977 Iowa women, 55–69 years of age in 1986, who had a valid driver’s licence and no previous history of cancer and who used the same water supply for > 10 years (87% > 20 years), 1986–98 linked to the Iowa Cancer Registry; response rate, 43%	Mailed baseline questionnaire in 1986 included tobacco smoking and other factors and a 126-item food-frequency questionnaire(adapted from the 1984 version of the Nurses’ Health Study); a follow-up questionnaire in 1989 included type of water system of current residence and duration of use	Average nitrate (mg/L nitrate-N)			Average nitrate: age, education, smoking, physical activity, body mass index, waist-to-hip ratio, total energy, vitamin C, E, diet nitrate, fruit and vegetables; nitrate in the diet: age, total energy	Nitrite in diet or interaction with vitamin C or smoking not evaluated
				< 0.36	31	1.0 (reference)		
				0.36–1.00	27	0.85 (0.49–1.47)		
				1.01–2.46	23	0.71 (0.39–1.29)		
				> 2.46	24	0.55 (0.29–1.04)		
				Nitrate in the diet (mg N/day)				
				< 11.6	37	1.0 (reference)		
	11.6–18.0	34	0.88 (0.55–1.40)					
	18.1–27.2	25	0.62 (0.37–1.04)					
	> 27.2	38	0.91 (0.56–1.46)					
	Average nitrate (mg/L nitrate-N)							
	< 0.36	27	1.0 (reference)					
	0.36–1.00	24	0.86 (0.48–1.55)					
	1.01–2.46	12	0.44 (0.20–0.95)					
> 2.46	31	1.12 (0.61–2.06)						
Nitrate in the diet (mg N/day)								
< 11.6	22	1.0 (reference)						
11.6–18.0	28	1.28 (0.73–2.24)						
18.1–27.2	23	1.07 (0.59–1.94)						
> 27.2	37	1.73 (1.00–3.00)						

CI, confidence interval; ICD, International Classification of Diseases

relationship was observed between average levels of nitrate in public supplies (median, 1.0 mg/L nitrate-N; interquartile range, 0.36–2.46 mg/L nitrate-N) and risk for leukaemia (94 cases) or non-Hodgkin lymphoma (105 cases). The relative risk for non-Hodgkin lymphoma decreased with increasing quartiles of average nitrate. The investigators did not evaluate the interaction with vitamin C or other factors that affect nitrosation. Use of private wells (> 10 years in 1989) was not associated with risk for either cancer. There was no trend in risk with increasing quartiles of dietary nitrate (highest quartile, > 27 mg per day), although the relative risk for leukaemia was marginally significant (1.73; 95% CI, 1.00–3.00).

The findings of case–control studies of nitrate in drinking-water and dietary intake of nitrate in relation to risk for non-Hodgkin lymphoma are summarized in Table 2.9. No case–control studies of other haematological malignancies have been conducted.

Three population-based case–control studies in the midwestern USA (Ward *et al.*, 1996, 2006; Freedman *et al.*, 2000) examined the relationship between average levels of nitrate in public water supplies over an average of 30 years and incident cases of non-Hodgkin lymphoma. The studies were similar in design, in that a history of source of residential water was collected and linked to data from public supply monitoring carried out by each town. The size of the population of the large majority of towns was < 50 000 and each study included over 100 utilites. Levels of nitrate were not monitored every year (the earliest year was 1935 for studies in Iowa and 1947 for the study in Nebraska) and values for missing years were imputed using weights based on the number of years since a measurement of nitrate. Data on measurements were most frequent by available after 1960. All studies evaluated potential confounding factors and analyses were limited to the populations who had spent 70–90% of their person–years in an area that had an estimate of nitrate during the exposure period to reduce misclassification by unknown (and probably higher) levels of nitrate from private wells. Two of the studies (Ward *et al.*, 1996, 2006) also evaluated measurements of nitrate in private wells. These studies also assessed dietary intake of nitrate using a food-frequency questionnaire and a database developed from published values for nitrate in foods.

Ward *et al.* (1996) linked 156 cases of non-Hodgkin lymphoma and 527 controls to the average level of nitrate in public water supplies in 1947–84 in eastern Nebraska, USA. The population for these analyses was limited to people who had spent 90% or more of their person–years in the exposure period in an area that had an estimate of nitrate. The median exposure was 1.8 mg/L nitrate-N (interquartile range, 1.7–3.8 mg/L). Increasing quartiles of average nitrate were associated with an increasing risk for non-Hodgkin lymphoma and risk was elevated in the highest quartile for both men and women. Another measurement of exposure (years with a supply \geq 10 mg/L nitrate-N versus no years at this level) was associated with a 50% increased risk (95% CI, 1.0–2.2). Those exposed to \geq 10 mg/L nitrate-N were generally exposed for a short time period (first and third quartiles of duration were 2 years) and the odds ratio decreased to 1.1 after adjustment for occupational use of organophosphate insecticides and family history of cancer. Usual intake of tap-water was assessed and intake of nitrate from drinking-water was calculated

Table 2.9. Case-control studies of nitrate in the drinking-water or nitrate and nitrite in the diet and non-Hodgkin lymphoma (ICD code 200, 202)

Reference, study location, study period	Characteristics of cases and controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments		
Ward <i>et al.</i> (1996), 66 counties of eastern Nebraska, USA, 1983–86	385 cases (white men and women) from Nebraska Lymphoma Study Group (population-based ascertainment of cases from hospitals and pathology laboratories across the state), aged 21 years and older; 40% of interviews with next-of-kin; 100% histologically confirmed; response rate, 90% 1432 population-based controls selected by random-digit-dialling for living controls aged < 65 years or from Health Care Finance Administration files and Nebraska mortality files for deceased controls; frequency-matched by race, age, sex, vital status; additionally matched by year of death	Interview-administered standardized FFQ designed to evaluate major sources of dietary nitrate, nitrite and vitamin C; lifetime history of water source; monitoring data on nitrate in 1947–84 from 138 public utilities in Nebraska; imputation of values for years without data on nitrate; analyses of nitrate in drinking-water: 156 cases, 527 controls with ≥ 90% person-years after 1947 with estimates of nitrate	Average nitrate (mg/L nitrate-N) in public supplies 1947–79				No interaction with vitamin C intake; average level of nitrate in 1965–79 (odds ratio, 1.12; 95% CI, 1.00–1.25) associated with risk but not 1947–64 (odds ratio, 0.99; 95% CI, 0.89–1.10); both metrics of nitrate in water showed similar results for men and women; self-reported and proxies.		
			Q1, < 1.6	21	1.0 (reference)	Age, gender, family history of cancer			
			Q2, 1.6–< 2.0	67	1.4 (0.8–2.5)				
			Q3, 2.0–< 4.0	19	1.5 (0.7–3.0)				
			Q4, ≥ 4.0	47	2.0 (1.1–3.6)				
			Average nitrate/vitamin C						Age, gender
			Q1/High vitamin C	5	1.0 (reference)				
			Q2/High vitamin C	12	1.5 (0.5–4.6)				
			Q3/High vitamin C	11	1.4 (0.5–4.6)				
			Q4/High vitamin C	15	2.0 (0.7–6.1)				
			Q1/Low vitamin C	10	1.4 (0.4–4.5)				
			Q2/Low vitamin C	16	2.4 (0.8–7.4)				
			Q3/Low vitamin C	16	2.2 (0.7–6.7)				
Q4/Low vitamin C	21	3.3 (1.1–9.9)							
Dietary nitrate (mg N/day)					Age, gender, family history of cancer, vitamin C, carotenes				
Q1, < 13	35	1.0 (reference)							
Q2, 13–< 19	38	1.1 (0.6–2.0)							
Q3, 19–26	20	0.8 (0.4–1.7)							
Q4, > 26	11	0.7 (0.3–1.9)							
Dietary nitrite (mg/day)					Results in text only for Q4 versus Q1, adjustment factors not stated				
Q4 versus Q1				0.9 (0.5–1.7)					

Table 2.9 (contd)

Reference, study location, study period	Characteristics of cases and controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Freedman <i>et al.</i> (2000), Minnesota, USA, excluding residents of the four largest cities, 1980-82	329 cases (white men) ascertained from Minnesota hospital and pathology laboratory records, aged ≥ 30 years; 100% histologically confirmed; response rate, 89% (reported in Cantor <i>et al.</i> , 1992) 642 population-based controls (white men), and selected by random-digit dialling for ages < 65 , a 1% random listing from Medicare files for living subjects aged 65 years and older and state death certificates for deceased cases; frequency-matched by age, vital status, state of residence	Interview-administered standardized questionnaire; lifetime history of water source; monitoring data on nitrate from 157 public utilities 1947-80; imputation of missing data (same method as Ward <i>et al.</i> , 1996); analysis of nitrate: 73 cases, 147 controls with $\geq 90\%$ person-years after 1947 with estimates of nitrate	Average nitrate (mg/L nitrate-N) in public supplies 1947-75 ≤ 0.05 $> 0.5-\leq 1.5$ > 1.5	41 19 3	1.0 (reference) 1.4 (0.7-2.5) 0.3 (0.1-0.9)	Age	Exposure range, 0.1-7.2 mg/L nitrate-N; no differences for proxy/self-respondents

Table 2.9 (contd)

Reference, study location, study period	Characteristics of cases and controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments	
Ward <i>et al.</i> (2006), Iowa, USA, 1998–2000	361 cases from Iowa Cancer Registry, aged 20–74 years; 100% histologically confirmed; response rate, 67% 276 population-based controls selected by random-digit dialling for ages < 65 and from Medicare eligibility files for ages 65–74 years; frequency matched by age, centre, race, gender	Interview-administered standardized questionnaire; complete history of water source; monitoring data on nitrate from public utilities and sample from private well at time of interview; analysis of nitrate: 181 cases, 142 controls with ≥ 70% person–years 1960 onwards with nitrate estimates; self-administered dietary questionnaire; modified version of Block FFQ with additional questions about high nitrate vegetables and processed meats	Average nitrate (mg/L nitrate-N) in public supplies, 1960–2000			Age, sex, education	Intake of tap-water did not modify association; no significant interaction with vitamin C (<90 mg/day; ≥90 mg/day), red meat, cured meat, smoking status. Among subgroup with estimates of nitrate in water and diet, nitrate in water contributed a median of 13% (men) and 11% (women) towards total intake of nitrate.	
			≤ 0.63	40	1.0 (reference)			
			0.63–1.36	49	1.3 (0.7–2.4)			
			1.37–2.89	46	1.0 (0.5–1.9)			
			≥ 2.90	46	1.2 (0.6–2.2)			
			Average nitrate in water (mg/day)/ vitamin C (mg/day)					
			High vitamin C/ < 3 mg nitrate	31	1.0 (reference)			
			Low vitamin C/ < 3 mg nitrate	38	1.1 (0.5–2.3)			
High vitamin C/ ≥ 3 mg nitrate	13	2.3 (0.7–7.0)						
Low vitamin C/ ≥ 3 mg nitrate	13	2.0 (0.6–5.9)						

Table 2.9 (contd)

Reference, study location, study period	Characteristics of cases and controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Ward <i>et al.</i> (2006) (contd)			Nitrate in the diet (mg/day)			Age, sex, education, study centre, race, dietary vitamin C, total energy	
			< 76	159	1.0 (reference)		
			76–113.9	116	0.75 (0.51–1.10)		
			114–169.9	111	0.71 (0.47–1.07)		
			≥ 170	80	0.54 (0.34–0.86)		
			Nitrite in the diet (mg/day)				
			< 0.71	82	1.0 (reference)		
			0.71–0.909	108	1.5 (1.0–2.3)		
			0.91–1.209	110	1.7 (1.1–2.7)		
			≥ 1.21	166	3.1 (1.7–5.5)		
			Nitrite in the diet from animal sources (mg/day)				
			< 0.16	101	1.0 (reference)		
			0.16–< 0.26	106	1.0 (0.7–1.5)		
0.26–< 0.42	123	1.0 (0.7–1.6)					
≥ 0.42	136	1.0 (0.6–1.7)					

CI, confidence interval; ICD, International Classification of Diseases; FFQ, food frequency questionnaire; Q, quartile

(in milligrams per day). Risks were higher among people who had a higher intake of nitrate from water (≥ 6.3 mg nitrate-N per day) and an intake of vitamin C below the median (< 130 mg per day) (odds ratio, 3.3; 95% CI, 1.1–9.9) compared with people whose intake of nitrate was < 2.5 mg nitrate-N per day and whose vitamin C intake was above the median; however, the statistical test for interaction was not significant. Increasing dietary intake of nitrate that came primarily from vegetables was associated with decreasing risks for non-Hodgkin lymphoma. Adjustment for vitamin C and carotenes attenuated the association and the inverse associations were not statistically significant.

Freedman *et al.* (2000) linked the average level of nitrate in Minnesota, USA, in 1947–75 (exposure was lagged 10 years) to 73 cases and 147 controls who lived in an area that had an estimate of nitrate for 90% of their person-years during the exposure period. Average levels of nitrate were low (highest exposure category, > 1.5 mg/L nitrate-N; median in this category, 2.4 mg/L nitrate-N) and were not associated with increasing risk; there was a significant inverse association for the highest category compared with ≤ 0.05 mg/L.

Ward *et al.* (2006) evaluated several indices of exposure to nitrate in Iowa, USA, including the average level of nitrate in 1960–2000 and the number of years that a public water supply with levels of nitrate ≥ 5 and ≥ 10 mg/L nitrate-N was used for 181 cases and 142 controls who lived in areas that had estimates of nitrate for 70% or more of their person-years during the exposure period. Increasing quartiles of the average level of nitrate were not associated with risk for non-Hodgkin lymphoma (median, 1.4; interquartile range, 0.6–2.9). Ten or more years of use of public supplies that had levels of > 5 mg/L nitrate-N was associated with a non-significant increased risk compared with no years of exposure at this level (odds ratio, 1.4; 95% CI, 0.7–2.9). One or more years of use of a supply that contained ≥ 10 mg/L nitrate-N was associated with a slight decrease in risk (odds ratio, 0.6; 95% CI, 0.2–1.5); exposure at or above this level was infrequent (11 cases, 10 controls). Levels of nitrate were measured in private wells at the time of the interview; 54 cases and 41 controls had used the well as their primary source of drinking-water for 5 or more years. Among this group, exposure levels were generally low; only eight cases and eight controls had levels of nitrate that were 5 mg/L nitrate-N or greater. Compared with those who had no detectable nitrate (< 0.2 mg/L nitrate-N), there was no association between levels of nitrate in private wells and risk for non-Hodgkin lymphoma (< 5 mg/L nitrate-N odds ratio, 1.7; 95% CI, 0.6–5.2; ≥ 5 mg/L nitrate-N odds ratio, 0.8; 95% CI, 0.2–2.5). For approximately half the study population, dietary intake was assessed using a 117-item food-frequency questionnaire that included questions about vegetables rich in nitrate and processed meats. Usual intake of nitrate from tap-water was also estimated. Among the subgroup that had estimates of nitrate from water and diet, nitrate from water contributed a median of 13% (men) and 11% (women) towards total intake of nitrate. Increasing quartiles of dietary intake were associated with an inverse risk for non-Hodgkin lymphoma after adjustment for dietary intake of vitamin C, education and study matching factors (age, sex, race and centre).

2.6.2 *Ingested nitrite*

Dietary nitrite was assessed in two case-control studies (Ward *et al.*, 1996, 2006; Table 2.9). Quartiles of dietary nitrate were not associated with risk for non-Hodgkin lymphoma in the first study. Only the odds ratio for the highest versus the lowest quartiles were presented and no cut-off points were given (Ward *et al.*, 1996). In the second study, quartiles of nitrite intake were significantly associated with an increasing risk for non-Hodgkin lymphoma; however, when animal and plant sources of nitrite were evaluated separately, the association was due only to plant and not to animal sources of nitrite (Ward *et al.*, 2006). [The authors interpreted these findings as not supportive of a role for nitrite *per se* because animal sources of nitrite would be expected to show a stronger association with risk according to the nitrosation hypothesis.]

2.7 **Oral, pharyngeal and laryngeal cancers**

2.7.1 *Ingested nitrate*

A cohort study of head and neck cancers in Finland (Knekt *et al.*, 1999) (described in detail in Section 2.2.1) estimated dietary intake of nitrate from a food-frequency questionnaire and a Finnish nutrient database (Table 2.10). Quartiles of dietary nitrate were not associated with an increased risk (48 cases).

Dietary intake of nitrate was evaluated in relation to oral and laryngeal cancers in one case-control study (Rogers *et al.*, 1995) (described in detail in Section 2.2.1) which included 351 cases of oral cancer and 169 cases of laryngeal cancer in the Seattle metropolitan area in the USA (Table 2.11). Levels of nitrate were estimated from a 125-item food-frequency questionnaire and were linked to a database developed from the published literature. Tertiles of dietary intake of nitrate (highest tertile, > 226 mg per day [51 mg per day as nitrate-N]) were associated with significant inverse trends in the risk for oral ($P = 0.001$) and laryngeal ($P = 0.005$) cancers.

2.7.2 *Ingested nitrite*

The cohort study in Finland (Knekt *et al.*, 1999) found no association between increasing quartiles of dietary nitrite and head and neck cancers, nor was NDMA intake associated with risk.

Tertiles of dietary intake of nitrite were not associated with the risk for oral or laryngeal cancers in the case-control study by Rogers *et al.* (1995); the odds ratios for the highest tertiles were below 1 (oral cancer, 0.66; 95% CI, 0.39–1.12; laryngeal cancer, 0.67; 95% CI, 0.34–1.34) (Table 2.11). The authors evaluated dietary intake of nitrate by level of vitamin C intake (> 195 versus < 195 mg per day) and by consumption of tea (less than once a week versus more than once a week), which are factors that inhibit endogenous nitrosation. Among persons who had a higher dietary intake of nitrate and lower consumption of either vitamin C or tea, no elevated risk was observed compared with

Table 2.10. Cohort studies of nitrate in the drinking-water and nitrate and nitrite in the diet and various cancers

Reference, study location, period	Organ site (ICD code)	Cohort description	Exposure assessment	Exposure categories	No. of cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Knekt <i>et al.</i> (1999), several regions of Finland, 1966–72	Head and neck (140–148, 150, 161) (n = 48)	Cohort of 9985 persons with no cancer screened by the mobile health clinic of the Social Insurance Institution in several regions; cancer incidence follow-up through the Finnish Cancer Registry to 1990	FFQ; nitrate, nitrite, levels of NDMA estimated from a Finnish database (see Dich <i>et al.</i> , 1996 for more details of estimations of nitrate and nitrite); reproducibility estimated at intervals of 4–8 months with intraclass correlation coefficient: 0.48, nitrate; 0.73, nitrite; 0.53, NDMA; and 0.36, 0.25, 0.26, respectively for long-term reproducibility (4–7 years)	Nitrate in the diet		1.0 (reference)	Age, sex, municipality, smoking, and energy intake	NDMA intake (Q2 versus Q1: odds ratio, 2.82; 95% CI, 1.11–7.11)
				Q1		0.72 (0.32–1.60)		
				Q2		0.50 (0.21–1.20)		
				Q3		0.84 (0.39–1.81)		
				Q4		0.95		
				<i>P</i> for trend				
				Nitrite in the diet		1.0 (reference)		
				Q1		0.46 (0.19–1.12)		
	Q2		0.76 (0.35–1.66)					
	Q3		0.83 (0.36–1.88)					
	Q4		0.77					
	<i>P</i> for trend							
	Nitrate in the diet		1.0 (reference)	NDMA intake (Q2 versus Q1: odds ratio, 2.12; 95% CI, 1.04–4.33)				
	Q1		1.01 (0.52–1.92)					
	Q2		0.98 (0.51–1.87)					
	Q3		1.04 (0.54–2.02)					
Q4		0.64						
<i>P</i> for trend								
Nitrite in the diet		1.0 (reference)						
Q1		0.82 (0.45–1.48)						
Q2		0.94 (0.50–1.78)						
Q3		0.74 (0.34–1.63)						
Q4		0.45						
<i>P</i> for trend								

Table 2.10 (contd)

Reference, study location, period	Organ site (ICD code)	Cohort description	Exposure assessment	Exposure categories	No. of cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments	
Weyer <i>et al.</i> (2001) (contd)	Rectum			Average nitrate (mg/L nitrate-N) 1955–88					
				< 0.36	33	1.0 (reference)			
				0.36–1.00	25	0.72 (0.41–1.25)			
				1.01–2.46	32	0.95 (0.56–1.62)			
				> 2.46	16	0.47 (0.24–0.92)			
				Nitrate in the diet (mg N/day)					
				< 11.6	28	1.0 (reference)			
				11.6–18.0	39	1.42 (0.87–2.31)			
	18.1–27.2	27	1.01 (0.59–1.73)						
	> 27.2	28	1.06 (0.61–1.83)						
	Pancreas				Average nitrate (mg/L nitrate-N) 1955–88				
					< 0.36	17	1.0 (reference)		
					0.36–1.00	13	0.88 (0.42–1.84)		
					1.01–2.46	20	1.45 (0.73–2.88)		
> 2.46					11	0.64 (0.27–1.56)			
Nitrate in the diet (mg N/day)									
< 11.6					19	1.0 (reference)			
11.6–18.0					15	0.79 (0.40–1.56)			
18.1–27.2	16	0.86 (0.44–1.69)							
> 27.2	19	1.02 (0.52–1.99)							

Table 2.10 (contd)

Reference, study location, period	Organ site (ICD code)	Cohort description	Exposure assessment	Exposure categories	No. of cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Weyer <i>et al.</i> (2001) (contd)	Oesophagus, stomach, small intestine, liver and bile ducts, gall-bladder, peritoneum/retroperitoneum, other digestive			Average nitrate (mg/L nitrate-N) 1955–88				
				< 0.36	11	1.0 (reference)		
				0.36–1.00	12	1.39 (0.58–3.30)		
				1.01–2.46	16	1.83 (0.78–4.31)		
				> 2.46	16	2.09 (0.88–4.94)		
				Nitrate in the diet (mg N/day)				
				< 11.6	17	1.0 (reference)		
				11.6–18.0	19	1.18 (0.61–2.27)		
18.1–27.2	20	1.32 (0.68–2.57)						
> 27.2	15	1.03 (0.50–2.13)						

CI, confidence interval; FFQ, food-frequency questionnaire; ICD, International Classification of Diseases; NDMA, *N*-nitrosodimethylamine; Q, quartile

Table 2.11. Case-control studies of nitrite and nitrate in drinking-water and the diet and lung, nasopharyngeal, oral and laryngeal cancer

Reference, study location, period	Organ site	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Goodman <i>et al.</i> (1992), Oahu, Hawaii, USA, 1983–85	Lung cancer	362 cases (226 men, 100 women), aged 30–84 years, residents of Oahu in five ethnic groups (Caucasian, Chinese, Japanese, Filipino, Hawaiian) identified by Hawaii Tumor Registry and diagnosed at seven major civilian hospitals; 100% histologically confirmed; response rates, 70% (men) and 63% (women)	In-person interview using standardized questionnaire; 130-item FFQ validated for this study	population; data sources for nitrite, nitrate and NDMA not specified	Nitrite in the diet ($\mu\text{g}/\text{week}$)		Age, ethnicity, smoking, β -carotene	Results consistent across ethnic groups; stronger associations (men) with cured meats among current versus past smokers; heavier smokers, squamous-cell versus adenocarcinoma; NDMA associated with stronger increased risks among men and women (<i>p</i> for trend: men, < 0.001; women, 0.04); dietary nitrate inversely associated with risk [data not shown]; 86% of intake from vegetables
					<i>Men</i>	<i>Women</i>		
				< 2000		1.0 (reference)		
				2000–< 4000		1.2 (0.6–2.3)		
				4000–< 7000		1.7 (0.9–3.3)		
				> 7000		2.0 (1.0–3.8)		
				<i>P</i> for trend		0.02		
				< 2000		1.0 (reference)		
				2000–< 4000		0.6 (0.2–1.2)		
				4000–< 7000		0.9 (0.4–2.1)		
				> 7000		1.5 (0.5–4.1)		
				<i>P</i> for trend		0.22		

Table 2.11 (contd)

Reference, study location, period	Organ site	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
De Stefani <i>et al.</i> (1996), Montevideo, Uruguay, 1994–95	Lung cancer	320 cases (307 men, 13 women) from participating hospitals, all ages; average age, 63.1 years; histologically confirmed; response rate, 100% 320 hospital controls excluding diseases related to tobacco use; most frequent conditions: fractures (36%), eye disorders (22%), abdominal hernia (15%), trauma (9%), appendicitis (5%); frequency-matched by age, sex, hospital, residence (Montevideo, other counties); response rate, 100%	Interview-administered standardized questionnaire; 70-item FFQ focusing on main sources of nitrite, NDMA	Dietary NDMA ($\mu\text{g/day}$) ≤ 0.13 0.14-0.18 0.19-0.26 ≥ 0.27		1.0 (reference) 0.88 (0.53–1.48) 1.77 (1.06–2.96) 3.14 (1.86–5.29)	Age, gender, residence, urban/rural status, family history of lung cancer, pack-years of tobacco smoking, total energy intake	Increasing risk with quartiles of NDMA for squamous-cell, small-cell and adenocarcinoma; strongest association for squamous-cell and adenocarcinoma; dietary nitrite was estimated but results were not presented.

Table 2.11 (contd)

Reference, study location, period	Organ site	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Ward <i>et al.</i> (2000), Taipei, Taiwan, China, 1991–94	Nasopharynx	375 cases identified from 2 referral hospitals in Taipei, aged < 75 years; 100% histologically confirmed; response rate, 99% of cases and 47% of mothers 327 population-based selected from national household registration system; matched by township/district, age, sex; response rate, 88% of controls and 63% of mothers	In-person interviews of cases and controls and their mothers; mothers asked about child's diet at age 10 and 3 years, and during weaning, and mother's diet during breast-feeding; cases and controls asked about diet as adult and age 10 years; nitrate, nitrite and nitrosamine levels (semi-quantitative) estimated from published values for Chinese foods and, when no values were available, from western foods	*Nitrite from soya bean products			Age, gender, ethnicity, vegetable intake	Results for mother's reports of intakes at age 3 years and weaning were similar (based on fewer foods);* intake at age 10 years as reported by mothers; nitrosamine intake from foods other than soya beans (as estimated by mothers) at ages 10 and 3 years, and weaning was positively associated with risk (weaning Q4 odds ratio, 3.9; 95% CI, 1.4–10.4)
				Q1 (low)	NR	1.0 (reference)		
				Q2	NR	0.6 (0.2–1.3)		
				Q3	NR	0.5 (0.2–1.1)		
				Q4 (high)	NR	0.6 (0.3–1.4)		
				*Nitrite from other foods				
				Q1 (low)	NR	1.0 (ref)		
				Q2	NR	1.7 (0.6–4.9)		
Q3	NR	3.5 (1.3–9.5)						
Q4 (high)	NR	2.0 (0.7–6.0)						

Table 2.11 (contd)

Reference, study location, period	Organ site	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Rogers <i>et al.</i> (1995), King, Snohomish and Pierce counties, Washington State, USA, 1983–87	Oral	351 cases from cancer surveillance system (part of NCI SEER); response rate, 73% (dietary component) 514 population-based controls selected by random-digit dialling; matched by age, gender; response rate, 76%	Personal interview; self-administered 125-item FFQ on foods high in nitrate, nitrite, NDMA; levels estimated from the published literature; nitrate in water estimated to be 1.3 mg/L	Dietary nitrate (mg/day)			All odds ratios adjusted for age, gender, education, pack-years of tobacco smoking, alcohol (drink-years), body mass index, total energy	No interaction of nitrite with vitamin C or tea consumption; NDMA tertiles associated with increasing risk (odds ratio highest versus lowest tertile, 1.82; 95% CI, 1.10–3.00)
				< 134 (T1)	113	1.0 (reference)		
				134–226 (T2)	109	0.66 (0.43–1.01)		
				> 226 (T3)	76	0.46 (0.28–0.76)		
				<i>P</i> for trend		0.001		
				Dietary nitrite (mg/day)				
				< 1.06 (T1)	112	1.0 (reference)		
				1.06–1.60 (T2)	92	0.96 (0.61–1.51)		
				> 1.60 (T3)	94	0.66 (0.39–1.12)		
				<i>P</i> for trend		0.099		
Nitrite (mg)/vitamin C	NR							
T1/high vitamin C		1.0 (reference)						
T2/high vitamin C		1.62						
T3/high vitamin C		1.01						
T1/low vitamin C		2.40						
T2/low vitamin C		1.64						
T3/low vitamin C		1.30						

95% CIs not given

Table 2.11 (contd)

Reference, study location, period	Organ site	Characteristics of cases and controls	Exposure assessment	Exposure categories	No. of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Rogers <i>et al.</i> (1995) (contd)	Larynx	169 cases from cancer surveillance system (part of NCI SEER)		Dietary nitrate (mg/day)				
				< 134 (T1)	67	1.0 (reference)		No interaction of nitrite with vitamin C, tea consumption; NDMA tertiles associated with increasing risk (odds ratio highest versus lowest tertile, 1.70; 95% CI, 0.91–3.18)
				134–226 (T2)	49	0.50 (0.29–0.88)		
				> 226 (T3)	35	0.42 (0.22–0.80)		
				<i>P</i> for trend		0.005		
				Dietary nitrite (mg/day)				
				< 1.06 (T1)		1.0 (reference)		
				1.06–1.60 (T2)		0.98 (0.54–1.79)		
				> 1.60 (T3)		0.67 (0.34–1.34)		
				<i>P</i> for trend		0.107		
Nitrite/vitamin C	NR							
T1/high vitamin C		1.0 (reference)		Age, gender, smoking, alcohol, body mass index, education, energy intake				
T2/high vitamin C		1.04						
T3/high vitamin C		0.65						
T1/low vitamin C		1.65						
T2/low vitamin C		1.22						
T3/low vitamin C		0.95						

CI, confidence interval; FFQ, food-frequency questionnaire; NCI SEER, National Cancer Institute Survey of Epidemiology and End Results; NDMA, *N*-nitrosodimethylamine; NR, not reported; Q, quartile; T, tertile

persons who had a low intake of nitrite and high intake of vitamin C or tea (95% CIs were not provided). The authors also estimated dietary intake of NDMA, which was associated with an 82% significant increased risk for oral cancer and 70% non-significant increased risk for laryngeal cancer.

2.7.3 Nitrate and nitrite in saliva

A small hospital-based study in Italy (Airoldi *et al.*, 1997), in which oral, pharyngeal and laryngeal cancers were combined (36 men), found no differences between concentrations of salivary nitrate, nitrite or *N*-nitroso compounds in cases and controls. Selection of controls was not specified in detail and it was indicated that they were often relatives of the cases.

Another small hospital-based study of oral cancer in Egypt (44 cases, 40 control volunteers) measured levels of nitrate and nitrite in saliva (Badawi *et al.*, 1998). Higher levels of salivary nitrite were associated with an increased risk for oral cancer (odds ratio, 4.3; 95% CI, 1.4–13.3 for > 40 µg/mL nitrite). [Levels in cases may have been affected by the disease; the controls were not population-based and did not appear to be matched to the cases by gender or age. Therefore, it was not possible to ascertain if their salivary levels are representative of the population.]

2.8 Nasopharyngeal cancer

No studies have evaluated nitrate ingested from drinking-water or diet and the risk for nasopharyngeal carcinoma (NPC).

2.8.1 Ingested nitrite

(a) Ecological studies

Two ecological studies (Wu *et al.*, 1993; Yi *et al.*, 1993) in areas of China where mortality from NPC was both high and low (rates for other cancers varied) used the NPRO test to evaluate the hypothesis that higher endogenous nitrosation is associated with a higher risk for NPC. The study by Wu *et al.* (1993) (described in detail in Section 2.2) evaluated cancer mortality rates in 69 counties in relation to urinary nitrate, nitrosation potential and excretion of three *N*-nitroso amino acids in urine. Mortality rates (ages 0–64 years in 1973–75) for NPC were significantly positively correlated with urinary nitrate ($r = 0.28$, $P < 0.05$) and one *N*-nitroso amino acid ($r = 0.26$, $P < 0.05$) but not with nitrosation potential ($r = 0.06$, $P > 0.05$).

Yi *et al.* (1993) evaluated endogenous nitrosation potential among men and women in high- ($n = 37$) and low-risk ($n = 40$) districts in Zangwu County, Gaungxi region in 1990. Incidence rates of NPC varied 10-fold across the districts. The methods used were similar to those of Wu *et al.* (1993) except that a baseline 12-h urine sample was collected to measure background levels of nitrate and *N*-nitroso amino acids after a normal evening

meal. Background levels did not differ between the two areas. Proline intake increased excretion of NPRO of people in the high-risk but not of those in the low-risk district.

(b) *Case-control studies*

One case-control study of NPC in Taiwan, China (Ward *et al.*, 2000), estimated dietary intake of nitrate during adulthood and at ages 3 and 10 years and maternal diet during pregnancy and breast-feeding (Table 2.11). Levels of nitrite were determined from the published literature using values for Chinese foods when available and from other published sources (mainly Japanese, USA and European data). Both the study subjects and their mothers were asked about the subject's diet at age 10 years. Intake of nitrite was assessed across all foods and results were presented separately for nitrite intake from soya bean products and from other foods (preserved meats, salted fish, preserved vegetables) because soya beans contain inhibitors of nitrosation. Based on self-reported adult dietary intakes and those at age 10 years, neither intake of nitrite from soya beans nor that from other foods was significantly associated with the risk for NPC. Based on maternal reports of the subject's diet at age 10 years, age 3 years and during weaning, intake of nitrite from soya bean products was inversely associated with risk, whereas, increasing intake of nitrite from other foods was positively associated with risk after adjustment for potential confounders, including vegetable intake. Dietary intake of nitrosamines was also estimated from the published literature and a semiquantitative level was assigned to each food. The results were similar to those for nitrite. Intake of nitrosamines from soya bean products was inversely associated with risk, whereas that from other foods was positively associated with the childhood diet based on maternal reports.

2.9 Colon and rectal cancers

2.9.1 *Ingested nitrate*

(a) *Ecological studies*

Gulis *et al.* (2002) (described in detail in Section 2.2) found a positive correlation between levels of nitrate in public water supplies and the incidence of colon but not of rectal cancer in Slovakia. Elevated levels of nitrate in public water supplies in 40 'ecodistricts' of Ontario, Canada, were positively correlated with age-standardized incidence ratios for colon cancer among women ($P = 0.048$) (described in detail in Section 2.2). In multiple variable regression analyses, age-standardized incidence ratios in women were no longer significantly associated with levels of nitrate in the drinking-water (Van Leeuwen *et al.*, 1999). In Spain (Morales-Suárez-Varela *et al.*, 1995) (described in detail in Section 2.2), no correlation was observed between levels of nitrate in 258 municipalities in 1968 (towns grouped by average nitrate level: 0–< 5.7, 5.7–11, > 11 mg/L) and mortality rates for colon cancer in 1975–80.

The study by Wu *et al.* (1993) in 69 counties of China (described in detail in Section 2.2) found no significant correlation between mortality rates (ages 0–64 years in 1973–75) for colorectal cancer and urinary concentrations of nitrate ($r = 0.16$).

(b) *Cohort and case-control studies*

A cohort study in Finland (Knekt *et al.* 1999) (described in detail in Section 2.2) enrolled men and women who were screened at mobile health clinics in 1966–72 and estimated dietary intake of nitrate from a food-frequency questionnaire and a Finnish database (Table 2.10). The incidence of colorectal cancer assessed through to 1990 (73 cases) was not associated with quartiles of dietary intake of nitrate.

A cohort study (described in detail in Section 2.4) of women in Iowa, USA (Weyer *et al.*, 2001), found an inverse association with long-term average levels of nitrate (1955–88) in the public water supplies that served the current residence (minimum duration, 11 years) and incidence of rectal cancer (Table 2.10). The highest quartile of the average level of nitrate was not associated with an increased risk for colon cancer; however, relative risks were significantly elevated in the second and third quartiles. The authors did not evaluate the interaction with vitamin C intake. Increasing quartiles of dietary intake of nitrate were not associated with risk for colon or rectal cancers.

A population-based case-control study of colon and rectal cancer in Iowa, USA (De Roos *et al.*, 2003), included 685 cases of colon cancer, 655 cases of rectal cancer and 2434 controls. The analyses of drinking-water were limited to those who had spent 70% or more of their person-years (1960–87) in an area with estimated levels of nitrate in public water supplies (376 colon cancers, 338 rectal cancers, 1244 controls). Overall, no relationship was observed between average levels of nitrate (1960–87) and years with a supply with >5 or >10 mg/L nitrate-N and risk for either colon or rectal cancer (Table 2.12). The authors evaluated potential interactions between exposure to nitrate in the drinking-water and factors that may affect endogenous nitrosation in the stomach or colon including vitamin C intake, meat intake and history of inflammatory bowel disease. For colon cancer, significant positive interactions were observed between exposures for 10 or more years to levels of above 5 mg/L nitrate-N and low vitamin C and high meat intake. Similar results were found for the metrics of nitrate in water. Quartiles of dietary intake of nitrate estimated from a food-frequency questionnaire and published levels in foods were inversely associated with risk for colon cancer and no association with rectal cancer was observed.

2.9.2 *Ingested nitrite*

A cohort study in Finland (Knekt *et al.*, 1999) and a case-control study of colon and rectal cancer in Iowa, USA (De Roos *et al.*, 2003), evaluated dietary intake of nitrite (Tables 2.10 and 2.12). Knekt *et al.* (1999) estimated dietary intake of both nitrite and NDMA. The incidence of colorectal cancer (73 cases) was not associated with increasing quartiles of dietary nitrite intake. However, the highest quartile of intake of NDMA was

Table 2.12. Case-control study of nitrate in drinking-water and nitrate and nitrite in the diet and colon and rectal cancers

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments		
De Roos <i>et al.</i> (2003), Iowa, USA, 1988–89	Colon	685 cases identified from Iowa Cancer Registry, aged 40–85 years; white only; 86% deceased or too ill with proxy respondents; cases confirmed by histology (348) or positive cytology (28); response rate, 88%	2434 population-based selected from driver's licence records (aged <65 years) or from Health Care Finance Administration listing (aged > 65 years); matched by age, sex; response rates, 82% (aged < 65) and 80% (aged > 65)	Postal questionnaire; lifetime residential water source history; 55-item food-frequency questionnaire assessed nitrate, nitrite, vitamin C; nitrate monitoring data from 1960–87 from public utilities in Iowa; imputation of values for years without nitrate data; drinking-water analyses: 376 colon cases, 338 rectal cases, 1244 controls with ≥ 70% person-years 1960 onward with nitrate estimates	Average nitrate (mg/L nitrate-N) in public supplies, 1960–89	≤ 1	172	1.0 (reference)	Age, gender	Significant interaction (<i>P</i> < 0.10) of water nitrate level with vitamin C, meat intake; odds ratio > 10 years nitrate at levels > 5 mg/L nitrate-N and > median meat intake, 2.2 (1.4–3.6) versus 0 years > 5 mg/L nitrate-N and low meat intake; no significant for interaction for water nitrate with tobacco smoking, history of bowel inflammation, beverage consumption or high-fibre food intake	
						> 1–≤ 3	116	1.0 (0.8–1.3)			
						> 3–≤ 5	27	0.7 (0.4–1.1)			
						> 5	61	1.2 (0.8–1.7)			
						Years with nitrate >5 mg/L nitrate-N					
						0	240	1.0 (reference)			
						1–≤ 10	65	0.8 (0.6–1.1)			
						> 10	71	1.2 (0.9–1.6)			
						Vitamin C/years > 5 mg/L					
						High vit C/0 year	74	1.0 (reference)			
						Low vit C/0 years	97	1.4 (1.0–1.9)			
						High vit C/1–10 years	22	0.9 (0.5–1.5)			
						Low vit C/1–10 years	28	1.1 (0.7–1.9)			
High vit C/> 10 years	24	1.1 (0.7–1.9)									
Low vit C/> 10 years	35	2.0 (1.2–3.3)									

Table 2.12 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
De Roos <i>et al.</i> (2003) (contd)	Colon (contd)				Dietary nitrate (mg/day)				
					≤ 59.3	89	1.0 (reference)		
					59.4–86.86	68	0.8 (0.6–1.2)		
					86.7–122	68	0.8 (0.5–1.1)		
					> 22	55	0.7 (0.4–1.0)		
					Dietary nitrite (mg/day)				
					≤ 0.705	90	1.0 (reference)		
					0.706–0.94	73	1.1 (0.8–1.6)		
					0.94–1.26	48	0.9 (0.6–1.3)		
					> 1.26	69	1.5 (1.0–2.1)		
					Average nitrate (mg/L nitrate-N) in public supplies, 1960–89				
					≤ 1	154	1.0 (reference)		
					> 1–≤ 3	98	0.8 (0.6–1.1)		
					> 3–≤ 5	30	0.7 (0.5–1.2)		
> 5	56	1.2 (0.8–1.8)							
Years with nitrate >5 mg/L nitrate-N									
0	222	1.0 (reference)							
1–≤ 10	57	0.8 (0.6–1.1)							
> 10	59	1.1 (0.7–1.5)							
	Rectum	655 cases identified from Iowa Cancer Registry, aged 40–85 years; white only; 86% deceased or too ill with proxy respondents; cases confirmed by histology (348) or positive cytology (28); response rate, 88%						Age, gender, years with chlorinated surface water	No significant interaction with vitamin C, meat intake, tobacco smoking, history of bowel inflammation, beverage consumption or high-fibre food intake

Table 2.12 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments	
De Roos <i>et al.</i> (2003) (contd)	Rectum (contd)				Vitamin C/years					
					> 5 mg/L					
					High vit C/0 year	87	1.0 (reference)			
					Low vit C/0 years	82	0.9 (0.7–1.3)			
					High vit C/1–10 years	15	0.5 (0.3–1.0)			
					Low vit C/1–10 years	24	0.8 (0.5–1.3)			
					High vit C/> 10 years	18	0.7 (0.4–1.3)			
					Low vit C/> 10 years	23	1.1 (0.6–1.8)			
					Dietary nitrate (mg/day)					
					≤ 59.3	56	1.0 (reference)			
					59.4–86.8	67	1.3 (0.9–1.9)			
					86.7–122	66	1.2 (0.8–1.8)			
					> 122	60	1.1 (0.8–1.7)			
					Dietary nitrite (mg/day)					
			≤ 0.705	74	1.0 (reference)					
			0.706–0.94	62	1.1 (0.7–1.6)					
			0.94–1.26	43	0.9 (0.6–1.4)					
			> 1.26	70	1.7 (1.1–2.5)					

CI, confidence interval; vit C, vitamin C

associated with a significant, 2.1-fold increase in risk. In the case-control study (De Roos *et al.*, 2003), the highest quartile of dietary intake of nitrite was associated with an increased risk for colon cancer (odds ratio for the highest versus lowest quartile, 1.5; 95% CI, 1.0–2.1) and rectal cancer (odds ratio for the highest versus lowest quartile, 1.7; 95% CI, 1.1–2.5).

Meta-analyses of epidemiological studies that evaluated consumption of meat and the occurrence of colorectal cancer (Sandhu *et al.*, 2001; Norat *et al.*, 2002) reported significantly increased risks for the consumption of red meats and cured meats such as frankfurters, bacon, ham and luncheon meat. Although most cured meats are usually treated with nitrite, few studies estimated dietary intake of nitrite directly.

2.10 Pancreatic cancer

2.10.1 Ingested nitrate

One cohort study (Weyer *et al.*, 2001) and one case-control study (Coss *et al.*, 2004) evaluated nitrate in both the drinking-water and diet and risk for pancreatic cancer. Two additional case-control studies (Howe *et al.*, 1990; Baghurst *et al.*, 1991) evaluated dietary intake of nitrate alone.

A cohort study of women in Iowa, USA (Weyer *et al.*, 2001; described in detail in Section 4.1), found no significant association with the long-term average levels of nitrate in public water supplies that served the current residence and the incidence of pancreatic cancer (Table 2.10); neither were quartiles of dietary intake of nitrate associated with risk.

A population-based case-control study in Canada of 249 cases and 505 controls (Howe *et al.*, 1990) evaluated the risk for pancreatic cancer in relation to dietary intakes of nitrate using a food-frequency questionnaire and a database of nitrate values obtained from the published literature (Table 2.13). Modelled as a continuous variable and adjusting for age, sex, tobacco smoking and intake of fibre and calories, no significant association was observed between intake of nitrate and risk for pancreatic cancer.

Baghurst *et al.* (1991) conducted a population-based case-control study of pancreatic cancer in Australia among 104 cases and 253 controls (Table 2.13). Dietary intake of nitrate was estimated from a food-frequency questionnaire with nitrate values provided by Howe *et al.* (1990). Increasing quartiles of dietary intake of nitrate were associated with decreasing risk (odds ratio for highest versus lowest quartile, 0.45; 95% CI, 0.22–0.94) after adjustment for total energy, tobacco smoking and alcohol consumption.

A population-based case-control study (Coss *et al.*, 2004) in Iowa, USA, included 376 cases of pancreatic cancer and 2034 controls. The analyses of drinking-water were limited to persons who had spent 70% of their person-years from 1960 onwards in an area with an estimate of levels of nitrate in the public water supply (189 cases, 1244 controls). The average level of nitrate in public water supplies (1960–87) and the number of years that the person used supplies with > 10 and > 7.5 mg/L nitrate-N were evaluated. No association was observed between any of the metrics of nitrate in the

Table 2.13. Case–control studies of nitrate in the drinking-water and nitrate and nitrite in the diet and pancreatic cancer

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Howe <i>et al.</i> (1990), metropolitan Toronto, Canada, 1983–86	Pancreas	249 diagnosed in 20 Toronto hospitals, aged 35–79 years, resident in greater metropolitan Toronto; 69% histologically confirmed, remainder clinically or radiologically; 194 interviews with proxies (62% spouse, 31% child); response rate, 46%	505 population-based selected from population rolls; matched by age, sex, residence in Toronto area; 194 interviews with proxies of selected controls (72% spouse; 19% child); response rate, 31%	Interview administered 200-item FFQ validated for the study population	Dietary nitrate, mean daily difference Q4 versus Q1: 35.1 mg		1.21 (0.72–2.02)	Age, sex, respondent status, total calories, fibre, cigarettes/day	Odds ratios estimated from continuous variable models; interaction with vitamin C not evaluated
					<i>P</i> for trend	0.48			
					Dietary nitrite, mean daily difference Q4 versus Q1: 1.6 mg		0.64 (0.34–1.22)		
					<i>P</i> for trend	0.17			

Table 2.13 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Baghurst <i>et al.</i> (1991), Adelaide, South Australia, 1984–87	Pancreas	104 (52 men, 52 women) from South Australian Cancer Registry; only 16 cases had next-of-kin respondents due to rapid enrolment; response rates for case series which included pancreas, gall-bladder and bile ducts was 62% for men and 63% for women	253 population-based from electoral rolls; matched on age, sex; response rates, 57% for men and 51% for women	Self-administered 179-item FFQ; validated for the study population; nitrate, nitrite, nitrosamine values from Howe <i>et al.</i> (1990; Canada) with local data for alcohol and cured meats from producers in Australia	Dietary nitrate (mg/day)			Total energy, alcohol, tobacco	Interaction with vitamin C or animal sources separately not evaluated; nitrosamines associated with non-significantly increased risk (<i>P</i> for trend = 0.11); [histological confirmation not mentioned]
					Q1	1.0 (reference)			
					Q2	0.90 (0.46–1.77)			
					Q3	0.73 (0.36–1.48)			
					Q4	0.45 (0.22–0.94)			
					<i>P</i> for trend	0.027			
					Dietary nitrite (mg/day)				
					Q1	1.0 (reference)			
					Q2	1.01 (0.51–1.99)			
					Q3	0.65 (0.32–1.30)			
					Q4	0.92 (0.46–1.84)			
					<i>P</i> for trend	0.49			

Table 2.13 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments	
Coss <i>et al.</i> (2004), Iowa, USA, 1985–87	Pancreas	376 (202 white men, 174 white women) identified from Iowa Cancer Registry, aged 40–85 years; 86% deceased or too ill with proxy respondents; cases confirmed by histology (348) or positive cytology (28); response rate, 88%	2034 population-based selected from driver’s licence records (aged < 65) or from Health Care Finance Administration listing (aged > 65); matched by age, sex; response rates, 82% (aged < 65) and 80% (aged > 65)	Postal questionnaire; lifetime residential water source history; 55-item FFQ; monitoring data for 1960–87 from public utilities in Iowa; imputed values for years without data; drinking water nitrate analyses: 189 cases, 1244 controls with ≥ 70% person–years 1960 onward with nitrate estimates	Average nitrate (mg/L nitrate-N) in public supplies, 1960–87				Age, gender, cigarette use	No significant associations with years using a public supply with ≥ 7.5 or 10 mg/L nitrate-N; no interaction with smoking or vitamin C intake [data not shown]; association not significant after excluding proxies who were not spouses; no association for plant sources of nitrite among men or women [data not shown]
					< 0.6	50	1.0 (reference)			
					0.6–< 1.3	62	1.2 (0.79–1.8)			
					1.3–2.8	28	0.54 (0.33–0.89)			
					> 2.8	49	0.99 (0.64–1.5)		Age, cigarette use, total calories	
					Dietary nitrate (mg/day)					
					<i>Men</i>					
					< 58	26	1.0 (reference)			
					58–82	33	1.1 (0.63–1.9)			
					83–117	39	1.2 (0.70–2.0)			
					> 117	43	1.0 (0.60–1.8)			
					<i>Women</i>					
< 63	39	1.0 (reference)								
63–90	33	0.99 (0.58–1.7)								
91–126	24	0.64 (0.36–1.1)								
> 126	26	0.53 (0.29–0.97)								

Table 2.13 (contd)

Reference, study location, period	Organ site	Characteristics of cases	Characteristics of controls	Exposure assessment	Exposure categories	No of exposed cases	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Coss <i>et al.</i> (2004) (contd)					Dietary nitrite (mg/day)				
					<i>Men</i>				
					< 0.75	15	1.0 (reference)		
					0.75–0.98	22	1.0 (0.52–2.0)		
					0.99–1.30	40	1.5 (0.81–2.9)		
					> 1.30	64	1.5 (0.79–3.0)		
					<i>Women</i>				
					< 0.56	18	1.0 (reference)		
					0.56–0.71	32	1.8 (0.94–3.4)		
					0.72–0.93	32	1.4 (0.72–2.6)		
					> 0.93	40	1.3 (0.65–2.5)		
					Dietary nitrite from animal sources (mg/day)				
					<i>Men</i>				
					< 0.22	9	1.0 (reference)		
					0.22–0.31	22	2.1 (0.95–4.8)		
					0.32–0.53	60	3.8 (1.8–8.0)		
				> 0.53	50	2.3 (1.1–5.1)			
				<i>Women</i>					
				< 0.13	13	1.0 (reference)			
				0.13–0.18	32	2.4 (1.3–4.7)			
				0.19–0.26	26	1.9 (0.94–4.0)			
				> 0.26	51	3.2 (1.6–6.4)			

CI, confidence interval, FFQ, food-frequency questionnaire; Q, quartile

drinking-water and risk for pancreatic cancer. No evidence of an interaction between the levels of nitrate in water and intake of vitamin C or tobacco smoking was found [results of analyses of interaction were not presented]. Increasing quartiles of dietary intake of nitrate were inversely associated with risk in women (odds ratio for the highest versus lowest quartile, 0.53; 95% CI, 0.29–0.97) but not in men (odds ratio, 1.0; 95% CI, 0.6–1.8). When analyses were limited to self-responding women and proxies who were husbands of the subjects, no significant association with risk was observed.

2.10.2 *Ingested nitrite*

The three case–control studies that evaluated dietary intake of nitrate also estimated dietary intake of nitrite using the same methods (Table 2.13).

Howe *et al.* (1990) modelled intake of nitrite as a continuous variable and found no association with the risk for pancreatic cancer. Baghurst *et al.* (1991) evaluated increasing quartiles of dietary intake of nitrite and found no association with the risk for pancreatic cancer; however, increasing quartiles of dietary intake of nitrosamines were associated with a non-significant positive trend in risk (P for trend = 0.11).

In the case–control study in Iowa (Coss *et al.*, 2004), a slight elevated risk for pancreatic cancer was observed for the high quartile of consumption of dietary nitrite (odds ratio for men, 1.5; odds ratio for women, 1.3). When animal sources of nitrite were evaluated separately, risks were higher (odds ratio for highest versus lowest quartile: men, 2.3; women, 3.2) and were statistically significant.

2.11 Lung

2.11.1 *Ingested nitrate*

One cohort study (Weyer *et al.*, 2001) (described in detail in Section 2.4) evaluated nitrate in the drinking-water and the diet and the risk for lung cancer among 252 cases. No significant relationship between average levels of nitrate (median, 1.0 mg/L nitrogen-N; interquartile range, 0.36–2.46 mg/L) in public water supplies and the risk for lung cancer was observed. Quartiles of dietary nitrate were not significantly associated with risk (odds ratio for highest versus lowest quartile, 0.78; 95% CI, 0.55–1.11).

One case–control study (Goodman *et al.*, 1992) evaluated dietary intake of nitrate and the risk for lung cancer and reported an inverse association [results not presented in the paper; 86% of intake was reported to be from vegetables].

2.11.2 *Ingested nitrite*

A population-based case–control study in Oahu, Hawaii, USA, of 326 cases and 865 controls (Goodman *et al.*, 1992) found elevated risks for lung cancer with increasing quartiles of dietary intake of nitrite among men and women (Table 2.11) with a significant positive trend among men (P for trend = 0.02). Risk estimates were adjusted

for ethnicity, tobacco smoking, and intake of β -carotene. Intake of nitrite was primarily from consumption of cured meat. The authors did not evaluate interactions with nitrite and vitamin C or other factors that affect endogenous nitrosation. The association with intake of individual cured meats was stronger among current smokers than among former smokers, and was somewhat stronger for squamous-cell carcinoma than for adenocarcinoma. Levels of NDMA were also estimated in this study and showed a stronger association with risk than nitrite among both men (P for trend < 0.001) and women (P for trend = 0.04).

2.12 Liver

No cohort or case-control studies have evaluated ingested nitrate and nitrite in relation to liver cancer.

Ecological studies

The study by Wu *et al.* (1993) in 69 counties of China (described in detail in Section 2.2.1) found a statistically significant positive correlation between mortality rates for liver cancer (ages 0–64 years in 1973–75) and urinary concentrations of nitrate ($r = 0.25$; $P < 0.05$). Mortality from these cancers was not significantly correlated with nitrosation potential, NPRO or specific *N*-nitroso amino acids.

An ecological study of cholangiocarcinoma in Thailand (Srianujata *et al.*, 1984) evaluated salivary and urinary concentrations of nitrate and nitrite in men and women in low- (12 subjects) and high-risk (32 subjects) areas who consumed regular meals. Multiple samples were taken within 2 h after eating. Levels of nitrate and nitrite in saliva were significantly higher (two to five fold) among those living in high-risk areas. Urinary levels of nitrate were higher at some time points, but all levels of nitrite were similar. Mitacek *et al.* (1999) measured the levels of volatile nitrosamines, nitrate and nitrite in foods typically consumed in high- and low-risk areas for liver cancer in Thailand. Concentrations of nitrate and nitrite were not correlated with volatile nitrosamines. Specific volatile nitrosamines tended to be found at higher concentrations in foods that were consumed in the high-risk areas; however, no formal statistical analyses were conducted.

2.13 References

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3. Studies of Cancer in Experimental Animals

3.1 Nitrate

3.1.1 *Mouse*

Oral administration

In a study of the effects of concurrent administration of sodium nitrate and piperazine, one group of 40 male strain A mice, 7–9 weeks of age, was given 12.3 g/L sodium nitrate in the drinking-water for 25 weeks and was killed 13 weeks later. Controls were untreated. Other groups were fed piperazine (18.75 g/kg) alone in the diet or received piperazine and nitrate. The incidence of lung adenomas did not differ statistically between the sodium nitrate-treated mice (7/40), untreated controls (12/37), mice fed piperazine alone (7/33) or mice fed piperazine and treated with nitrate (15/37). The authors noted that less than 2.5% of the administered nitrate was reduced to nitrite *in vivo* in mice (Greenblatt & Mirvish, 1973).

Groups of 10 male and 10 female ICR mice, 8 weeks of age, were fed an experimental diet containing 0, 2.5 or 5.0% sodium nitrate for 2 years. No significant differences in food intake, body weight or tumour incidence were observed between untreated control and treated animals (Sugiyama *et al.*, 1979). [The Working Group noted the small number of animals per group.]

Groups of 100 female NMRI mice, weighing 27–31 g, were given drinking-water that contained 0, 100 or 1000 mg/L (0, 30 or 300 mg/kg body weight (bw)) calcium nitrate daily for 18 months. Survival of the high-dose group was lower than that of controls. No increase in tumour incidence was observed in the nitrate-treated groups (Mascher & Marth, 1993).

3.1.2 *Rat*

Oral administration

Groups of 15 male and 15 female MRC-derived rats, 8–10 weeks of age, were given 0 or 5 g/L sodium nitrate in the drinking-water for 84 weeks and served as controls in a study of nitrilotriacetic acid. The animals were killed 20 weeks later. Tumour incidences did not differ between nitrate-treated males and females and the untreated control groups (Lijinsky *et al.*, 1973a). [The Working Group noted the short duration of exposure.]

Groups of 10 male Sprague-Dawley rats, 2 months of age, received 0 or 4000 mg/L sodium nitrate in the drinking-water for 14 months. Body weights of the nitrate-treated group were lower than those of untreated controls. The nitrate-treated rats had a higher incidence of lung lesions compared with controls (4/4 versus 1/5). The nature of lung lesions was not described (Chow *et al.*, 1980). [The Working Group noted the small

number of animals, the short duration of exposure and the lack of histopathological examination.]

Groups of 50 male and 50 female Fischer 344 rats, 8 weeks of age, were fed a diet containing 0, 2.5 or 5% sodium nitrate for 2 years [equivalent to 0, 1250 or 2500 mg/kg bw sodium nitrate per day or 0, 910 or 1820 mg/kg bw per day expressed as nitrate ion]. Thereafter, tap-water and basal diet were given to all experimental groups and observation was continued until week 123. The survival rate of the treated animals was significantly higher ($P < 0.05$) than that of controls. Full histopathology was performed. The incidence of mononuclear-cell leukaemia was reduced ($P < 0.01$, χ^2 test) in the treated group compared with that in controls (males: control, 36%; low-dose, 4%; and high-dose, 2%; females: control, 28%; low-dose, 0%; and high-dose, 2%). No significant differences were observed in the incidence of any other types of tumour (Maekawa *et al.*, 1982). [The Working Group noted that Fischer 344 rats have a high incidence of spontaneous mononuclear-cell leukaemia].

Two groups of locally bred male white rats, weighing 120–140 g [age unspecified], received daily either a 0.05% solution of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBNA) in the drinking-water for 30 days alone (18 effective animals) or, after treatment with BBNA was completed, 4 g/L sodium nitrate also in the drinking-water for 273 days (34 effective animals). A third group was treated with sodium nitrate alone at the same dose for 273 days (20 effective animals) and 20 effective animals in the fourth group served as untreated controls. All surviving rats were killed 10 months after the start of the experiment. Urinary bladders of all animals were examined histologically. BBNA alone induced urinary bladder carcinoma in one effective animal (5.6%), papillomas in four animals (22.2%) and papillary and nodular hyperplasia in eight animals (44.4%). Combined treatment with BBNA and sodium nitrate resulted in the development of carcinoma in six (17.6%; $P < 0.05$), papilloma in five (14.7%) and papillary and nodular hyperplasia of the urothelium in 22 rats (65%). No morphological changes of the urothelium were observed in males treated with sodium nitrate alone or in control animals. No spontaneous tumours of the urinary bladder were reported in historical controls of this strain of animal. The authors concluded that sodium nitrate significantly promoted bladder carcinogenesis induced by BBNA (Pliss & Frolov, 1991).

3.2 Nitrite

In most of the studies reviewed, mice or rats that were exposed to nitrite alone in the diet, by gavage or in the drinking-water did not have a higher incidence of tumours compared with untreated controls. It was noted that the negative findings may be due to the low doses of nitrite used, the short duration of exposure or the instability of nitrite. In some instances, when carcinogenic activity of nitrite was observed, the investigators noted the formation of *N*-nitroso compounds in the diet mix or in the stomach contents.

3.2.1 *Mouse*

(a) *Oral administration in the diet*

Groups of 20 male and 20 female C57BL/6 mice, 6 weeks of age, were fed 0.5% sodium nitrite in the diet (prepared freshly each week) for 1 year. Groups of 100 males and 100 females were untreated. The treated animals were observed for 120 days and did not exhibit a higher tumour incidence (males, 2/11; females, 2/12) than the untreated controls (males, 8/95; females, 17/92), however, when a third group of 50 males and 50 females was fed 0.58% butylurea and a fourth group of 50 males and 70 females was treated with 0.5% sodium nitrite and 0.58% butylurea, an increase in tumour incidence was observed in the latter groups (males, 35/39; females, 27/40) compared with animals treated with butylurea alone (males, 7/26; females, 4/24). The tumours reported in the latter groups were mainly malignant lymphomas (Krishna Murthy *et al.*, 1979). [The Working Group noted the small number of nitrite-treated animals. The effective number of animals represents the number of animals evaluated histologically rather than the number necropsied.]

(b) *Oral administration in the drinking-water*

Six groups of male and female Swiss mice, 6–11 weeks of age, were administered secondary amines (6.25 g piperazine/kg of diet, 40 males and 40 females; 6.33 g morpholine/kg of diet, 20 males and 20 females; or 1.95 g *N*-methylaniline/kg of diet, 20 males and 20 females) in the feed (continuously) with or without sodium nitrite in the drinking-water (1.0 g/L, 5 days per week) for 28 weeks and were killed 12 weeks later. Forty male and 40 female mice received 1.0 g/L sodium nitrite in the drinking-water alone and served as nitrite controls and 80 male and 80 female mice were given tap-water and served as untreated controls. An increased incidence of lung adenomas was observed in male and female (combined) mice exposed to piperazine (48/75 versus 10/68), morpholine (20/35 versus 5/38) or *N*-methylaniline (23/38 versus 6/36) in the diet and sodium nitrite in the drinking-water compared with their respective controls. Sodium nitrite alone (14/74) or the secondary amines alone produced no increased incidence of lung adenomas compared with that in untreated controls (20/144). The authors concluded that the increased incidence of lung adenomas probably resulted from in-vivo nitrosation of secondary amines by nitrite (Greenblatt *et al.*, 1971).

In a study that was designed to evaluate the carcinogenicity of concurrent administration of amino acids (proline, hydroxyproline and arginine) and sodium nitrite, 30 male and 30 female Swiss mice, 5–7 weeks of age, received 1 g/L sodium nitrite in the drinking-water for 26 weeks and were killed at 38 weeks. Sixty untreated male and female controls were given tap-water. No increase in the incidence of lung adenomas was seen in nitrite-treated mice compared with untreated controls. No increase in the incidence of lung adenomas was seen in mice treated with a mixture of nitrite and proline, hydroxyproline or arginine in the drinking-water compared with their respective controls (Greenblatt & Lijinsky, 1972).

Swiss mice [number unspecified], 6–11 weeks of age, were given 1.0 g/L sodium nitrite in the drinking-water for 28 weeks while being fed methylurea or ethylurea. After an observation period of 12 weeks, they developed an increased incidence of lung adenomas (nitrite + methylurea, 105 adenomas, 16/26 mice [$P < 0.001$], 4.0 ± 4.4 adenomas/mouse [$P < 0.001$]; nitrite + ethylurea, 134 adenomas, 25/31 mice [$P < 0.001$], 4.3 ± 3.6 adenomas/mouse [$P < 0.001$]) compared with groups fed alkylureas alone. Two control groups were either given 1.0 g/L sodium nitrite in the drinking-water on 5 days per week for 28 weeks and were observed for another 12 weeks before they were killed or remained untreated. These were the same controls as those described in Greenblatt *et al.* (1971). No difference in the incidence of tumours was observed between the untreated and nitrite-treated groups (untreated, 26 adenomas, 20/144 mice, 0.2 ± 0.4 adenomas/mouse; nitrite-treated, 16 adenomas, 14/74 mice, 0.2 ± 0.5 adenomas/mouse) (Mirvish *et al.*, 1972).

Groups of 40 male strain A mice (locally bred), 7–9 weeks of age, were given 1.0 g/L sodium nitrite in the drinking-water on 5 days per week for 25 weeks and were killed 13 weeks later. In another study, groups of 40 male strain A mice were given 2.0 g/L sodium nitrite in the drinking-water on 5 days per week for 20 weeks and were killed 10 weeks later. Untreated controls were given tap-water. The incidence of lung adenomas in both groups of nitrite (1.0 or 2.0 g/L)-treated animals (11/37 or 7/39) was similar to that in the untreated controls (12/37 and 5/39). However, when the mice were exposed to piperazine at 6.25 g/kg in the diet and sodium nitrite in the drinking-water at levels of 0, 0.05, 0.25, 0.5, 1.0 or 2.0 g/L, the incidence of lung adenomas increased with increased intake of sodium nitrite in the drinking-water (11/39, 10/39, 20/40 ($P < 0.001$), 30/39 ($P < 0.001$), 37/37 ($P < 0.001$) and 39/40 ($P < 0.001$), respectively). Sodium nitrite may exert a dose-related carcinogenic effect in mice by nitrosating piperazine *in vivo* (Greenblatt & Mirvish, 1973).

In a study that was designed to evaluate the carcinogenicity of nitrilotriacetic acid, groups of 40 male and 40 female Swiss mice, 8 weeks of age, received 1 g/L sodium nitrite in the drinking-water on 5 days per week for 26 weeks or sodium nitrite in combination with 5 g/L nitrilotriacetic acid in the drinking-water and were killed at 37–38 weeks. Groups of 40 males and 40 females that were given tap-water served as untreated controls. No difference in weight, survival or tumour incidence was observed between the nitrite-treated, nitrite/nitrilotriacetic acid-treated and untreated control mice (Greenblatt & Lijinsky, 1974).

Groups of male and female Swiss mice were exposed transplacentally to sodium nitrite with or without the pesticide carbendazim. Pregnant mice were given 0 or 0.05% sodium nitrite in the drinking-water with or without intragastric intubation with five doses of 500 mg/kg bw carbendazim [frequency unspecified] throughout pregnancy. Offspring were killed at 252 days of age. A fourth group served as untreated controls. Of the offspring exposed to carbendazim and sodium nitrite, 7/10 (70%) developed lymphosarcomas. Only 1/256 (0.4%) untreated mice developed lymphosarcomas. No malignant lymphomas were found in the 82 sodium nitrite-exposed or 85 carbendazim-exposed offspring mice (Börzsönyi *et al.*, 1976).

Groups of pregnant Swiss mice, 4–6 weeks of age, received a single intragastric dose of 0 (control) or 10 mg/kg bw sodium nitrite in distilled water followed by 0 (control) or 0.1% sodium nitrite in the drinking-water on days 15–21 of pregnancy. The animals (parents and offspring) were killed 10 months after the beginning of the experiment. No increase in tumour frequency was observed in either parent animals (19 nitrite-treated, 18 control) or their offspring (62 male and 71 female nitrite-exposed, 70 male and 62 female control) (Börzsönyi *et al.*, 1978). [The Working Group noted the short duration of exposure.]

Groups of 50 male and 50 female ICR mice, 8 weeks of age, were exposed to 0.125, 0.25 or 0.5% sodium nitrite in the drinking-water for 109 weeks. A group of 20 males and 20 females served as untreated controls. No difference in tumour incidence, latency period or tumour type was observed between the nitrite-exposed and control mice (Inai *et al.*, 1979).

In a study that was designed to evaluate the carcinogenicity of cimetidine and nitrite combined, the effects of transplacental, transmammmary and oral exposure to nitrite for life was studied in (C57BL/6 × BALB/c)F₁ mice (also called B6CF₁ mice). Female C57BL/6 and male BALB/c parent mice were exposed to 0.184 or 1.84 g/L nitrite in the drinking-water (starting 2 weeks before conception) and their offspring were also given nitrite in the drinking-water for life after delivery. Controls received nitrite-free water. Neither C57BL/6 dams (15–20/group) treated before and during pregnancy and until death nor female B6CF₁ mice treated from conception with the low or high dose of sodium nitrite developed more neoplasms than untreated controls. However, male B6CF₁ mice exposed to sodium nitrite from conception through gestation, lactation and life at a dose of 0.184 g/L had an increased incidence ($P < 0.029$) of lymphomas (16/52 versus 7/52 untreated controls) and an increased incidence ($P < 0.017$) of lung tumours [benign and malignant combined] (41/52 versus 30/52 untreated controls). The dose of 1.84 g/L had no effect on tumour incidence, survival or body weight (Anderson *et al.*, 1985). [The Working Group noted the absence of an increase in tumour incidence at the high dose.]

Male and female CBA and male and female C57Bl mice were subdivided into groups of 25, 30 or 40 male or female animals and were treated daily for the duration of the experiment (96 weeks) with sodium nitrite in the drinking-water (total dose consumed, ~0.02, 0.2, 1.6 or 2.0 g/mouse) alone or in combination with morpholine (total dose, 230 mg/mouse) added to the feed twice a week. One group of 30 male and 30 female C57Bl mice received a total dose of 230 mg morpholine alone in the feed. Two groups of 60 male and 60 female CBA mice and 30 male and 30 female C57Bl mice served as untreated controls. All tumours were examined histologically. Compared with untreated controls, statistically significant increases in the incidence of benign liver tumours (15/40 versus 9/53) in male CBA mice treated with 1.6 g sodium nitrite alone, of haemoblastosis (11/32 versus 3/53) in male CBA mice treated with 0.2 g sodium nitrite and morpholine and of malignant liver tumours (16/34 versus 11/57) in female CBA mice treated with 0.02 g sodium nitrate plus morpholine were observed (Iurchenko *et al.*, 1986). [The Working Group noted the limited reporting of the study.]

VM mice are susceptible to the spontaneous development of cerebral gliomas. A group of 100 male and female VM mice (equal sex ratios for all groups) were exposed to sodium nitrite *in utero* while suckling (pregnant mice were given 0.2% sodium nitrite in the drinking-water throughout pregnancy and suckling) and throughout their adult lives (0.2% sodium nitrite in the drinking-water). Another group of 200 male and female VM mice was administered 0.2% sodium nitrite in the drinking-water from the time of weaning. A third group of 200 male and female mice received distilled water from weaning and served as controls. The mice were maintained until their natural death. No excess incidence of nervous system tumours occurred in the nitrite-exposed groups (0/100 and 2/200) compared with controls (1/200). Survival and body weights were similar among the groups (Hawkes *et al.*, 1992).

Groups of 50 male and 50 female B6C3F₁ mice, approximately 6 weeks of age, received drinking-water that contained 0, 750, 1500 or 3000 mg/L sodium nitrite (equal to average daily doses of 0, 60, 120 or 220 mg/kg bw for males and 0, 45, 90 or 160 mg/kg bw for females) for 2 years. The survival rate of the treated groups was similar to that of the controls. The mean body weights of females at the highest dose were lower than those of controls throughout the study, and the treated groups generally consumed less water than controls. In female mice, the incidence of squamous-cell papilloma and carcinoma (combined) of the forestomach (1/50, 0/50, 1/50 and 5/50) showed a positive trend ($P = 0.011$). The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in males at the highest dose than in controls (0/50, 0/50, 2/50, 10/50; $P \leq 0.01$, Poly-3 test) (National Toxicology Program, 2001).

(c) *Gavage studies*

A group of male (C57BL \times C3H)F₁ mice, 15 days of age, was given a single intragastric dose of 50 μ g/g bw sodium nitrite and animals were killed periodically up to 110 weeks. Controls were gavaged with distilled water (0.1 mL/g bw). Two groups that were given a dose of diethylamine hydrochloride (50 μ g/g bw) or a dose of diethylamine hydrochloride (50 μ g/g bw) followed by a dose of nitrite (50 μ g/g bw) were also included in the study. There were from 30 to 31 mice per group. The incidence of hepatic tumours was: control, 2/17 (all cancers); nitrite, 2/11 (one haemangioma and one adenoma); diethylamine hydrochloride, 5/15 (two cancers and three adenomas); and diethylamine hydrochloride/nitrite, 14/23 [four cancers and 10 adenomas; $P < 0.05$ versus the nitrite-treated group; $P < 0.01$ versus the control group]. The authors suggested that carcinogens were formed *in vivo* by interaction of the two compounds (Rijhsinghani *et al.*, 1982).

Groups of 30 male and 30 female ICR mice, 5 weeks of age, were administered 10 weekly doses of ethylthiourea and sodium nitrite by gavage with the following combinations of treatment (ethylthiourea versus sodium nitrite, mg/kg bw per week): 0 versus 0 (untreated controls), 100 versus 0 (ethylthiourea-treated group), 0 versus 70 (nitrite-treated group), 25 versus 17.5 (low-dose ethylthiourea/nitrite-treated group), 50 versus 35 (mid-dose ethylthiourea/nitrite-treated group) and 100 versus 70 (high-dose ethylthiourea/nitrite-treated group). Mice were allowed to live without further treatment

up to 18 months after the first administration. No increase in tumour incidence was observed in the nitrite-treated or ethylthiourea-treated groups compared with untreated controls. An increased incidence of malignant lymphoma in the high-dose ethylthiourea/nitrite-treated group compared with the ethylthiourea-treated group in both males (13/30 versus 3/30; $P < 0.05$) and females (19/30 versus 7/30; $P < 0.05$), of lung adenoma and adenocarcinoma combined in the mid- and high-dose ethylthiourea/nitrite-treated group compared with the ethylthiourea-treated group in both males (22/30 and 25/30 versus 9/30; $P < 0.05$) and females (16/30 and 21/30 versus 4/30; $P < 0.05$), of lung adenocarcinoma in the high-dose ethylthiourea/nitrite-treated group compared with the ethylthiourea-treated group in both males and females (5/30 versus 0/30; $P < 0.05$), of forestomach squamous-cell carcinoma and squamous-cell papilloma/carcinoma combined in the high-dose ethylthiourea/nitrite-treated group compared with the ethylthiourea-treated group in both males (5/30 and 12/30 versus 0/30; $P < 0.05$) and females (5/30 and 8/30 versus 0/30; $P < 0.05$) and of uterine adenocarcinomas in the high-dose ethylthiourea/nitrite-treated group compared with the ethylthiourea-treated group (6/30 versus 0/30; $P < 0.05$) were observed (Yoshida *et al.*, 1993). [The Working Group noted the use of low doses.]

(d) *Oral administration with known carcinogens*

The effect of sodium nitrite administered in the drinking-water to CC57Br and AKR/J mice on the development of leukaemia and thymic lymphoma was investigated. Groups of 2-day-old CC57Br mice were infected with the Mazurenko leukaemia virus and were given 0 (control), 50 or 500 mg/L sodium nitrite in the drinking-water beginning 1 month after infection until death. The incidence of leukaemia and mean lifespan of leukaemic mice were, respectively: 19/24 and 229±28 days, 29/34 ($P < 0.05$) and 183±14 days ($P < 0.05$), and 26/34 and 194±6 days ($P < 0.05$). Groups of 2-month-old AKR/J mice were infected with the Gross leukaemia virus and given 0 (control) or 2000 mg/L sodium nitrite in the drinking-water until death. The incidence of thymic lymphoma and mean lifespan of mice with lymphoma were, respectively: 30/40 and 312.2±24 days and 36/40 and 246±38 days ($P < 0.05$). The authors concluded that sodium nitrite enhances the carcinogenic effect of leukaemia viruses *in vivo* (Ilnitsky & Kolpakova, 1997).

3.2.2 *Rat*

(a) *Oral administration in the diet*

(i) *Long-term carcinogenicity studies*

Groups of 30 male and 30 female weanling Wistar rats were fed a diet (prepared freshly each week) containing 40% canned meat and 0, 0.02 or 0.5% sodium nitrite (0, 200 or 5000 mg/kg of diet), with or without 1% glucono- δ -lactone (to lower the pH), for 2 years. Full histopathology was performed. No differences were observed in the

incidence of tumours or preneoplastic changes between the sodium nitrite-treated and control rats (van Logten *et al.*, 1972).

Groups of F₀ pregnant Sprague-Dawley rats were fed diets containing 0 or 1000 mg/kg sodium nitrite with or without 1000 mg/kg morpholine from mating onwards. The diets were prepared freshly each week, and the concentration of sodium nitrite decreased gradually; the weekly average concentration was 263 mg/kg. Randomly selected F₁ and F₂ offspring were maintained on the same diets and the experiment was terminated at week 125 of life of the F₂ generation. Results were presented for the F₁ and F₂ generations combined. No difference in survival was observed among the groups. Rats ($n = 96$) fed sodium nitrite in the diet developed a higher incidence (61%) of total tumours compared with 156 untreated controls (18%). The incidence of lymphoreticular tumours was also higher in sodium nitrite-treated (27%) than in untreated groups (6%). Rats ($n = 159$) fed sodium nitrite and morpholine combined developed a higher incidence (97%) of liver-cell carcinoma compared with 104 morpholine-treated controls (3%) (Shank & Newberne, 1976).

Four groups of male Wistar rats, 5 weeks of age, were fed a pelleted diet (CE2, CLEA) containing sodium nitrite (0.16%) and methylguanidine (0.16%) (Group 1; 20 rats), the pelleted diet containing 0.16% sodium nitrite (Group 2; five rats), the diet containing 0.16% methylguanidine (Group 3; five rats) or the pelleted diet alone (Group 4; 10 rats). The diets [storage conditions not specified] were used within 5 months after their preparation, and the study lasted 480 days. *N*-Nitroso compounds in the diet were determined with a thermal energy analyser. After 1 month, the basal diet contained only 5 ppb total *N*-nitroso compounds and less than 4 ppb NDMA, while *N*-nitrosopyrrolidine (NPYR) was not detectable. In the Group 2 diet, the concentrations of *N*-nitroso compounds were 2.52 ppm and 3.60 ppm at 1 and 5 months after preparation of the diet, respectively, those of NDMA were 1.86 and 3.36 ppm, respectively, and those of NPYR were 130 ppb and 110 ppb, respectively. No tumours were found in Group 4 rats. One rat in Group 2 died at 11 months with no detectable tumour. Among the remaining rats in Group 2, the incidence of liver tumours was 3/4 ($P < 0.02$); more precisely, the incidence of liver haemangioma was 2/4 and that of bile duct adenoma was 3/4. The authors concluded that the data demonstrated that *N*-nitroso compounds were formed in the feed during storage and possibly in the rat stomach (Matsukura *et al.*, 1977). [The Working Group noted the very small number of animals.]

In a follow-up report, three groups of 24 male Wistar rats, 8 weeks of age, were fed a pelleted diet mixed with 0 (control), 800 or 1600 mg/kg sodium nitrite for 646 days. The diets were prepared every 3 months and were kept at -10°C . The incidence of tumours was 0/19 in the control group, 1/22 (one hepatocellular adenoma) in the low-dose group and 5/19 ($P < 0.05$; two hepatocellular adenomas, one haemangioendothelioma, one hepatocellular carcinoma and one haemangioendothelial sarcoma) in the high-dose group. However, the nitrite diets were found to contain NDMA (1.26–2.01 ppm in the low-dose and 4.08–4.74 ppm in the high-dose diets) and NPYR (0.04–0.06 ppm in the low-dose and 0.13–0.11 ppm in the high-dose diets). *N*-Nitroso compounds were not detectable in

the control diet. The authors concluded that these preformed nitrosamines were probably the principal cause of the liver tumours (Aoyagi *et al.*, 1980).

Groups of male and female Fischer 344 rats, 6 weeks of age, were given 0 (50 males and 55 females) or 0.5% (20 males and 20 females) sodium nitrite in the feed for 1 year and were observed for a further 120 days. The incidence of neoplasms observed in controls was: males, 30/50 (60%); and females, 20/44 (45%); that in nitrite-treated groups was: males, 8/16 (50%); and females, 6/16 (37%). Although sodium nitrite in the feed did not cause a higher tumour incidence in male or female Fischer 344 rats, when 0.5% sodium nitrite and 0.58% butylurea were mixed together in the feed and given to 50 males and 50 females, a higher incidence of tumours (mainly Zymbal gland carcinoma, lung adenoma and squamous-cell papilloma and carcinoma of the forestomach) occurred (43/46 males and 44/45 females) compared with 50 male and 50 female rats fed 0.58% butylurea only (7/16 males and 5/16 females) (Krishna Murthy *et al.*, 1979). [Despite the short duration of exposure, a high incidence of treatment-related tumours was observed. The effective number of animals represents the number of animals histologically evaluated rather than the number necropsied.]

Feeding BD rats by gastric intubation from day 13 to day 23 of pregnancy with L-citrulline (100 mg/kg bw) and sodium nitrite (50 mg/kg per day) induced malignant tumours of the kidney (diagnosed as Wilms tumour) in 6/22 of the offspring. Neither of the controls given either compound alone developed tumours (Ivankovic, 1979). [The Working Group noted the limited reporting of the study.]

Groups of 68 male and 68 female Sprague-Dawley rat offspring [age unspecified] were administered sodium nitrite in the diet for up to 26 months. Groups 1–5 received 0, 250, 500, 1000 or 2000 ppm (mg/kg) sodium nitrite in an agar gel casein diet; Group 8 received urethane in agar diet; Groups 9–11 were fed commercial chow that contained 0, 1000 or 2000 ppm nitrite; Group 12 received urethane in chow; Groups 13–14 were fed an agar gel casein diet in a dry form that contained 0 or 1000 ppm nitrite. For Groups 1–14, the diets had been given to dams beginning 5 days before birth. Groups 15 and 16 were the respective dams of Groups 1 and 4 rat offspring that received 0 and 1000 ppm nitrite in agar diet. Groups 17–18 were only exposed to 0 or 1000 ppm nitrite in agar diet beginning at weaning. The experiment was terminated at 26 months. The incidence of malignant lymphomas was increased in nitrite-treated Groups 2–5 compared with control rats (Group 1) fed agar gel diet and increases were also observed in Groups 10, 11, 14, 16 and 18 compared with their respective control (i.e. Groups 9, 13, 15 and 17). The incidence of lymphoma in all control groups (combined) was 5.4% and that in all the treated groups (combined) was 10.2%. The increase was significant (Newberne, 1979). The findings were not confirmed following a review of the histology by a governmental interagency working group submitted to the US Food and Drug Administration. That working group arrived at different diagnoses and reported a smaller number of malignant lymphomas. Some of the lesions diagnosed as lymphomas by Newberne (1979) were classified as extramedullary haemopoiesis, plasmocytosis or histiocytic sarcomas. It was concluded that the incidence of lymphomas and other types of tumour was similar to that

arising spontaneously in Sprague-Dawley rats and no evidence existed that an increased incidence of tumours was induced by ingestion of sodium nitrite (Walker, 1990).

A group of 20 male and 20 female Fischer 344 rats, 8–9 weeks of age, was fed a mixture of 0.1% disulfiram with 0.2% sodium nitrite in a powdered diet for 78 weeks. The diet was mixed at first every 2 weeks and then weekly and was stored at 4°C until placed in the feeders every 2–3 days. Analysis of the food showed the presence of traces of *N*-nitrosodiethylamine (NDEA) after 7 days of storage and no more than 200 µg/kg after 17 days. Control groups of 20 male and 20 female rats were fed 0.2% sodium nitrite. A control group of 50 males and 50 females fed disulfiram only from a previous bioassay was also used (National Toxicology Program, 1979). The animals were observed until death. It was estimated that male rats consumed 30 mg disulfiram and 60 mg sodium nitrite per day and females 20 mg disulfiram and 40 mg sodium nitrite per day. Benign and malignant tumours were observed in the oesophagus (males, 7/20; females, 11/20), tongue (males, 0/20; females, 2/20), forestomach (males, 3/20, females, 0/20) and nasal cavity (olfactory adenocarcinomas; males, 2/20; females, 2/20). None of these tumours were observed in rats fed either disulfiram (National Toxicology Program, 1979) or sodium nitrite alone at similar doses. The authors hypothesized that these tumours were due to the reaction of disulfiram and nitrite in the stomach, which resulted in the formation of NDEA (Lijinsky & Reuber, 1980).

In a series of two experiments, the second of which began 3 months after the first, groups of 24 male and 24 female Fischer 344 rats, 7–8 weeks of age, were fed 0 or 2000 mg/kg (2000 ppm) sodium nitrite in the diet (the mixture was prepared weekly and no evidence of instability of sodium nitrite was found) for 104 weeks and observed until experimental week 127–130. The incidence of hepatocellular neoplasms in the two female groups (combined) exposed to sodium nitrite was significantly higher than that in the untreated controls (27/48 versus 8/48; $P < 0.05$); that in treated and untreated males was similar (9/48 versus 8/48). A decrease in the incidence of monocytic leukaemia was observed in treated males (6/48 versus 24/48) and females (4/48 versus 18/48). No treatment-related increases in other tumour types were observed (Lijinsky *et al.*, 1983). [This study lacked data for life parameters including growth curve and daily food consumption as well as intake of sodium nitrite. Thus, the effect of nutritional condition on the reduction in the incidence of leukaemia could not be measured.]

In a study of the effects of amines with sodium nitrite, groups of 20–24 male and 20–24 female Fischer 344 rats, 7–8 weeks of age, were fed 0 or 2000 ppm sodium nitrite in the diet for 106 weeks with or without 0.1% chlorpheniramine maleate, 0.2% diphenhydramine hydrochloride or 0.2% allantoin and were observed until death. Based on food intake, the males were estimated to have consumed 42 g and females 28 g sodium nitrite. Survival was similar in all groups. An increase in the incidence (13/24 versus 4/24; $P = 0.015$) of hepatocellular adenoma and carcinoma (combined) was reported in females but not in males (3/24 versus 5/24) treated with nitrite compared with untreated controls; the incidence of hepatocellular carcinoma was 3/24 versus 0/24 female controls. In male rats given diphenhydramine or chlorpheniramine maleate concurrently with sodium

nitrite, there was a significant increase in the incidence of hepatocellular adenoma and carcinoma combined (diphenhydramine + nitrite, 11/24 versus 4/24, $P < 0.05$; chlorpheniramine maleate + nitrite, 14/24 versus 3/24, $P < 0.05$) compared with their respective control groups given the amine alone; the incidence of hepatocellular carcinoma was 2/24 and 8/24 ($P = 0.0039$) versus 0/24 male controls, respectively (Lijinsky, 1984). [This study lacked data on life parameters including body weight and feed/water consumption during the experiment.]

In a two-generation study, male and female Wistar rats [age unspecified] were fed a diet of cured meat that had been treated with 0, 200, 1000 or 4000 mg/kg potassium nitrite (expressed as sodium nitrite) and were allowed to breed 10 weeks later. The diets were canned and stored for 1 month at room temperature and then at 4°C before use. The presence of volatile *N*-nitroso compounds was demonstrated only in meat to which 4000 mg/kg nitrite had been added: up to 30 µg/kg NDMA were found during the experiment. Reproduction outcomes were similar among the F_0 groups. After weaning, the F_1 generation rats (140, 120, 120 and 132 males and females) were fed the same diets as the F_0 generation and were killed at experimental week 132. There was no significant difference in survival or body weight among the groups. In males, the total incidence (all organ sites examined) of malignant tumours was increased with a significantly positive trend using the Armitage-Cochran test (males: 9/70, 10/60, 10/60 and 16/66; females: 15/70, 12/60, 11/60 and 15/66) [no P values provided]. The authors hypothesized that the increase in tumour incidence may be attributed to preformed nitrosamines present in the diet (Olsen *et al.*, 1984).

Two groups of 50 male Fischer 344 rats, 5–6 weeks of age, were fed 0.2 or 0.5% (w/w) sodium nitrite mixed in a reduced-protein diet for up to 115 weeks; the diets were prepared weekly. A control group of 20 male rats received the reduced-protein diet only. Body weight gain was decreased ($P < 0.05$) [~25%; read from figure] in the 0.5% nitrite-treated group. A dose-related decrease was noted in both the time of onset and the incidence of lymphomas (15/20, 14/50 ($P < 0.001$) and 10/49 ($P < 0.001$)), mononuclear-cell leukaemia (9/18, 7/39 and ($P < 0.05$) and 3/40 ($P < 0.001$)) and testicular interstitial-cell tumours (16/20, 31/48 ($P < 0.05$) and 7/50 ($P < 0.001$)) (Grant & Butler, 1989). [The Working Group noted the reduction in body weight.]

Three groups of 13 Sprague-Dawley rats [sex unspecified], 6 weeks of age, were fed Purina rat chow that contained wheat (10%), squid (10%, dehydrated at 60°C and then crushed to powder) or squid (10%) plus sodium nitrite (0.3%) for 10 months [no information on the frequency of preparation of the mixture or storage provided]. Levels of nitrosamines in the diets were not measured. Squid contains high levels of naturally occurring amines, such as trimethylamine-*N*-oxide and dimethylamine. The squid- and nitrite-treated rats had lower body weights compared with wheat-treated controls. The incidence of hepatocellular carcinoma was 0/13 (0%) in rats fed the diet that contained 10% wheat, 2/12, (16%) in rats fed the diet that contained 10% squid and 4/12 (33%) in rats fed the diet that contained 10% squid and 0.3% sodium nitrite (Lin & Ho, 1992). [The Working Group noted the small number of animals used.]

(ii) *Combined administration of nitrite and nitrosatable compound*

Seven groups of F₀ female Sprague-Dawley rats were fed from conception and for life a diet (prepared freshly each week) that contained nitrite or morpholine: Group 1, diet with no addition; Group 2, 1000 ppm nitrite and 1000 ppm morpholine; Group 3, 1000 ppm nitrite and 50 ppm morpholine; Group 4, 1000 ppm nitrite and 5 ppm morpholine; and Group 5, 50 ppm nitrite and 1000 ppm morpholine. After weaning, the F₁ generation rats were fed the same diet for life. The results for F₀ females and F₁ males and females were combined. Sodium nitrite at 1000 ppm combined with 50 or 1000 ppm morpholine (Groups 2 and 3) induced a dose-related increase in the incidence of hepatocellular carcinoma (24/122 and 156/159 versus 0/160), liver angiosarcoma (11/122 and 38/159 versus 0/160) and lung angiosarcoma (2/122 and 37/159 versus 0/169) compared with controls (Group 1). The incidence of metastatic hepatocellular carcinoma (10/122 and 109/159 versus 0/169) to the lung was also increased. The authors concluded that the data provided evidence of in-vivo nitrosation, presumably under acidic conditions in the rat stomach (Newberne & Shank, 1973). [The Working Group noted the absence of a group treated with morpholine only].

(b) *Oral administration in the drinking-water*

(i) *Long-term carcinogenicity studies*

Lijinsky *et al.* (1973a) reported negative results in a 2-year study when groups of 15 male and 15 female MRC rats, 8–10 weeks of age, were given 0 (untreated control) or 2000 ppm sodium nitrite in the drinking-water at a daily rate of 20 mL/rat with or without nitrilotriacetic acid for 84 weeks.

Groups of male and female MRC Wistar rats, 8 weeks of age, received 0 (control) or 3 g/L sodium nitrite in the drinking-water on 5 days per week for life (total dose, 63 g/kg). The incidence of forestomach squamous papillomas was significantly greater ($P < 0.002$ by the life-table method) in sodium nitrite-treated animals (18%; 5/22 males and 3/23 female) than in control animals (2%; 2/47 males and 0/44 female), which suggested that sodium nitrite was tumorigenic in this experiment (Mirvish *et al.*, 1980).

Three groups of 68 male and 68 female Sprague-Dawley rat offspring [age unspecified] were administered 0, 1000 or 2000 ppm sodium nitrite in the drinking-water for up to 26 months. Dams had been given 0, 1000 or 2000 ppm sodium nitrite in a semi-synthetic diet beginning 5 days before birth. The incidence of lymphomas was 5/136 (3.7%), 16/136 (11.8%), and 14/136 animals (10.3%) in the 0-, 1000- and 2000-ppm nitrite-treated groups, respectively. The incidence of other types of tumours was not increased (Newberne, 1979). A US governmental interagency working group, however, came to different conclusions following a review of the histology. That working group diagnosed only a small number of lesions as lymphomas and assessed an incidence of approximately 1% in both treated and control groups. This discrepancy concerned the differentiation between the lymphomas diagnosed by Newberne (1979) and the

extramedullary haemopoiesis, plasmacytosis or histiocytic sarcomas diagnosed by the interagency working group (Walker, 1990).

Groups of 50 male and 50 female Fischer 344 rats, 8 weeks of age, received 0, 0.125 or 0.25% sodium nitrite in the drinking-water for 2 years. Thereafter, tap-water and basal diet were given to all experimental groups and observation was continued until week 120. No carcinogenic effects were observed. The survival rate of the treated males was higher ($P < 0.05$) than that of controls. In females treated with 0.25%, mean body weight was decreased by more than 10% during the experiment relative to controls [statistics not provided]. There was a significant decrease in overall tumour incidence in high-dose females compared with controls: 73% (35/48) versus 92% (45/49; $P < 0.05$ by the χ^2 test). This reduced tumour incidence was due in part to a reduction in the incidence of mononuclear-cell leukaemias in treated animals and a high incidence in controls. [This neoplasm has a rather high spontaneous incidence in this rat strain.] Haematopoietic tumour incidence was 27% (13/49) in untreated females versus 8% (8/48) in 0.25% sodium nitrite-treated females ($P < 0.05$ by the χ^2 test). Treated males had a reduced incidence of haematopoietic tumours (most of which were mononuclear-cell leukaemias): 35% (16/46) in untreated males versus 10% (5/50) in 0.25% sodium nitrite-treated males and 10% (5/49) in the 0.125% sodium nitrite-treated males ($P < 0.05$ by the χ^2 test) (Maekawa *et al.*, 1982).

Groups of 24 male and 24 female Fischer 344 rats, 7–8 weeks of age, received 0 or 2000 ppm sodium nitrite in the drinking-water for 2 years. Survival of treated animals was slightly increased (average of time of death: 107 weeks for untreated males and 122 weeks for untreated females; 116 weeks for treated males and 130 weeks for treated females). The incidence of mononuclear-cell leukaemia was: control males, 12/24; control females, 11/24; treated males, 5/24; and treated females, 1/24 ($P = 0.0018$ versus untreated females by two-tailed Fischer test). No treatment-related differences were observed in other tumour types, while a slight, but a non-significant increase ($P = 0.060$) in the incidence of liver neoplasms (carcinomas and neoplastic nodules) was observed in females (control females, 0/24 carcinoma and 4/24 neoplastic nodules; treated females, 1/24 carcinoma and 10/24 neoplastic nodules) (Lijinsky *et al.*, 1983; Lijinsky, 1984). [This study lacked data on life parameters including growth curve, daily food consumption and intake of sodium nitrite. Thus, the effect of nutritional condition on the reduction in the incidence of leukaemia could not be measured.]

Groups of 50 male and 50 female Fischer 344/N rats, approximately 6 weeks of age, were exposed to 0, 750, 1500 or 3000 ppm sodium nitrite (equivalent to average daily doses of approximately 0, 35, 70, or 130 mg/kg bw for males and 0, 40, 80 or 150 mg/kg bw for females) in the drinking-water for 105 weeks. The survival rate of the treated groups was similar to that of controls. The mean body weights and water consumption of males and females exposed to 3000 ppm were less than those of controls throughout the study. The incidence of hyperplasia of the forestomach epithelium in both sexes at 3000 ppm was significantly greater than that in the control groups (males, 12/50 at 0 ppm versus 44/50 at 3000 ppm; females, 8/50 at 0 ppm versus 40/50 at 3000 ppm; $P < 0.01$ by

the Poly-3 test). In females, the incidence of fibroadenoma (multiple and single combined) of the mammary gland was increased only at 1500 ppm (31/50 versus 21/50; $P=0.029$) and exceeded the historical range for National Toxicology Program (NTP) controls given NTP-2000 diet (all routes) (108/299; $36.1\pm 6.2\%$). The incidences in the 750-ppm (54%) and 3000-ppm (50%) groups also exceeded the range for the NTP controls and the incidence in the control group (42%) equaled the highest incidence in the NTP-2000 diet historical control database. The incidence of multiple fibroadenoma was also increased at both 750 and 1500 ppm; however, these neoplasms occur with a high background incidence, and no increase was seen at 3000 ppm (multiple fibroadenomas: 7/50, 13/50, 13/50 and 5/50 at 0, 750, 1500 and 3000 ppm, respectively). The incidence of mononuclear-cell leukaemia was significantly decreased in both sexes at 1500 ($P\leq 0.01$) and 3000 ppm ($P\leq 0.001$) (incidence in males: 17/50, 12/50, 7/50 and 3/50 at 0, 750, 1500 and 3000 ppm, respectively; incidence in females: 15/50, 10/50, 1/50 and 1/50 at 0, 750, 1500 and 3000 ppm, respectively) and were less than the historical ranges for NTP controls (all routes) given NTP-2000 diet (130/299; $43.5\pm 9.6\%$) (National Toxicology Program, 2001).

(ii) *Combined administration of nitrite and nitrosatable compounds*

In a study of electron spin resonance signal in the liver of rats treated with hepatocarcinogens, tumour induction was examined in male Holtzman rats [age unspecified, but it was stated that rats weighed about 140 g and were maintained on standard diet for 1–3 weeks before the experiment began] that were fed synthetic or chow diet with or without 0.067% 2-acetylaminofluorene (AAF) in the presence or absence of nitrite (40 mg/L nitrite-N) in the drinking-water for up to 8 months. A reduction in liver tumours was found in the group treated with nitrite and AAF–synthetic diet (24/38) compared with the group treated with AAF and maintained on distilled water (25/26). The authors concluded that nitrite inhibits the hepatocarcinogenicity of AAF (Commoner *et al.*, 1970) [statistical analysis was not performed].

Induction of malignant tumours was examined in rats following administration of secondary or tertiary amines together with sodium nitrite in the drinking-water. Groups of 15 male and 15 female Sprague-Dawley rats [age unspecified] were given drinking-water on 5 days a week that contained 2000 ppm heptamethyleneimine hydrochloride + 2000 ppm sodium nitrite for 28 weeks, 1000 ppm aminopyrine + 1000 ppm sodium nitrite for 30 weeks or 250 ppm aminopyrine + 250 ppm sodium nitrite for 50 weeks, and were observed until death. Control groups of 15 male and 15 female rats were each given 2000 ppm heptamethyleneimine hydrochloride, 1000 ppm aminopyrine or 2000 ppm sodium nitrite. Treatment with aminopyrine + nitrite (1000 ppm) resulted in the death of all animals within 1 year and induced haemangiosarcomas of the liver in most cases (29/30). Of the 30 animals that received the lower dose of aminopyrine + nitrite, 14 died within the first year, 12 of which had liver haemangiosarcomas and two had other types of tumour. In the group treated with heptamethyleneimine hydrochloride + sodium nitrite, 23/30 animals died in the first year of treatment, and 20 had developed primary tumours

at one or more site. The most prevalent tumours were oesophageal papillomas (17 animals) and squamous-cell carcinoma of the lung (13 cases). Of the control animals, only one died with a large mammary tumour. The authors suggested that the interaction of secondary and tertiary amines with nitrite in the stomach may represent an important facet in the etiology of human cancer by generation of nitrosoamines in the stomach (Lijinsky *et al.*, 1973b).

Groups of 15 male and 15 female Sprague-Dawley rats, 8–10 weeks of age, were given approximately 20 mL of a drinking-water solution that contained either 0.2% heptamethyleneimine hydrochloride or this salt together with 0.2% sodium nitrite on 5 days a week for 28 weeks. Another group of 27 male and 30 female rats was given 0.2% sodium nitrite solution alone for 104 weeks. Most of the animals given heptamethyleneimine hydrochloride or sodium nitrite alone survived 2 years or more after the beginning of the treatment, and no tumours that could be attributed to the treatment were seen at death; tumours were those of endocrine origin that are found commonly in untreated controls. In the group that received the combined treatment, most females had died by 50 weeks and most males had died by 80 weeks, 27/30 of which had tumours that were not seen in either control group: a total of 16 had squamous-cell carcinomas of the lung; 25 had tumours (mostly papillomas) of the oropharynx, tongue, oesophagus and forestomach; eight had squamous-cell carcinomas of the nasal cavity; and four had metaplasias or papillomas of the larynx and trachea (Taylor & Lijinsky, 1975a). [Although tumour induction was evident following combined administration of heptamethyleneimine and sodium nitrite, statistical analysis for a comparison of the tumour incidence was not provided.]

Groups of 15 male and 15 female Sprague-Dawley rats, 8 weeks of age, were given combinations of 0.1% or 0.025% aminopyrine or 0.1% oxytetracycline with or without 0.1% or 0.025% sodium nitrite in the drinking-water. The experiment was terminated in experimental week 104 (aminopyrine-treated groups) or 130 (oxytetracycline-treated groups). Of 30 animals that received 0.1% aminopyrine and 0.1% sodium nitrite for 30 weeks, 29 developed haemangiosarcomas of the liver. The same tumour developed in 26/30 animals that received 0.025% aminopyrine and 0.025% sodium nitrite for 50 weeks. No animals in the control group that received 0.1% aminopyrine for 30 weeks developed this tumour. After treatment with 0.1% oxytetracycline and 0.1% sodium nitrite for 60 weeks, liver tumours were present in 4/30 rats (three hepatocellular tumours [tumour type not further specified] and one cholangioma). No liver tumours were observed in the controls treated with oxytetracycline for 60 weeks (Taylor & Lijinsky, 1975b).

The possible formation of *N*-nitroso compounds from ingested secondary or tertiary amines and nitrite was tested with 13 amino compounds that were administered to groups of 15 male and 15 female Sprague-Dawley rats, 8–10 weeks of age, on 5 days per week in the drinking-water as follows: 0.1% arginine with 0.2% sodium nitrite, 0.2% chlordiazepoxide with 0.2% sodium nitrite, 0.2% chlorpromazine alone or 0.1% chlorpromazine with 0.2% sodium nitrite, 0.1% cyclizine with 0.2% sodium nitrite, 0.18%

dimethyldodecylamine with 0.2% sodium nitrite, 0.1% dimethylphenylurea with or without 0.2% sodium nitrite, 0.1% hexamethylenetetramine with or without 0.2% sodium nitrite, 0.14% lucanthone with or without 0.2% sodium nitrite, 0.1% methapyrilene with 0.2% sodium nitrite, 0.1% methylguanidine with 0.1% sodium nitrite, 0.09% piperidine with or without 0.2% sodium nitrite, 0.1% tolazamide with or without 0.2% sodium nitrite or 0.08% trimethylamine oxide with or without 0.2% sodium nitrite. Duration of treatment was 50 weeks except for cyclizine and dimethyldodecylamine (80 weeks) and methapyrilene (90 weeks). After the end of treatment, the animals were kept until they died spontaneously or until they became moribund and were killed. Survival rates differed little between the various groups and only a few of the amines, either alone or in combination with nitrite, induced a significant incidence of malignant tumours. Chlordiazepoxide plus nitrite induced nervous system tumours in 4/30 effective animals (one spinal cord tumour in 15 effective females and one brain glioma, one malignant neurinoma of spinal nerves and one heart neurofibrosarcoma in 15 effective males); methapyrilene plus nitrite induced liver tumours in 9/29 effective animals (four cholangiocarcinomas, one hepatocellular carcinoma and one liver haemangiosarcoma in 14 females and one cholangiocarcinoma and two hepatocellular carcinomas in 15 males); and dimethyldodecylamine plus nitrite induced tumours of the urinary bladder in 3/24 effective animals (one papilloma in nine females and one transitional-cell carcinoma and one leiomyosarcoma in 15 males). Sodium nitrite-treated controls [described in Taylor & Lijinsky (1975a)] developed three thyroid adenomas, one thyroid adenocarcinoma, one multiple myeloma, one pancreatic islet-cell adenoma, one thymic lymphoma and one squamous-cell carcinoma of the ear in 30 females and two thyroid adenomas and two adenocarcinomas, one brain ependymoma, two pancreatic islet-cell adenomas and two carcinomas, one hepatocellular carcinoma, one parotid-gland squamous-cell carcinoma, one forestomach carcinoma and two gut adenocarcinomas in 26 males. The authors concluded that these results provide further evidence that ingestion of secondary and tertiary amines together with nitrite can lead to the formation of significant amounts of carcinogenic *N*-nitroso compounds in the stomach (Lijinsky & Taylor, 1977). [Statistical analysis was not provided and the control group of a previously published study in which a 0.2% sodium nitrite solution was given for 2 years to 26 male and 30 female rats was used as the concurrent control (Taylor & Lijinsky, 1975a).]

Two groups of 24 male and 24 female Fischer 344 rats, 7–8 weeks of age, were given drinking-water that contained 0.1% *N,N*-dimethyldodecylamine-*N*-oxide (DDAO) with or without 0.2% sodium nitrite on 5 days per week for 93 weeks. Two other groups of 24 male and 24 female control rats were given untreated drinking-water or 0.2% sodium nitrite in the drinking-water. At the end of the treatment period, the rats were observed until death. Any survivors were killed between week 127 and week 130 of the experiment. There was no marked life-shortening effect in any treatment group. Administration of DDAO alone did not induce an increased incidence of any tumour in comparison with the untreated control group. In male rats given DDAO concurrently with nitrite, a significant increase in the incidence of liver neoplasms (hepatocellular

carcinomas and neoplastic nodules, 10/24 animals versus 3/24 untreated controls; $P = 0.049$ by Fisher's exact probability test) was observed. The author suggested that the ingestion of DDAO under conditions where it could be nitrosated with nitrite in the stomach might present an increased carcinogenic risk (Lijinsky, 1984). [This study lacked data on life parameters including body weight and feed/water consumption during the experiment].

The carcinogenic activity of endogenously synthesized *N*-nitroso-bis(2-hydroxypropyl)amine (NDHPA) was investigated in rats administered 1% bis(2-hydroxypropyl)amine (DHPA) mixed into a powdered diet and 0.15% or 0.3% sodium nitrite dissolved in distilled water for 94 weeks (Yamamoto *et al.*, 1989). Groups of 20–28 male Wistar rats, 6 weeks of age, were divided into untreated controls (group 1) and groups treated with 0.15% sodium nitrite (group 2), 0.3% sodium nitrite (group 3), 1% DHPA (group 4), 1% DHPA + 0.15% sodium nitrite (group 5) or 1% DHPA + 0.3% sodium nitrite (group 6). The experiment was terminated at experimental week 94. Three to five animals from groups 1, 3, 4 and 6 were used to investigate urinary excretion of endogenously synthesized NDHPA at experimental weeks 24, 34 and 80. As a result, endogenous synthesis of NDHPA was clearly observed in rats given 1% DHPA and 0.3% sodium nitrite at any time-point (0.97–1.5 $\mu\text{mol}/\text{rat}$ per dose), but not in the groups that received either of these precursors alone. Tumours of the nasal cavity, lung, oesophagus, liver (hepatocellular carcinoma) and urinary bladder (carcinoma) were found in rats treated with 1% DHPA and 0.15% or 0.3% sodium nitrite. In rats given 1% DHPA and 0.3% sodium nitrite, the incidence of tumours of the nasal cavity (papilloma and carcinoma, combined), lung (benign and malignant, combined) and oesophagus (papilloma) was 74% (14/19, $P < 0.01$ versus groups 1–5), 58% (11/19, $P < 0.01$ versus groups 1–4 and $P < 0.05$ versus group 5) and 11% (2/19), respectively. The tumour distribution was almost the same as that seen in rats given NDHPA (Konishi *et al.*, 1976; Mohr *et al.*, 1977; Pour *et al.*, 1979), indicating that endogenously synthesized NDHPA has similar carcinogenic activity to exogenously administered NDHPA in rats (Yamamoto *et al.*, 1989; Konishi *et al.*, 1990).

Induction of cancers and precancerous lesions of the oesophagus was examined in rats following the administration of precursors of *N*-nitrososarcosine ethyl ester. A total of 90 non-inbred male Wistar rats, 6 weeks of age, were given 2 g/kg bw sarcosine ethyl ester hydrochloride and concurrently 0.3 g/kg bw sodium nitrite, which are precursors of *N*-nitrososarcosine ethyl ester, in 2% sucrose as drinking-water. Group 1 (28 rats) received the precursors twice a week for 6 weeks followed by 8 weeks of observation and group 2 (34 rats) received the precursors once every 3 days for 7 weeks followed by 26 weeks of observation. Group 3 (28 rats) served as untreated controls. At the end of the treatment period, no tumour had developed in the oesophagus of three rats in group 1 that were killed. On subsequent observation, papillomas appeared in 1/3 rats in group 1 that were killed and carcinomas in 1/3 rats in group 2 that were killed within 4 weeks. The tumours induced in group 1 at the end of the experiment were mostly papillomas (all tumours, 12/20) and rarely carcinomas (3/20). When the observation was prolonged in

group 2, 100% (10/10) of the animals had carcinomas at observation week 20 (Xiang *et al.*, 1995). [No tumour incidences were reported for the untreated group.]

The effects of oral administration of ethylenethiourea (ETU) and sodium nitrite on the development of endometrial adenocarcinoma was investigated in female Donryu rats. A total of 119 females were divided into four groups. At 10 weeks of age, groups 1 and 3 received a single dose of polyethylene glycol (333 $\mu\text{L}/\text{kg}$ bw) into the uterine cavity, while groups 2 and 4 were given *N*-ethyl-*N*-nitrosourea (ENU; 15 mg/kg bw), dissolved in polyethylene glycols in the same manner. ETU (80 mg/kg bw) and sodium nitrite (56 mg/kg bw) dissolved in distilled water were given orally to animals in groups 3 and 4 once a week by gavage from 11 to 51 weeks of age. Groups 1 and 2 received the vehicle alone. At termination (52 weeks of age), endometrial adenocarcinomas were observed in 29 (6/21), 13 (4/31) and 57% (21/37) of rats in groups 2, 3 and 4, respectively, but not in group 1 (0/21) (group 2 versus group 1, $P < 0.01$; group 4 versus group 1, $P < 0.001$; group 4 versus group 2, $P < 0.05$; and group 4 versus group 3, $P < 0.001$). Persistent estrus appeared earlier in groups 3 and 4 than in group 1. In groups 3 and 4, forestomach squamous-cell hyperplasias (16/31 and 12/37, respectively) and papillomas (14/31 and 19/37, respectively) were also observed, but with no difference between the two groups. All of the incidences were statistically increased ($P < 0.01$) versus group 1. The authors concluded that the results indicate that *N*-nitroso ETU formed in the stomach by concurrent oral administration of ETU and sodium nitrite induces endometrial adenocarcinomas by its mutagenic action, as well as promoting their development after initiation with ENU, presumably by influencing the hormonal balance as shown by the early appearance of persistent estrus (Nishiyama *et al.*, 1998). [Methods of statistical analysis were not shown.]

Six groups of 50 male and 50 female Fischer 344 rats, 6 weeks of age, were fed diets (basal diet) supplemented with 64%, 32% or 8% fish meal and were simultaneously given 0.12% sodium nitrite in the drinking-water (males, groups 1–3; and females, groups 7–9) or tap-water (males, groups 4–6; and females, groups 10–12). At experimental week 104, all surviving animals were killed and examined histopathologically. Treatment with fish meal dose-dependently increased the incidences and multiplicities of atypical tubules [tubular hyperplasia], renal-cell adenomas and renal-cell carcinomas in sodium nitrite-treated males. The incidence and multiplicity of each renal proliferative lesion in males were: atypical tubules: 73.4% ($P < 0.05$) and 1.95 ± 1.82 ($P < 0.01$) in group 1, 42.2% ($P < 0.05$) and 0.61 ± 0.84 ($P < 0.01$) in group 2 and 4.0% and 0.04 ± 0.19 in group 3; adenoma: 67.3% ($P < 0.05$) and 1.40 ± 1.33 in group 1, 25.5% ($P < 0.05$) and 0.38 ± 0.73 in group 2 and 2.0% and 0.02 ± 0.14 in group 3; and carcinoma: 57.1% ($P < 0.05$) and 1.61 ± 1.98 ($P < 0.01$) in group 1 and 14.8% ($P < 0.05$) and 0.19 ± 0.58 in group 2. Females were less susceptible than males to the induction of renal tumours: atypical tubules: 45.8% ($P < 0.05$) and 0.92 ± 1.30 ($P < 0.01$) in group 7, 11.6% and 0.12 ± 0.33 in group 8 and 6.3% and 0.07 ± 0.26 in group 9; adenoma: 16.6% ($P < 0.05$) and 0.23 ± 0.56 ($P < 0.01$) in group 7, 2.3% and 0.03 ± 0.16 in group 8 and 2.1% and 0.02 ± 0.14 in group 9; and carcinoma: 2.0% and 0.02 ± 0.14 in group 7 and 0% and 0 in groups 8 and 9 (statistical

analysis by Fisher's exact probability test and one-way analysis of variance; comparison was made with the respective groups 4, 5, 6, 10, 11 or 12). The authors concluded that these results clearly indicate that concurrent administration of fish meal and sodium nitrite induces renal epithelial tumours (Furukawa *et al.*, 2000).

(c) *Gastric intubation*

Seven pregnant BD IX rats were treated on days 13–23 after conception with 100 mg/kg ethylurea and 50 mg/kg sodium nitrite daily by gavage. Ten of 13 newborn F₁ rats that died 77–211 days after delivery developed nine tumours of the nervous system (malignant neurinomas, mixed gliomas, medulloblastoma) and one myeloid leukaemia. Ethylurea only- and nitrite only-treated controls were available. No tumour development was observed in the treated mothers or in the ethylurea only- or nitrite only-exposed F₁ rats (Ivankovic & Preussmann, 1970). [This short communication does not provide any information on the number of animals in the control groups.]

A total 205 male and 195 female Wistar rats, 12 weeks of age, were divided into four groups and served as untreated controls (55 males and 45 females) or were treated by gastric intubation with aminopyrine alone (50 males and 50 females; 12.1 µmol; 2.8 mg/0.5 mL), with sodium nitrite alone (50 males and 50 females; 100 µmol; 6.9 mg/0.5 mL) or with aminopyrine plus sodium nitrite (50 males and 50 females; doses identical to those of individual compounds) three times a week for 29 months. The males and females treated with aminopyrine plus nitrite had an increased incidence of various types of liver tumours (37/45 males and 27/42 females; $P < 0.05$ versus other groups by Fisher's exact probability test; untreated controls, 9/48 males and 3/41 females; aminopyrine alone, 3/44 males and 5/46 females; sodium nitrite alone, 13/44 males and 3/44 females). Unlike the other groups, more than half of the tumours in the aminopyrine plus nitrite-treated group were malignant (mainly hepatocellular carcinomas and cholangiocarcinomas). The incidence of tumours other than that of the liver did not differ between groups (Scheunig *et al.*, 1979).

Male Wistar rats, 50 days of age, were divided into two groups; treated animals ($n = 15$) were given extracts from homogenates of the mackerel pike (*Sanma hiraki* in Japanese) that had been incubated with sodium nitrite at pH 3.0 by stomach tube three times a week for 6 months. Control animals ($n = 10$) were given extracts from fish homogenates that had not been incubated with nitrite. The weekly amount of extract given was calculated to have mutagenic activity by the Ames test equal to that of a weekly oral dose of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), which is active in inducing adenocarcinomas of the glandular stomach in rats. The animals were held for an additional observation period of 18 months. Twelve to 18 months after administration, 8/12 treated animals developed tumours (two adenomas, two adenocarcinomas and one adenosquamous carcinoma of the glandular stomach, two squamous-cell carcinomas of the forestomach, two adenomas and one adenocarcinoma of the pancreas and one adenocarcinoma of the small intestine). Furthermore, preneoplastic lesions (including intestinal metaplasia and glandular hyperplasia of the glandular stomach as well as

squamous-cell hyperplasia of the forestomach) were noted in virtually all of the treated animals. No tumours were seen in eight control rats given the untreated fish extract. The authors concluded that extract of nitrite-treated fish was demonstrated to induce cancers of the glandular stomach in rats [although statistical analysis was not performed] (Weisburger *et al.*, 1980).

Transplacental carcinogenesis and chemical determination of 1-butyl-1-nitrosourea (BNU) in stomach content was assessed after simultaneous oral administration of 1-butylurea and sodium nitrite (Maekawa *et al.*, 1977). Solutions of either 100 mg/kg bw 1-butylurea and 50 mg/kg bw sodium nitrite (seven dams) or 1-butylurea alone (five dams) were administered daily by stomach tube to pregnant ACI/N rats (> 12 weeks of age at mating) from day 13 to day 21 of gestation. Neurogenic tumours were induced in their offspring. In the group treated with 1-butylurea plus sodium nitrite, the incidence of tumours and mean survival time of rats with nervous system tumours were 64% (23/36 male and female pups) and 309 (189–672) days, respectively. Thirty-nine neurogenic tumours developed in these 23 rats. Fourteen tumours of the peripheral nerves were all neurinomas. Among 25 tumours found in the central nervous system, gliomas were the most frequent (20/25). Localization and histological findings of the nervous system tumours were similar to those observed previously in rats whose dams had received BNU during pregnancy (Maekawa & Odashima, 1975). Neurogenic tumours did not develop in the offspring (0/23 male and female pups) of the dams that received 1-butylurea alone. In-vivo formation of BNU in the stomach content after intubation of 1-butylurea and sodium nitrite was determined. Four groups of 3–4 female ACI/N rats served as untreated controls (distilled water) or were treated with sodium nitrite alone, 1-butylurea alone or 1-butylurea plus sodium nitrite. In the group treated with 1-butylurea plus sodium nitrite, BNU was detected in the stomach content at levels of 25 ppm at 30 min and 23 ppm at 60 min after administration. The concentration of BNU in the stomach content corresponded to 48.3 and 29.2 µg/rat, respectively. BNU was not detected in the groups of untreated controls, or those treated with sodium nitrite alone or 1-butylurea alone (Maekawa *et al.*, 1977). [Statistical analysis was not performed for comparison of the data between groups.]

(d) *Studies of interactions with carcinogens or antioxidants*

In a lifetime study in which the effect of sodium ascorbate on tumour induction was examined in rats treated with morpholine and sodium nitrite or with *N*-nitrosomorpholine, a group of 40 male MRC Wistar rats, 8 weeks of age, was fed morpholine (10 g/kg of diet) on 5 days a week for 2 years and received 3 g/L sodium nitrite in the drinking-water (group 1). Another group of 40 rats was fed sodium ascorbate (22.7 g/kg of diet) in addition to this treatment (group 2). An untreated control group of 50 rats was also available. When ascorbate was ingested, the liver tumours induced by morpholine and nitrite showed a longer induction period (54 weeks in group 1 versus 93 weeks in group 2; $P < 0.001$), a slightly lower incidence (24/37 in group 1 versus 19/39 in group 2; statistically non-significant) and an absence of liver tumour metastases in the lungs

[statistical difference between groups 2 and 1 not provided], indicating, according to the authors, that ascorbate had inhibited the in-vivo formation of *N*-nitrosomorpholine. The incidence of forestomach tumours in group 2 was 21/39, including 7/39 squamous-cell carcinomas, whereas no forestomach tumours were found in group 1. The authors hypothesized that ascorbate may promote the action of *N*-nitrosomorpholine in this organ (Mirvish *et al.*, 1976). [The Working Group noted that statistical methods used in this study were unclear and the absence of sodium nitrite- or morpholine-treated control groups.]

Three groups of 40 male Sprague-Dawley rats, 4 weeks of age, were fed powdered basal diet and administered 0.1% aminopyrine and 0.1% sodium nitrite in the drinking-water (group A) on 5 days a week for 40 weeks, when the experiment was terminated. Animals in group B were similarly treated but the diet was supplemented with 0.7% ascorbic acid. Rats in group C were fed basal diet and tap-water *ad libitum*. With respect to survival, 12 animals died or were killed when moribund in group A, one animal accidentally died in group B and all group C animals survived in good condition. The tumour incidence was 31/37 and 14/39 in the groups A and B, respectively ($P < 0.001$ between the two groups) [method of statistical analysis not provided]. In group A, tumours were detected in the lungs, liver and kidneys of 25, 12 and 10 animals, respectively. In group B, tumours were found in the lung and kidneys of 13 and seven animals, respectively. Tumours were mainly lung adenocarcinomas, hepatocellular carcinomas and renal adenomas. These results suggested that ascorbic acid protected rats against the induction of liver tumours by aminopyrine and sodium nitrite, but that protection against the induction of lung and kidney tumours was not complete. The authors considered that the mechanism was in part due to the blockage of in-vivo nitrosation (Chan & Fong, 1977).

Groups of 40 male MRC-Wistar rats, 8 weeks of age, were fed 10 g/kg of diet morpholine and received 2 g/L sodium nitrite in the drinking-water for life without (group 1) or with (group 2) the addition of 22.7 g/kg of diet sodium ascorbate. Group 3 was untreated. No liver tumours were observed in rats in group 3. Rats in group 2 had a lower incidence of liver tumours (15/37) with a longer latency than group 1, with a survival period of 108 ± 12 weeks in group 2 versus an incidence of 37/39 ($P < 0.05$ by the life-table method reviewed by Peto) and 55 ± 12 weeks of survival in group 1, which made the authors consider that ascorbate inhibited the in-vivo formation of *N*-nitrosomorpholine (NMOR). Liver tumours were mainly hepatocellular carcinomas. In contrast, the incidence of forestomach papillomas was 1/30 in group 1, 14/37 in group 2 and 3/39 in group 3. The difference between groups 1 and 2 was not significant due to the shorter life-span of rats in group 1. Rats in group 1 and group 2 had a higher incidence of forestomach hyperplasia than those in group 3 ($P < 0.01$ for group 1 and $P < 0.05$ for group 2 versus group 3). The authors concluded that ascorbate may have enhanced induction of these lesions because of an action synergistic with that of NMOR. However, they found that it was more probable that the reduced dose of NMOR and concomitantly

increased survival produced by the ascorbate were solely responsible for the increased incidence of forestomach papillomas and other lesions in group 2 (Mirvish *et al.*, 1983).

Thioprolin, which is readily nitrosated to form nitrosothioprolin, is expected to act as a nitrite scavenger. The effect of thioprolin as an inhibitor of the carcinogenesis induced by *N*-nitroso-*N*-benzylmethylamine precursors was examined. Two groups of male Fischer 344 rats, 6 weeks of age, were fed diets that contained 0.25% *N*-benzylmethylamine (group I, eight rats) or 0.25% *N*-benzylmethylamine plus thioprolin (0.25% thioprolin until week 17 and then 0.5%; group II, 10 rats). Both groups were given drinking-water that contained sodium nitrite (0.1% until week 17 and then 0.2%). The experiment was continued for 717 days. Squamous-cell carcinomas of the forestomach developed in 7/7 [6/7 in publication abstract] rats in group I and in significantly fewer (2/9 rats) in group II ($P < 0.01$ by the χ^2 test). The degree of tumour invasion was also lower in group II rats that were given thioprolin than in group I rats (Tahira *et al.*, 1988). [The Working Group noted the lack of an *N*-benzylmethylamine-treated group.]

Groups of male non-inbred Swiss mice, 45 days of age, were given 1000 ppm dibutylamine (DBA) in combination with 2000 ppm sodium nitrite in the drinking-water, with or without either 30% soya bean in the diet or 5000 ppm ascorbic acid in the drinking-water, for a maximum of 12 months. An untreated control group was included. In each experimental group, some animals were killed after 7–9 or 10–12 months of treatment. Microscopical examination of the tumours observed in the urinary bladder and liver was performed. No tumours of the urinary bladder or liver were observed in the animals treated with DBA plus sodium nitrite plus soya bean for 7–9 months or 10–12 months (43 rats), in the animals treated with DBA plus sodium nitrite plus ascorbic acid for 7–9 months (20 rats) or in untreated controls (60 rats). Following treatment with nitrite plus DBA, the incidence of hepatomas was 3/20 (7–9 months of exposure) and 4/15 (10–12 months of exposure) and that of urinary bladder papillomas was 4/20 (7–9 months of exposure) and 6/20 (10–12 months of exposure). The authors concluded that a clear carcinogenic effect of the nitrosamine precursor (DBA) and sodium nitrite was demonstrated, and that soya bean and ascorbic acid had a protective effect (Mokhtar *et al.*, 1988)

Groups of 10 male Fischer 344 rats, 10 weeks of age, were treated with 0 or 0.3% sodium nitrite in the drinking-water and fed either 0.8% catechol or 2% 3-methoxycatechol in the diet for 24 weeks, when the experiment was terminated. Simultaneous administration of sodium nitrite with 3-methoxycatechol increased the formation of forestomach papillomas (5/10 versus 0/10, $P < 0.05$) compared with the groups treated with 3-methoxycatechol alone. In contrast, induction of adenomas in the glandular epithelium of the stomach was reduced (1/10 versus 10/10, $P < 0.01$; 2/10 versus 7/10 [$P < 0.05$]) compared with the respective catechol- and 3-methoxycatechol-treated groups (Hirose *et al.*, 1990).

Groups of 15 male Fischer 344 rats, 6 weeks of age, were given a single intraperitoneal injection of 100 mg/kg bw NDEA, four intraperitoneal injections of

20 mg/kg bw *N*-methylnitrosourea, four subcutaneous injections of 40 mg/kg bw dimethylhydrazine, 0.05% BBNA in the drinking-water for the first 2 weeks and 0.1% 2,2'-dihydroxy-di-*n*-propylnitrosamine in the drinking-water for the next 2 weeks of the initial 4-week initiation period. Starting 3 days after the completion of these treatments, animals were fed diets that contained 2% butylated hydroxyanisole, 0.8% catechol, 2% 3-methoxycatechol or basal diet either alone or in combination with 0.3% sodium nitrite until week 28, when a complete autopsy was performed. Sodium nitrite enhanced the development of forestomach lesions but inhibited that of glandular stomach lesions in rats that were given catechol or 3-methoxycatechol simultaneously with or without prior exposure to carcinogens. The incidence of carcinomas *in situ* (7/14 versus 1/15, $P < 0.05$ by Fisher's exact probability test) and squamous-cell carcinomas (14/14 versus 5/15, $P < 0.001$) of the forestomach was increased by sodium nitrite in addition to catechol and carcinogen treatment; the incidence of papillomas of the forestomach (5/10 versus 0/10, $P < 0.05$) was increased by sodium nitrite in addition to 3-methoxycatechol without carcinogen treatment. The incidence of submucosal hyperplasias and/or adenomas of the glandular stomach was decreased by sodium nitrite in addition to catechol or 3-methoxycatechol with or without carcinogen treatment. However, reductions in body weight, that probably reflected the decreased food consumption following co-administration of sodium nitrite, may have been a factor in the modification of the development of glandular stomach lesions. The authors concluded that sodium nitrite can stimulate cell proliferation and enhance carcinogenesis, particularly in the forestomach epithelium (Hirose *et al.*, 1993).

Groups of 15–20 male Fischer 344 rats, 5 weeks of age, were treated with an intragastric dose of 150 mg/kg bw MNNG and, starting 1 week later, were fed 2.0% butylated hydroxyanisole, 0.8% catechol or 2.0% 3-methoxycatechol in the diet or basal diet either alone or in combination with 0.2% sodium nitrite in the drinking-water until they were killed at week 52. All three antioxidants significantly enhanced forestomach carcinogenesis without any effect of additional treatment with sodium nitrite. The incidence of forestomach papillomas varied from 74 to 95% in the six treated groups: 75% (15/20) in the group treated with sodium nitrite and basal diet (not significant) compared with 50% (9/18) in the group fed basal diet only. The incidence of forestomach squamous-cell carcinoma varied from 85 to 100% in the two butylated hydroxyanisole- and two catechol-treated groups, was 42 and 50% in the 3-methoxycatechol- and the 3-methoxycatechol and sodium nitrite-treated groups, respectively, and was 25 and 33% in the groups given sodium nitrite and basal diet and basal diet alone, respectively. In the absence of MNNG pretreatment, there was no significant increase in the incidence of forestomach papillomas or squamous-cell carcinomas, except that a statistically non-significant increase in forestomach papillomas was observed in the group treated with 3-methoxycatechol and sodium nitrite compared with the 3-methoxycatechol-treated group (10/15 versus 4/15). A statistically non-significant increase in forestomach papillomas was also observed in the group treated with catechol and sodium nitrite compared with

the catechol-treated group (4/15 versus 0/15). Sodium nitrite had no effect on catechol- or 3-methoxycatechol-induced glandular stomach carcinogenesis (Kawabe *et al.*, 1994).

The effects on forestomach carcinogenesis of combined treatment with sodium ascorbate or ascorbic acid and sodium nitrite, with or without pretreatment with MNNG, were examined. Groups of 20 or 15 male Fischer 344 rats, 6 weeks of age, were given a single intragastric administration of 150 mg/kg bw MNNG in dimethyl sulfoxide:water (1:1) or vehicle alone, respectively. One week later, the animals received supplements of 1% sodium ascorbate or 1% ascorbic acid in the diet and 0.3% sodium nitrite in the drinking-water, alone or in combination, or basal diet until the end of week 52. In MNNG-treated animals, the incidence of forestomach papillomas and squamous-cell carcinomas was significantly enhanced in the group that received sodium nitrite alone (16/19 and 9/19, $P < 0.05$ and $P < 0.01$, respectively, by Fisher's exact probability test) compared with the group that received basal diet (6/20 and 2/20, respectively); a further significant increase in the incidence of carcinomas occurred following additional treatment with sodium ascorbate (15/19, $P < 0.01$ versus basal diet group, $P < 0.05$ versus sodium nitrite group by Fisher's exact probability test) or ascorbic acid (17/20, $P < 0.01$ versus basal diet group, $P < 0.05$ versus sodium nitrite group by Fisher's exact probability test). With no MNNG treatment, all animals that received sodium nitrite demonstrated mild hyperplasia, but no papillomas were observed. Additional administration of sodium ascorbate or ascorbic acid remarkably enhanced the grade of hyperplasia, and resulted in a 53% (8/15, $P < 0.01$ versus basal diet or sodium nitrite groups) and a 20% (3/15, not significant) incidence of papillomas, respectively. The authors concluded that these results demonstrate that sodium nitrite exerts a promoting effect on forestomach carcinogenesis and that sodium ascorbate and ascorbic acid act as co-promoters; also, combined treatment with sodium ascorbate or ascorbic acid and sodium nitrite may induce forestomach carcinomas (Yoshida *et al.*, 1994).

Groups of 15 male Fischer 344 rats, 6 weeks of age, were treated with an intragastric dose of 150 mg/kg bw MNNG and, starting 1 week later, were fed 0.5% *tert*-butylhydroquinone (TBHQ), 1% α -tocopherol or 1% propyl gallate in the diet or basal diet with or without 0.2% sodium nitrite in the drinking-water until they were killed at the end of week 36. In MNNG-treated animals, combined administration of α -tocopherol or propyl gallate with sodium nitrite significantly increased the areas ($P < 0.001$) and numbers ($P < 0.01$) of macroscopic forestomach nodules per animal (0.88 ± 0.52 cm² and 8 ± 5 ; 1.47 ± 1.22 cm² and 7 ± 3) compared with the respective values for animals treated with antioxidants alone (0.30 ± 0.59 cm² and 3 ± 2 ; 0.47 ± 1.01 cm² and 3 ± 3), and combined administration of α -tocopherol with sodium nitrite compared with administration of α -tocopherol alone significantly increased the multiplicity of forestomach papillomas (1.0 ± 1.1 versus 0.2 ± 0.4 , $P < 0.05$) and tended to increase their incidence (9/15 versus 3/15, statistically non-significant). In rats not treated with MNNG, combined treatment with antioxidants and sodium nitrite significantly increased the incidence of mild or moderate hyperplasia of the forestomach (TBHQ, $P < 0.001$; α -tocopherol, $P < 0.05$; propyl gallate, $P < 0.01$). In the glandular stomach, atypical hyperplasias were observed

in all the MNNG-treated groups. Although the incidence showed a non-significant tendency to decrease with TBHQ treatment, additional administration of sodium nitrite caused a significant increase (60% compared with 13% in rats treated with TBHQ alone, $P < 0.05$). The authors concluded that co-administration of sodium nitrite with α -tocopherol, TBHQ or propyl gallate promotes forestomach carcinogenesis (Miyachi *et al.*, 2002).

The effect of the antioxidants, 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ) and ascorbic acid, on carcinogenesis induced by administration of aminopyrine and sodium nitrite was examined using a rat multiorgan carcinogenesis model. Groups of 20 male Fischer 344 rats, 6 weeks of age, were treated sequentially, during the first weeks, with an initiation regimen of NDEA (100 mg/kg bw, single intraperitoneal dose at commencement), *N*-methylnitrosourea (20 mg/kg bw, intraperitoneal injections on days 2, 5, 8 and 11), BBNA (0.05% in the drinking-water during weeks 1 and 2), *N,N'*-dimethylhydrazine (40 mg/kg bw, subcutaneous injections on days 14, 17, 20 and 23) and 2,2'-dihydroxy-di-*n*-propylnitrosamine (0.1% in the drinking-water during weeks 3 and 4). Initiation was followed by treatment with aminopyrine plus sodium nitrite, aminopyrine plus sodium nitrite plus HTHQ or ascorbic acid, sodium nitrite plus HTHQ or ascorbic acid, each of the individual chemicals alone or basal diet and tap-water as a control. Aminopyrine and sodium nitrite were given in drinking-water at a dose of 0.05% and HTHQ and ascorbic acid were given in the diet at a dose of 0.25%. All surviving animals were killed at experimental week 28, and major organs were examined histopathologically. In the group treated with aminopyrine and sodium nitrite, the incidence of hepatocellular adenomas and haemangiosarcomas was 19/20 and 7/20, respectively (both lesions, $P < 0.01$ versus untreated control by Fisher's exact probability test). When HTHQ, but not ascorbic acid, was administered with aminopyrine and sodium nitrite, the incidence decreased to 11/19 and 2/19 (adenomas, $P < 0.01$ versus aminopyrine plus sodium nitrite group by Fisher's exact probability test). In contrast, in the group treated with aminopyrine and sodium nitrite and the group treated with sodium nitrite alone, when HTHQ, but not ascorbic acid, was administered simultaneously, the incidence of forestomach squamous-cell carcinomas significantly increased in the aminopyrine plus sodium nitrite plus HTHQ-treated group (5/19, $P < 0.05$) and in the sodium nitrite plus HTHQ-treated group (8/20, $P < 0.01$), compared with 0/20 in the groups treated with aminopyrine plus sodium nitrite and sodium nitrite alone. The authors suggested that HTHQ can prevent [liver] tumour production induced by aminopyrine and sodium nitrite more effectively than ascorbic acid. In contrast, an enhancing or possibly carcinogenic effect of simultaneous administration of HTHQ and sodium nitrite only on the forestomach was suggested, while simultaneous treatment with the same dose of ascorbic acid and sodium nitrite may not be carcinogenic to the forestomach or other organs (Yada *et al.*, 2002).

Using a model of rat mammary carcinogenesis induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) as an initiator, chemoprevention by synthetic and naturally occurring compounds of the carcinogenic effect was investigated in a series of

experiments. In one experiment, two groups of 6-week-old female Sprague-Dawley rats were given eight intragastric doses of 100 mg/kg bw PhIP during an initial 4-week period alone (20 rats) or simultaneously with 0.2% sodium nitrite in the drinking-water (10 rats). A third group of 10 rats received sodium nitrite alone for 4 weeks and a fourth group of 10 rats received basal diet alone. At the end of experimental week 48, all animals were killed and mammary tumours were examined histopathologically. No tumour was observed in the groups that received sodium nitrite or basal diet alone. The first tumour in the PhIP-treated group appeared at week 13, whereas this was delayed until week 46 in the group treated with PhIP and sodium nitrite. Although the final incidence of mammary fibroadenomas and adenocarcinomas was not significantly different between the two groups (PhIP: adenocarcinomas, 11/20; fibroadenomas, 3/20; PhIP plus sodium nitrite: adenocarcinomas, 6/10; fibroadenomas, 2/10), the average volume of tumours was significantly reduced in the PhIP plus sodium nitrite-treated group compared to that in the group that received PhIP alone (0.3 ± 0.4 versus 4.5 ± 15.1 cm³ [method of statistical analysis not provided]) (Hirose *et al.*, 2002).

3.2.3 Hamster

Groups of F₀ pregnant Syrian golden hamsters and their offspring [number and age unspecified] were fed various concentrations of nitrite (5, 50 or 1000 ppm) or morpholine (5, 50 or 1000 ppm) in an agar-gel diet from the time of conception. The F₁ generation was fed the same diet as the dams: a group of 30 hamsters was fed the diet containing sodium nitrite at 1000 ppm, a group of 22 hamsters was fed the diet containing morpholine at 1000 pm, another group of 23 hamsters was fed the untreated diet only and served as controls and groups of 16–40 animals were fed diets that contained combinations of the various concentrations of nitrite and morpholine. The survivors were killed when the group number was reduced to 20% of the initial population. No increase in tumour incidence was found between the groups, except that liver-cell carcinomas were found in 5/16 (31%) hamsters treated with 1000 ppm nitrite and 1000 ppm morpholine compared with 0/22 hamsters treated with 1000 ppm morpholine alone (Shank & Newberne, 1976). [The Working Group noted that hamsters appear to be less sensitive than rats to the carcinogenicity of nitrite.]

3.3 References

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4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Introduction

The biochemistry and pharmacology of nitrate and nitrite have been under active investigation for hundreds of years (Gladwin *et al.*, 2005), and several aspects, e.g. the endogenous synthesis of nitrate, have been discovered (Mitchell *et al.*, 1916) and rediscovered (Green *et al.*, 1981; Leaf *et al.*, 1987). The recognition in the 1980s of the ubiquitous role of nitric oxide in mammalian biochemistry appeared to clarify the endogenous formation of nitrite and nitrate simply as potentially hazardous end-products of nitric oxide production. Indications of additional complexity often went unheeded. Doyle *et al.* (1981), for example, noted that nitrite reacted *in vitro* with deoxygenated haemoglobin to form nitric oxide, and Cortas and Wakid (1991), while investigating the oral biochemistry of nitrate and nitrite, noted an earlier suggestion by Wolff and Wasserman (1972) that production of nitrite by the oral flora may protect against the growth of clostridia. A growing body of research is now revealing that nitrate and nitrite participate in a metabolic cycle in which nitrite and nitric oxide can be interconverted depending on physiological needs and conditions.

The following sections cover absorption, distribution, metabolism and excretion of nitrate and nitrite. However, these processes are not independent since nitrate and nitrite, together with nitric oxide and other important compounds such as carbon dioxide, superoxide and hydrogen peroxide, participate in a complex set of regulatory pathways that have important consequences for both normal and abnormal physiology and biology. These pathways relate to—among others—neural signalling (Garthwaite 1995), regulation of blood pressure (Hunter *et al.*, 2004; Crawford *et al.* 2006), inflammation (Stuehr & Marletta, 1985; Clancy *et al.*, 1998) and ischaemia (Zweier *et al.*, 1995; Dybdahl *et al.*, 2005).

4.1.2 Absorption

Ingested nitrate enters the general circulation from the gastrointestinal system, which leads to maximum plasma concentrations within about 1 h, followed by clearance with a half-life of about 5 h (Wagner *et al.*, 1983; Cortas & Wakid, 1991; McKnight *et al.*, 1997). Above a certain level, about 25% of an oral dose of nitrate is re-circulated into the saliva, of which 20%—i.e. 5% of ingested nitrate—is converted into nitrite by oral bacteria (Spiegelhalder *et al.*, 1976; National Research Council, 1981).

Exposure to nitrate/nitrite is primarily through ingestion and endogenous synthesis. Under normal conditions, endogenous synthesis of nitrate/nitrite occurs in many places in

the body, e.g. the liver, brain, blood vessels and sites of inflammation, by the conversion of arginine to nitric oxide which is mediated by a combination of constitutive and inducible nitric oxide synthases, followed by the reaction of nitric oxide with other cellular constituents, e.g. oxygen or peroxynitrite (Rodkey, 1976; Leaf *et al.*, 1989a; Ignarro, 1990; Marletta *et al.*, 1988, 1990; Moncada & Higgs 1991; Koppenol *et al.*, 1992; Edwards & Plumb, 1994; Marletta, 1994; Pryor & Squadrito, 1995; Kelm, 1999). Nitrate/nitrite that is formed via this set of pathways is absorbed from the generating organ or tissue into the general circulation and constitutes a more or less steady-state baseline level in the body, which is then episodically supplemented by ingested nitrate/nitrite.

4.1.3 *Distribution*

Distribution of ingested nitrate and nitrite would be expected to reflect to some extent the levels of production in individual tissues and compartments. ¹³N-Labelled nitrate administered to rats by gavage or intravenous injection was found to be distributed throughout the body, including the stomach (~20%), intestines (~20%), urinary bladder and kidney (~5%) and the remainder largely in the carcass within 1 h after dosing, which suggests fairly rapid distribution (Witter *et al.*, 1979). When ¹³N-labelled nitrite was given in the drinking-water to germ-free Sprague-Dawley rats for 48 h, detectable levels were found in the stomach, small intestine, caecum and large intestine but not in the urinary bladder (Witter & Balish, 1979). Parks *et al.* (1981) administered ¹³N-labelled nitrate and nitrite intravenously or intratracheally to mice and rabbits and found that the label via both routes of administration was distributed uniformly throughout most of the body. In experiments with two human volunteers, most of an oral dose of [¹³N]nitrate remained in the stomach for up to 1 hour (Witter *et al.*, 1979).

While some earlier reports of nitrate plasma levels noted that nitrite was undetectable (Cortas & Wakid, 1991; Meulemans & Delsenne, 1994), recent advances in analytical techniques have allowed reasonably precise measurements of circulating plasma nitrite levels in mammals, which are generally in the range of 150–600 nM (Tsikas *et al.*, 1998; Gladwin *et al.*, 2000; Tsikas & Frölich, 2002; Kleinbongard *et al.*, 2003).

In addition to saliva and plasma, nitrate and nitrite have been measured in the human brain, pancreas, lung, thyroid, kidney, liver, spleen and cerebrospinal fluid, generally at high nanomolar to mid-micromolar levels for nitrite and mid-to-high micromolar levels for nitrate (Friedberg *et al.*, 1997; Goodman *et al.*, 2002). Overall, based on analyses in animals and humans, nitrate and nitrite are widely distributed throughout the body—predominately as nitrate—although with large local variations.

4.1.4 *In-vivo formation of NPRO and other nitrosamino acids*

Ohshima and Bartsch (1981) introduced the use of excretion of NPRO in the urine as a measure of in-vivo nitrosation. They gave a volunteer 325 mg nitrate in beet juice and

500 mg L-proline, and determined NPRO in the urine collected over the next 24 h. They found 160 nmol (23 µg) NPRO in the 24-h urine. Ascorbic acid or α -tocopherol given simultaneously inhibited the formation of NPRO.

This was the first clear demonstration that humans can form *N*-nitroso compounds *in vivo*. NPRO is formed by gastric nitrosation of proline, absorbed into the blood and excreted quantitatively in the urine. The test is considered to be safe because NPRO is non-carcinogenic and is excreted quantitatively. Because of its carboxyl group, nitrosation of proline proceeds maximally at pH 1.5–2.7, rather than pH 3.0–3.3 which is usual for amines, but its rate of nitrosation is proportional to the square of the nitrite concentration, similarly to other amines, and its rate constant is intermediate compared with that of other amines (Mirvish, 1975). The NPRO test measures the nitrosating capacity of the stomach but not the gastric levels of amines and amides.

(a) *Application of the NPRO test*

Bartsch *et al.* (1990) summarized their research on the endogenous formation of *N*-nitroso compounds as measured by the NPRO test. The test (in which proline alone or proline plus vitamin C were given) has been applied to subjects from areas at high and low risk for cancer of the stomach in Colombia, Costa Rica, Japan and Poland, for squamous-cell cancer of the oesophagus in northern China and for nasopharyngeal cancer in southern China (Lu *et al.*, 1986; Kamiyama *et al.*, 1987; Zatonski *et al.*, 1989; see also Mirvish 1994). In most cases, excretion of NPRO was greater in high-risk areas for these cancers than in low-risk areas. Patients infested with liver flukes in Thailand, who are at risk for liver cholangiocarcinoma, also showed increased excretion of NPRO (Bartsch *et al.*, 1992). In most of these studies, vitamin C was found to reduce the formation of NPRO.

(b) *Correlation of the NPRO test with gastric pH*

In a study of patients who had gastric lesions, the yields of NPRO were highest when gastric pH was 1.5–2.0. The yield did not increase in cases who had advanced gastric lesions. The number of nitrate-reducing bacteria correlated positively with pH but not with urinary NPRO. These results indicate that NPRO was produced only in an acid-catalysed reaction (Hall *et al.*, 1987).

In another study (Wagner *et al.*, 1984a), excretion of NPRO by human volunteers was expressed after subtracting the amount of NPRO ingested in the diet. Mean basal excretion was 26 ± 10 nmol per day, which was not reduced by ascorbic acid (2 g per day) or α -tocopherol (400 mg per day). After subjects ingested nitrate and proline, the mean excretion of NPRO was 100 ± 81 nmol/day. When they were fed [^{15}N]nitrate, [^{15}N]NPRO was formed. Ascorbic acid inhibited [^{15}N]NPRO formation by 80%. Formation of another non-carcinogenic compound (*N*-nitrosothiazolidine-4-carboxylic acid (NTCA); see below) after ingestion of nitrate was completely inhibited by ascorbic acid. These results

indicated that a portion of urinary NPRO was produced by a mechanism that is not inhibited by ascorbic acid.

It has been suggested that there are two types of NPRO synthesis *in vivo* (Tannenbaum, 1987). One type incorporates ^{15}N from nitrite and is inhibited by ascorbic acid while the other type does not incorporate [^{15}N]nitrite, is not inhibited by ascorbic acid and does not, therefore, entail acid-catalysed nitrosation by nitrite (Wagner *et al.*, 1985).

(c) *NPRO formation in subjects with high levels of nitrate in their drinking-water*

Urinary excretion of NPRO was increased in 44 men from a rural area in the USA, who drank well-water that contained levels of nitrate below or above 10 ppm nitrate-N. For 5 days, the subjects followed a diet low in NPRO and nitrate and (on days 4 and 5) ascorbate. The 24-h urine was collected on day 4 while normal activities were followed and on day 5 after an overnight fast and ingestion of 500 mg proline. Saliva samples were also collected. The concentration of nitrate in the drinking-water (< 10 or > 10 ppm nitrate-N) was significantly associated with urinary NPRO (< 1500 or > 1500 ng/day) on both days, with urinary nitrate, and with nitrite and nitrate in the saliva (Mirvish *et al.*, 1992).

A similar study of 285 residents in rural Denmark also involved the ingestion of 500 mg proline and analysis of 12-h urine samples. The 24-h diet was recorded and analysed for nitrate. The concentration of nitrate in the drinking-water was weakly associated ($P = 0.08$) with an increase in the excretion of NPRO. Nitrate consumption in food was also measured. The risk for excreting excess NPRO increased significantly with total nitrate intake in food and water, and with tobacco smoking. Tea consumption reduced the excretion of NPRO, whereas the intake of vegetables did not have a clear effect. These findings show that a high intake of nitrate in water can increase the formation of NPRO (Møller *et al.*, 1989).

In a study of subjects who consumed proline under controlled conditions, factors that affected excretion of NPRO were studied in healthy subjects after 5 days on a diet low in NPRO, nitrate, proline and (on days 4 and 5) ascorbate (Mirvish *et al.*, 1995). In the standard test, subjects took 400 mg nitrate (as potassium nitrate) 1 h before a standard meal that contained 500 mg proline. Mean excretion of NPRO determined in 24-h urine was 26 ± 2 (mean \pm standard error (SE)) nmol compared with 5 ± 1 nmol when subjects ingested proline alone. Similar results were observed in men and women. Doses of 200 and 100 mg nitrate produced mean NPRO excretory levels of 9 ± 1 and 11 ± 2 nmol/24 h, respectively. Nitrate produced the highest level of NPRO when given 1 h before the test meal. This time of 1 h reflects the 1–2 h required for salivary nitrite to peak after a dose of nitrate is taken (Spiegelhalter *et al.*, 1976; Tannenbaum *et al.*, 1976). When nitrate was given 15 min or 2 h before the meal, the NPRO yields were 17 ± 2 and 19 ± 4 nmol/24 h, respectively. Gavage of fasting subjects with nitrate and proline increased the formation of NPRO from three- to fourfold compared with when a meal was taken. Sodium ascorbate showed maximum inhibition of NRPO excretion from 2 h before to 1 h

after the test meal. When the dose of ascorbic acid was varied under the same test conditions as those described above, mean inhibitions compared with no ascorbic acid were 28, 62 and 60% for doses of 120, 240 and 480 mg ascorbic acid, respectively (Mirvish *et al.*, 1998). As little as 100 mg ascorbic acid significantly reduced NPRO formation when taken with a meal containing proline. [The Working Group noted that these results strongly support the view that NPRO is produced by acid-catalysed nitrosation in the stomach.]

(d) *Nitrosamino acids other than NPRO*

In addition to NPRO, human urine contains NTCA (*N*-nitrosothioprolin) and 2-methyl-NTCA. The precursor amines of these compounds are formed in the stomach by acid-catalysed condensation of formaldehyde and acetaldehyde, respectively, with cysteine (Tsuda *et al.*, 1983). These nitrosamino acids are acid-labile and hence require appropriate analytical conditions. Excretion of NTCA and methyl-NTCA was increased in smokers (Tsuda *et al.*, 1987). Thioprolin is nitrosated about 1000 times faster than proline *in vitro*, and NTCA is probably not carcinogenic (Tsuda & Kurashima, 1991), but it seems less suitable than NPRO as a measure of gastric nitrosation because its formation depends on the levels of aldehydes and cysteine as well as nitrite.

(e) *Other NPRO tests and related assays*

Excretion of NPRO was reduced in patients who had elevated gastric pH and higher than normal bacterial counts in the stomach caused by premalignant gastric conditions or by treatment with cimetidine (Bartsch *et al.*, 1989). This confirms that NPRO is produced in the stomach and suggests that, if products of gastric nitrosation initiate gastric cancer, they do so before premalignant conditions associated with achlorhydria develop.

Excretion of NPRO after ingestion of proline but not nitrate, was higher in groups of 25–50 healthy individuals from several areas with high cancer incidence than in similar groups from neighbouring areas with low incidence. This was shown for gastric cancer in Japan (Bartsch *et al.*, 1989), Poland (Zatonski *et al.*, 1989), Colombia (Stillwell *et al.*, 1991) and Costa Rica (Sierra *et al.*, 1993), for oesophageal cancer in northern China (Lu *et al.*, 1986; Bartsch *et al.*, 1989) and for nasopharyngeal cancer in southern China (Yi *et al.*, 1993). In the NPRO tests conducted by Bartsch *et al.* (1989), 100 mg proline taken 1 h after the evening meal or after each of three daily meals and ascorbic acid (100–200 mg taken with proline) was shown to inhibit the formation of NPRO. Wu *et al.* (1993) examined pooled urines from 30 men from each of two villages in 69 counties in China with different cancer incidences. Significant associations were found between (a) NPRO yield and the decrease in NPRO yield after ingestion of ascorbic acid (the ‘nitrosation potential’) and (b) the incidences of cancer of the oesophagus and leukaemia, but not of gastric cancer or several other cancers. The urinary excretion of *N*-nitrosarcosine was strongly correlated with the incidence of oesophageal cancer ($P < 0.001$).

Excretion of NPRO was also elevated in patients with schistosomiasis in Egypt (Tricker *et al.*, 1989) and those with liver fluke infestation in Thailand (Srivatanakul *et*

al., 1991; Ohshima & Bartsch, 1994). Presumably, these infestations promoted the synthesis of nitrogen oxide, which was converted to nitrate and hence increased nitrite levels and nitrosation in the stomach, as was demonstrated in Syrian hamsters infected with liver flukes (Ohshima *et al.*, 1994).

Shapiro *et al.* (1991) examined the urinary excretion of NPRO and NTCA by subjects who had ingested sodium nitrate and proline, and compared these with results before and after use of an oral antiseptic (*Peridex*). This mouthwash solution reduced the number of nitrate-reducing bacteria in saliva by 94%, strongly reduced the reduction of nitrate to nitrite by saliva *in vitro* and inhibited the excretion of NPRO and NTCA by 62 and 74%, respectively. Individuals who had high levels of nitrate-reducing oral bacteria in their saliva showed significantly increased excretion of NPRO. These results suggest that gastric or oral nitrosamine formation is increased in individuals with poor oral hygiene.

Four studies showed that cigarette smoking increases the formation of NPRO, probably because of catalysis by thiocyanate or increased gastric acid secretion due to irritation by the smoke (Bartsch *et al.*, 1989). Subjects who chewed quids of betel nuts and/or tobacco, but did not ingest proline or nitrate, showed increases in mean salivary and urinary NPRO, but the effects were not significant (Nair *et al.*, 1985). When nitrate was given 1 h before a standard meal containing proline, chewing of gum and tobacco did not significantly affect the yield of NPRO after correcting for preformed NPRO derived from the tobacco. Chewing did not affect levels of nitrate but reduced levels of nitrite in the saliva, probably because of the increased flow of saliva (Mirvish *et al.*, 1995).

Endogenous formation of *N*-nitrosamines – other than NPRO – following ingestion of nitrate in combination with an amine-rich diet was investigated by Vermeer *et al.* (1998, 1999).

A group of 25 female volunteers consumed during seven consecutive days a fish-meal rich in amines as nitrosatable precursors. The diet was supplemented with an aqueous solution of potassium nitrate (170 mg nitrate anion in 100 mL; the acceptable daily intake level). During one week before and one week after the test period a low-nitrate diet was consumed. The intake of nitrate caused a significant increase of mean nitrate concentrations in saliva and urine. *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopiperidine (NPIP) were detected as volatile *N*-nitrosamines in the urine. While excretion of NPIP was not directly correlated with nitrate intake and composition of the diet, the excretion of NDMA was highly correlated with nitrate excretion and with salivary nitrate and nitrite concentrations. This study shows that nitrate intake in combination with a diet containing nitrosatable precursors increases the formation of carcinogenic *N*-nitrosamines (Vermeer *et al.*, 1998).

In a further study with the same experimental design, the effects of ascorbic acid and green tea on NDMA excretion were investigated. Intake of 250 mg or 1 g of ascorbic acid per day resulted in a significant decrease in urinary NDMA excretion during days 4–7, but not during days 1–3 of the test week. Likewise, consumption of four cups of green tea per day (2 g) caused a significant decrease in excretion of NDMA during days 4–7, but not during days 1–3. In contrast, consumption of eight cups of green tea per day (4 g)

significantly increased the excretion of NDMA during days 4–7, again not during days 1–3. This may be a result of catalytic effects on nitrosation of the polyphenols in the tea. The results indicate that intake of ascorbic acid and moderate consumption of green tea can reduce endogenous formation of NDMA (Vermeer *et al.*, 1999).

(f) *Ascorbic acid and the NPRO test*

Ascorbic acid inhibits the formation of *N*-nitroso compounds from nitrite and amines or amides, because it reduces nitrite to nitric oxide while it is itself oxidized to dehydroascorbic acid (Mirvish, 1975, 1994). Partly on the basis of these findings, all nitrite-preserved meat in the USA is currently prepared with 550 ppm sodium erythorbate (a non-vitamin isomer of ascorbate that also reduces nitrite to nitric oxide), and the level of nitrite in such foods has decreased accordingly (see Section 1).

Inhibition of NPRO formation by vegetable and fruit juices was shown to be due to both ascorbic acid and the phenolic fraction (Helser *et al.*, 1992; Helser & Hotchkiss, 1994). Two inhibitory phenols in tomatoes were identified as *para*-coumaric and chlorogenic acids. The normal median level of ascorbic acid plus dehydroascorbic acid is 50 mg/L human gastric juice compared with 7 mg/L plasma, which indicates active secretion of 0.4 mg ascorbic acid per h into the stomach; however, this level is only 3.4 mg/L gastric juice in patients with chronic atrophic gastritis (O'Connor *et al.*, 1989; Rathbone *et al.*, 1989).

Gradually increasing doses of ascorbic acid were fed with a standard dose of proline on consecutive days to subjects on a low-ascorbic acid diet. Sodium nitrate (5.24 mmol) was given 2 h after the last meal; 30 min later, a 4.35-mmol dose of proline was given and the 24-h urine was analysed for nitrate, NPRO and total ascorbic acid. Nitrate balance was monitored using [¹⁵N]nitrate. It was estimated from the dilution of urinary nitrate by [¹⁴N]nitrate that nitrate synthesis was 1.28 mmol/day. NPRO excretion was reduced by 6 nmol/day for each increment of 0.05 mmol (9 mg) dietary ascorbic acid (Leaf *et al.*, 1987).

4.1.5 *Metabolism (other than formation of N-nitroso compounds)*

The metabolism of nitrate and nitrite under normal physiological conditions has been investigated and reviewed extensively (Tannenbaum *et al.*, 1978; Lundberg *et al.*, 2004); the following is a brief summary.

Nitrate, either ingested or secreted into the mouth, is partly reduced by oral bacteria into nitrite (Spiegelhalter *et al.*, 1976; Tannenbaum *et al.*, 1976); the resulting mixture is swallowed and passes into the gastrointestinal system from which most is absorbed into the general circulation. Nitrite in plasma is converted by a rapid reaction with oxyhaemoglobin into nitrate (Rodkey, 1976) and this cycle then continues. Plasma nitrate and plasma nitrite levels are typically in the micromolar and nanomolar range, respectively (Kleinbongard *et al.*, 2003). Thus, in the classic model, nitrate/nitrite exists

primarily as nitrate, which is the end-product of what has often been considered to be a detoxification and/or elimination mechanism for nitric oxide and nitrite.

The details of nitrite metabolism became a more complex when it was recognized that conversion of nitrite into nitric oxide can occur under certain physiological conditions such as hypoxia (Dejam *et al.*, 2004; Gladwin *et al.*, 2005; Kim-Shapiro *et al.*, 2005). This represents a reversal of the well-known nitric oxide-to-nitrite/nitrate pathways. As noted earlier, experiments in the 1980s showed that the nitrite ion could react with deoxygenated haemoglobin to release nitric oxide (Doyle & Hoekstra, 1981; Doyle *et al.*, 1981), but these experiments were carried out *in vitro* and their potential physiological relevance was not apparent. Zweier *et al.* (1995) reported that, in a perfused heart model for ischaemia, nitrite ion was converted *in vivo* directly into nitric oxide, which demonstrated that the earlier observations did in fact have biochemical implications. These studies have been followed by several related experiments indicating a renewed interest in the biochemistry of nitrite/nitrate. A model for nitrite/nitrate metabolism has emerged based on extensive and sometimes subtle interactions among ingested and endogenously synthesized nitrate, nitrite, nitric oxide and some related species, and the physiology of the organism. The details of these are beyond the scope of this monograph, but are discussed in several reviews (McKnight *et al.*, 1997; Dejam *et al.*, 2004; Lundberg *et al.*, 2004; Gladwin *et al.*, 2005; Kim-Shapiro *et al.*, 2006).

4.1.6 Excretion

As detailed in previous sections, nitrate and nitrite are interconverted, primarily via bacterial reduction of nitrate in the mouth and the gut and oxidation of nitrite in the blood. The steady-state balance favours nitrate. Experiments with isotopically labelled nitrate indicate that about 60% of dietary nitrate appears in the urine within 48 h (Wagner *et al.*, 1984b). Excretion, therefore, is mainly as urinary nitrate, typically in the approximate range of 0.4–3.5 mmol/day, with values towards the lower end for low-nitrate diets and towards the higher end for normal diets, although there can be large geographical variations (Lee *et al.*, 1986; Hill *et al.*, 1996; van Maanen *et al.* 1996). Patients with inflammatory bowel disease (Melichar *et al.*, 1994), rheumatoid arthritis (Stichtenoth *et al.*, 1995) and multiple sclerosis (Giovannoni *et al.*, 1999), among other disorders, have been found to have elevated levels of urinary nitrate. Newborn infants had higher levels of urinary nitrate than adults when excretion was corrected for reduced kidney function and body size, which suggests that newborns produce more intravascular nitric oxide than adults (Honold *et al.*, 2000). Urinary nitrite can be detected in the urine of people with bacterial infections, but this is due to bacterial reduction of nitrate to nitrite in the bladder (Tricker *et al.*, 1991; Lundberg *et al.*, 1997) and analysis of urinary nitrite has been suggested as a diagnostic aid for urinary tract infections (Liptak *et al.*, 1993).

In summary, nitrate and nitrite participate in a dynamic interchange that involves ingestion, endogenous synthesis and excretion, mainly as nitrate in the urine. Dietary exposure is primarily as nitrate, approximately 5% of which is reduced in the mouth to

nitrite, which then passes into the stomach; some of the nitrate is absorbed into the circulation. Under acidic gastric conditions, the newly formed nitrite is converted partially to nitric oxide that participates in gastric functioning. Some of the gastric nitrite also passes into the general circulation where it is oxidized by haemoglobin into nitrate that then re-enters the cycle. Endogenous synthesis is mainly via the arginine-to-nitric oxide pathway followed by ultimate conversion of nitrogen oxide to nitrate/nitrite. Although this is the predominant relationship between nitrite and nitric oxide, it is now clear that, under some physiological conditions, e.g. acidic hypoxia, nitrite can be converted in the reverse direction to nitric oxide. There is evidence that nitrite may function partly as a storage system for nitric oxide, and that it may be involved in other important processes in the body.

4.1.7 *Endogenous formation of N-nitroso compounds*

Most of the evidence that nitrite or *N*-nitroso compounds may induce cancer at a particular site is based on the presence or formation of *N*-nitroso compounds at or near these sites, e.g. in the lumen of the stomach and colon, or on the excretion of *N*-nitroso compounds in urine or faeces. This section also discusses the formation of other potential carcinogens and of deaminated DNA bases from nitrite.

Nitrate is a potential hazard because it can be reduced to nitrite *in situ* in foods, as well as endogenously. This nitrite can react with amines or amides to form potentially carcinogenic *N*-nitroso compounds. In the human body, the oral cavity is the main site of reduction of nitrate to nitrite through high nitrate concentrations and high levels of nitrate-reducing bacteria. Thus, 25% of ingested nitrate is secreted into the saliva and 20% of the secreted nitrate (5% of ingested nitrate) is reduced to nitrite by oral bacteria (National Research Council, 1981). Although the majority of nitrate in the body arises from ingested nitrate (except during inflammation), there is—as noted elsewhere in this monograph—a substantial contribution from endogenous synthesis (Green *et al.*, 1981). This is primarily via the arginine–nitric oxide pathway (Marletta *et al.*, 1988; Rassaf *et al.*, 2005), but some may arise via other precursors, e.g. ammonia, as has been found in rats (Saul & Archer, 1984).

(a) *In-vivo nitrosation due to nitrite, dinitrogen tetroxide, bacterial action and inflammation*

This subject has been reviewed (National Research Council, 1981). Tannenbaum (1987) listed three types of in-vivo nitrosation: acid-catalysed nitrosation in the stomach, the reaction of inhaled nitrogen dioxide (or, more correctly, dinitrogen tetroxide) in the lung and nitrosation mediated by macrophages and bacteria.

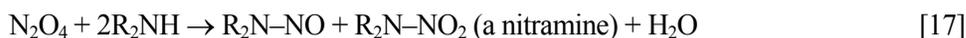
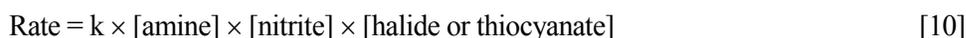
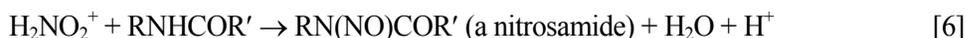
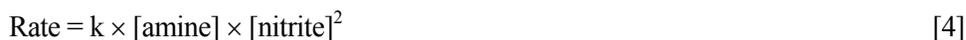
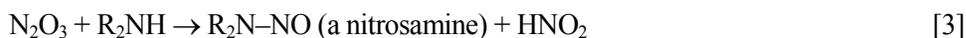
(i) *Acid-catalysed nitrosation in the stomach*

Gastric nitrosation is thought to be brought about by nitrite that arises from salivary reduction of nitrate. Since most saliva is swallowed, about 80% of gastric nitrite in the normal, acidic stomach is formed through reduction of ingested or endogenous nitrate. The remaining 20% of gastric nitrite arises from ingested nitrite in nitrite-preserved meat, fish and other foods. Gastric nitrosation may be catalysed by thiocyanate (Mirvish, 1983; National Research Council, 1981). In the achlorhydric stomach, bacteria proliferate and can reduce large amounts of nitrate to nitrite. Mean nitrite levels were 0.1–2.6 and 26–54 μM in fasting gastric juice of $\text{pH} < 5$ and > 5 , respectively (Xu & Reed, 1993).

(ii) *Chemical acid-catalysed nitrosation*

The principal reactions involved in chemical acid-catalysed nitrosation have been reviewed (Mirvish, 1975; National Research Council, 1981), and are shown in the equations below. For nitrite to produce nitrosation, it must first be acidified to form nitrous acid (HNO_2 ; Equation [1]). This dimerizes with loss of water to form nitrous anhydride (N_2O_3 ; Equation [2]), which can react with amines to form nitrosamines ($\text{R}_2\text{N}-\text{NO}$; Equation [3]). For nitrosation of amines in the absence of catalysts (Equations [2] and [3]), the reaction rate is proportional to the square of the nitrite concentration (Equation [4]) and is maximal at $\text{pH} 3.3$ (the acid dissociation constant of nitrous acid). Nitrous acid can also be protonated to form the nitrous acidium ion (H_2NO_2^+ ; Equation [5]), which preferentially reacts with amides to form nitrosamides ($\text{RN}(\text{NO})\text{COR}'$; Equation [6]). The rate of this reaction is proportional to the nitrite concentration and increases 10-fold for each one-unit drop in pH (Equation [7]). Because of the initial acidification step (Equation [1]), these reactions occur *in vivo* mainly in the acidic stomach (Mirvish, 1975; Leaf *et al.*, 1989b). When catalysts such as iodide and thiocyanate are involved, the catalyst—taking thiocyanate (NCS^-) as an example—reacts with nitrous anhydride to form the active intermediate ($\text{ON}-\text{NCS}$; Equation [8]) that reacts with amines to form nitrosamines (Equation [9]). In this case, the nitrosation rate is proportional to the concentration of nitrite (Equation [10]).

In other reaction pathways, nitrous anhydride can be oxidized to give dinitrogen tetroxide (N_2O_4 ; Equation [11]). It can also reversibly decompose to yield nitrogen oxide and nitrogen dioxide (Equation 12). The nitrogen oxide generated in this way, or produced from arginine by nitrogen oxide synthase, can react with oxygen to form nitrogen dioxide (Equation [13]), which dimerizes to form dinitrogen tetroxide (N_2O_4 ; Equation [14]). Dinitrogen tetroxide reacts with secondary amines to produce nitrosamines (Equation [15]), with water to yield a mixture of nitrous and nitric acids (Equation [16]) or with amines to form a mixture of nitrosamines and nitramines ($\text{R}_2\text{N}-\text{NO}_2$), the latter of which are weak carcinogens (Equation [17]). Nitrous acid decomposes spontaneously in strong acid, especially at concentrations above 25 mM, to give nitrogen oxide and nitric acid (Turney & Wright, 1959; Equation [18]).



In the rate equations [4], [7] and [10], the square brackets refer to molar concentration, and the named compounds refer to total concentration irrespective of the state of ionization. Equations that give the particular ions involved, e.g. nitrite anion, nitrous acid, non-protonated amine and protonated amine, have been discussed in detail elsewhere (Mirvish, 1975; National Research Council, 1981). Equations [4] and [7] apply to secondary amines and Equation [10] pertains to *N*-alkylamides (Mirvish, 1975). The rate constants (*k* values) for at least Equations [1] and [2] may vary by factors of five to six orders of magnitude depending on the particular amine or amide that is nitrosated.

(iii) *Bacterial nitrosation in the stomach and intestine*

Bacterial nitrosation proceeds at neutral pH, is most likely to take place in the achlorhydric stomach and is mediated most efficiently by bacteria that can carry out denitrification (reduction of nitrate to nitrogen) (Leach *et al.*, 1987; Calmels *et al.*, 1991). In an in-vitro study, *Escherichia coli* reduced nitrite to nitric oxide and small amounts of

nitrogen dioxide under anaerobic conditions. When air was admitted, nitrosating agents were produced, which were presumably nitrous anhydride and dinitrogen trioxide that reacted with amines to form nitrosamines. The nitric oxide produced from nitrite was oxidized by dissolved oxygen to nitrogen dioxide, which dimerized to dinitrogen tetroxide or reacted with more nitric oxide to form nitrous anhydride. These reactions were inhibited by nitrate and azide, which indicates that the nitrite was reduced by nitrite reductase. The potential for nitrosamine formation was determined by the nitrosation of 2,3-diaminonaphthalene to form fluorescent naphthotriazole (Ji & Hollocher, 1988a,b; Misko *et al.*, 1993).

In an in-vitro study on nitrosation by intestinal bacteria, more than 50 different strains were investigated. *E. coli* A10 catalysed the formation of nitrosamine from nitrite plus dimethylamine and other secondary amines. Other aerobic intestinal bacteria also catalysed these reactions, for which the optimum pH was 7.5. In this study, only one of 32 anaerobic species of bacteria catalysed nitrosamine formation (Suzuki & Mitsuoka, 1984).

Bacterial catalysis of nitrosation in the achlorhydric rat stomach was demonstrated when rats were treated with omeprazole to suppress gastric acid secretion and then gavaged with nitrosation-proficient *E. coli* A10 bacteria. Control rats did not receive omeprazole or *E. coli*. The rats were given nitrate and thiazolidine-4-carboxylic acid (the precursor of NTCA). Excretion of NTCA in the urine was 4.8 ± 1.2 nmol/day in control rats, 2.8 ± 0.7 nmol/day in rats treated with omeprazole and 19.2 ± 3.5 nmol/day in rats that received omeprazole and *E. coli* A10 bacteria (Calmels *et al.*, 1991).

In a study of the composition of gastric juice and urine in 45 patients on long-term omeprazole treatment—which would raise the level of gastric bacteria—and in 13 untreated controls, gastric pH was raised in the patient groups (60% of gastric juice samples had pH > 6) and gastric nitrite level was slightly elevated by the omeprazole treatment, but the concentrations of volatile nitrosamines in the stomach were not affected. Urinary excretion of NDMA was significantly higher in patients than in controls. Patients who tested positive for *Helicobacter pylori* showed somewhat higher gastric nitrite levels and urinary NDMA excretion than did uninfected patients (Vermeer *et al.*, 2001).

(iv) *Nitrosamines in the blood*

Ellen *et al.* (1982) analysed the blood and urine of patients who took 2.5–9.0 g ammonium nitrate daily to prevent redevelopment of calcium phosphate kidney stones for the presence of volatile nitrosamines. NDMA and NDEA were not detected in the blood, whereas traces of NPYR of around 0.1 µg/L were found in six of 23 urine samples.

(v) *Nitrate and nitrite levels and nitrosation in the normal colon*

Chemical nitrosation in the human and rodent colon is not likely to occur because of the near-neutral pH and the low levels of nitrite. Normal faecal levels were 0–0.9 mg/kg

for nitrate and 0.3–0.9 mg/kg for nitrite (Saul *et al.*, 1981). *N*-Nitroso compounds can be found endogenously within the colon because amines and amides produced mainly through bacterial decarboxylation of amino acids can be *N*-nitrosated in the presence of a nitrosating agent, mediated by anaerobic bacteria at an optimum of pH 7.5 (Mirvish, 1995; Calmels *et al.*, 1988; Rowland *et al.*, 1991; Bingham *et al.*, 2002).

(vi) *Nitrite levels in the inflamed colon*

Ulcerative colitis was associated with elevated nitrite levels and decreased pH in colonic dialysates, which were collected by placing dialysis tubing filled with water or buffer in the colon of patients for 1 h (Roediger *et al.*, 1986, 1990). Median values were 10.2 nmol nitrite/mL dialysate (mean pH, 7.1) in patients and 5.3 nmol/mL (mean pH, 7.5) in controls (this value in controls is not consistent with the very low levels in faeces reported by Saul *et al.*, 1981). This nitrite probably derives from nitrogen oxide produced by inflammation in the mucosa and could yield *N*-nitroso compounds in the lumen. Nitrosating species derived from nitrogen oxide could also deaminate DNA bases directly. These reactions may help explain the risk for colon cancer in cases of ulcerative colitis (Gyde *et al.*, 1988; Bernstein, 2000).

In a population-based case–control study, patients who had inflammatory bowel disease had a higher incidence of colon cancer if they drank water with high levels of nitrate (De Roos *et al.*, 2003). In another study, levels of NDMA were measured in the faeces of patients with inflammatory bowel disease, who were given liquid nutrition at the hospital or had an unrestricted diet at home. Seventeen ulcerative colitis patients and 17 healthy controls were examined. NDMA was determined by extraction followed by gas chromatography–mass spectrometry. Mean levels of NDMA (as ng/g faeces) were: all controls, 1.4; all patients, 10.9; non-hospitalized patients, 14.3; and hospitalized patients, 2.4. Hence, diet can affect the level of NDMA in such cases. It was proposed that diet would modulate the risk for colon cancer in patients with inflammatory bowel disease, who should avoid foods that contain high levels of *N*-nitroso compound precursors, e.g. nitrite-processed meat (de Kok *et al.*, 2005).

The amounts of faecal *N*-nitroso compounds increased 1.9-fold in mice that had acute colitis induced by dextran sulfate sodium compared with untreated mice (Mirvish *et al.*, 2003).

4.2 Genetic and related effects

4.2.1 *Humans*

Kleinjans *et al.* (1991) studied chromosomal damage in the peripheral lymphocytes of human populations exposed to low (0.13 mg nitrate/L), medium (32.0 mg/L) and high (56.0–311.0 mg/L; mean 133.5±68.5 mg/L) levels of nitrate in the drinking-water in the Netherlands. The high nitrate levels were from private water wells. Nitrate contamination

of drinking-water caused dose-dependent increases in nitrate body-load as monitored by 24-h urinary nitrate excretion, but was not associated with an increase in the frequencies of sister chromatid exchange in peripheral lymphocytes.

van Maanen *et al.* (1996) compared the frequency of *HPRT* (hypoxanthine–guanine phosphoribosyltransferase) variants in the peripheral lymphocytes of human populations exposed to various levels of nitrate in the drinking-water (0.02 and 17.5 mg/L from public water supplies, 14 and 21 subjects, respectively; 25 and 135 mg/L from private water wells, six and nine subjects, respectively) in the Netherlands. Higher nitrate intake via drinking-water resulted in a dose-dependent increase in 24-h urinary nitrate excretion and in increased levels of salivary nitrate and nitrite. The mean log frequency of *HPRT* variants was significantly higher in the group with medium exposure from well-water (25 mg/L) than in the groups with low and medium exposure from tap-water (0.02 and 17.5 mg/L, respectively). Multiple regression analysis showed a significant correlation between frequency of *HPRT* variants and 24-h urinary nitrate excretion ($P = 0.02$) and salivary nitrite levels ($P = 0.03$) and between 24-h urinary excretion of NPYR (measured by gas chromatography-mass spectrometry) and 24-h urinary nitrate excretion ($P = 0.02$). [The Working Group noted that the volatile nitrosamines reported to be present in urine are generally metabolized and not excreted.] A small number of the well-water samples were mutagenic in the *Salmonella typhimurium* mutagenicity test after concentration over XAD-2 resin.

Tsezou *et al.* (1996) examined the frequency of sister chromatid exchange and chromatid/chromosomal aberrations in the peripheral blood lymphocytes of 70 children (12–15 years of age) who were exposed to elevated concentrations of nitrates (55.7–88.0 mg/L) in the drinking-water and 20 children from areas with low nitrate concentrations (0.7 mg/L) in Greece. The mean numbers of chromatid and chromosome breaks were significantly increased in children exposed to nitrate concentrations above 70.5 mg/L ($P < 0.01$), but there was no increase in the mean level of sister chromatid exchange. [The Working Group noted that other genotoxic agents present in the drinking-water could be responsible for the increase in chromosomal aberrations.]

4.2.2 *Experimental systems*

(a) *Genotoxicity and mutagenicity*

(i) *Nitrate* (see Table 4.1 for details and references)

Potassium nitrate and sodium nitrate were not mutagenic in the Ames test in several strains of *S. typhimurium*.

Sodium nitrate did not induce DNA single-strand breaks in Chinese hamster V79 cells *in vitro* as measured with an alkaline elution assay. Positive results were reported in an *in-vitro* test for chromosomal aberrations in Chinese hamster fibroblasts with

Table 4.1. Genetic and related effects of nitrate

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
<i>Salmonella typhimurium</i> TA1535, TA100, reverse mutation	–	–	20.0 mg/plate ^c	Ishidate <i>et al.</i> (1984)
<i>Salmonella typhimurium</i> TA1535, TA100, reverse mutation	–	–	5 mg/plate	Ishidate <i>et al.</i> (1984)
DNA single-strand breaks, Chinese hamster V79 cells <i>in vitro</i>	–	–	1 mM, 2 h ^d	Görsdorf <i>et al.</i> (1990)
Chromosomal aberrations, Chinese hamster fibroblast cells <i>in vitro</i>	–	NT	1 mg/mL ^c	Ishidate <i>et al.</i> (1984)
Chromosomal aberrations, Chinese hamster fibroblast cells <i>in vitro</i>	+	NT	4 mg/mL, 24 h, 48 h	Ishidate <i>et al.</i> (1984)
Unscheduled DNA synthesis, mid-spermatids	–	–	600, 1200 ig, 3 days ^e	Alavantić <i>et al.</i> (1988)
Gene (8-azaguanine-resistance and ouabain-resistance C3HX101 mice, <i>in vivo</i>) mutation, hamster embryonic cells, <i>in utero</i>	–	–	500, ig × 1 ^d	Inui <i>et al.</i> (1979)
Micronucleus formation, hamster embryonic cells <i>in utero</i>	–	–	500, ig × 1 ^d	Inui <i>et al.</i> (1979)
Micronucleus formation, male Swiss mouse, polychromatic erythrocytes cells <i>in vivo</i>	± ^f	–	78.5, ig × 2	Luca <i>et al.</i> (1985)
Chromosomal aberrations, hamster embryonic cells <i>in utero</i>	–	–	500, ig × 1 ^d	Inui <i>et al.</i> (1979)
Chromosomal aberrations, male Wistar rat bone-marrow cells <i>in vivo</i>	– ^g	–	2120, ig × 2	Luca <i>et al.</i> (1985)
Chromosomal aberrations, male Wistar rat bone-marrow cells <i>in vivo</i>	+ ^g	–	78.5, ig × 2 weeks	Luca <i>et al.</i> (1985)
Chromosomal aberrations, male Swiss mouse bone-marrow cells <i>in vivo</i>	± ^h	–	706.6, ig × 2 weeks	Luca <i>et al.</i> (1985)
Chromosomal aberrations, male C57Bl mouse bone-marrow cells <i>in vivo</i>	+	–	50, 100, ip ^e	Rasheva <i>et al.</i> (1990)
Morphological transformation, hamster embryonic cells <i>in utero</i>	–	–	500, ig × 1 ^d	Inui <i>et al.</i> (1979)
Sperm-head abnormality test	– ⁱ	–	600, 1200 ig, 3 days ^e	Alavantić <i>et al.</i> (1988)

^a +, positive; –, negative; ±, positive at certain concentrations only; NT, not tested

^b LED, lowest effective dose; HID, highest ineffective dose; ig, intragastric; ip, intraperitoneal; doses are converted to µg/mL for in-vitro experiments and mg/kg per day for in-vivo experiment unless otherwise specified.

^c Potassium nitrate was used. All other experiments were carried out with sodium nitrate.

^d Only one dose was examined.

^e Only two doses were examined.

^f 6 h after the last dose of nitrate; the higher doses (706.6 and 2120 mg/kg ip × 2) were negative.

^g 24 h after the last dose of nitrate

^h The higher dose (2120 mg/kg ig × 2 weeks) was negative.

ⁱ 11 and 17 days after the last dose of nitrate

sodium nitrate, whereas potassium nitrate gave negative results. As sodium chloride was also positive in the same assay, the chromosomal aberrations induced by sodium nitrate could be due to the high osmotic pressure and sodium ion concentration.

No micronucleus formation, chromosomal aberrations, morphological or malignant cell transformation or drug-resistant mutation were observed in embryonic cells obtained from Syrian golden hamsters that had been given oral doses sodium nitrate on days 11 and 12 of pregnancy.

Significant increases in the frequency of chromosomal aberrations were reported in the bone marrow of rats treated intragastrically with sodium nitrate for 2 weeks. The response was much weaker in mice. Similarly, increased cytogenetic changes were reported in the bone marrow of mice injected intraperitoneally with sodium nitrate.

When sodium nitrate was given to mice by gastric intubation, no increased in-vivo genotoxicity, as assayed by unscheduled DNA synthesis in early-to-mid-spermatids, and no sperm-head abnormality were observed.

(ii) *Nitrite* (see Table 4.2 for details and references)

Below pH 5.5, nitrite is converted to nitrous acid which can react with amino groups. Primary amines could thus easily be deaminated and secondary amines react with nitrous acid to form *N*-nitroso compounds. Early studies on the reaction of nitrous acid with RNA and DNA and on genetic effects of nitrous acid have been reviewed (Zimmermann, 1977). Nitrous acid can deaminate various nucleobases, including guanine, adenine and cytosine which are converted to xanthine, hypoxanthine and uracil, respectively. Nitrous acid can induce point mutations mainly by deamination of cytosine and adenine, but not of guanine because its deamination is rapidly lethal. More complex events may also be caused by nitrous acid-induced DNA–DNA and DNA–protein crosslinks (Zimmermann, 1977).

Sodium and potassium nitrite are mutagenic in *S. typhimurium* (strains TA100, TA1530, TA1535 and TA1950 with or without metabolic activation). Sodium nitrite gave weakly positive results in the *umu* test and negative results in the SOS chromotest.

Chronic feeding of 3-day-old larvae of *Drosophila melanogaster* with sodium nitrite at a concentration of 72.5 mM produced small single wing spots in the somatic mutation and recombination test.

Sodium nitrite did not induce DNA single-strand breaks in cultured mouse mammary carcinoma cells or in Chinese hamster V79 cells. However, it induced chromosomal aberrations in cultured mouse mammary carcinoma cells, hamster fibroblasts, Syrian hamster embryo cells, monkey fetal liver cells and HeLa cells *in vitro*. Sodium nitrite up to a concentration of 1 mM did not induce DNA single-strand breaks in Chinese hamster V79 cells *in vitro*, as measured with an alkaline elution assay. Sodium nitrite induced 6-thioguanine-resistant mutants in V79 hamster cells *in vitro*, and 8-azaguanine-resistant mutants in mouse mammary carcinoma cells *in vitro*. Incubation of newborn hamster cells *in vitro* with sodium nitrite resulted in aneuploidy, chromosomal aberrations and cell

Table 4.2. Genetic and related effects of nitrite

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SOS chromotest, <i>Escherichia coli</i> strain PQ37	–	–	6.9 mg/mL	Brams <i>et al.</i> (1987)
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	10 mg/plate	Ishidate <i>et al.</i> (1984)
<i>Salmonella typhimurium</i> TA100, reverse mutation	NT	+	1 mg/plate	Brams <i>et al.</i> (1987)
<i>Salmonella typhimurium</i> TA100, reverse mutation	NT	+	10 mg/plate ^c	Rubenchik <i>et al.</i> (1990)
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	1 mg/plate ^d	Prival <i>et al.</i> (1991)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	1 mg/plate	Zeiger <i>et al.</i> (1992); National Toxicology Program (2001)
<i>Salmonella typhimurium</i> TA100, TA1530, TA1535, reverse mutation	+	+	1 mg/plate	Balimandawa <i>et al.</i> (1994)
<i>Salmonella typhimurium</i> TA102, YG1024, DJ400, DJ460, reverse mutation	–	–	5 mg/plate	Balimandawa <i>et al.</i> (1994)
<i>Salmonella typhimurium</i> TA98, TA97, reverse mutation	NT	–	1 mg/plate	Brams <i>et al.</i> (1987)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–	–	10 mg/plate	Zeiger <i>et al.</i> (1992)
<i>Salmonella typhimurium</i> TA1950, reverse mutation	NT	+	10 mg/plate ^c	Rubenchik <i>et al.</i> (1990)
<i>umu</i> test, <i>Salmonella typhimurium</i> TA1535/pSK1002, reverse mutation	(+)	NT	2.2 mg/mL, 2 h	Nakamura <i>et al.</i> (1987)
<i>Drosophila melanogaster</i> , somatic mutation, wing spot test	+	–	72.5 mM, 48 h ^e	Graf <i>et al.</i> (1989)
DNA single-strand breaks, C3H mouse mammary carcinoma cells <i>in vitro</i>	–	NT	100 mM, 24 h	Kodama <i>et al.</i> (1976)
DNA single-strand breaks, Chinese hamster V79 cells <i>in vitro</i> , alkaline elution	–	–	1 mM, 2 h ^e	Görsdorf <i>et al.</i> (1990)
Gene (8-azaguanine-resistant) mutation, C3H mouse mammary carcinoma cells <i>in vitro</i>	+	NT	1 mM, 48 h	Kodama <i>et al.</i> (1976)
Gene (6-thioguanine-resistant) mutation, Chinese hamster V79 cells <i>in vitro</i>	+	NT	0.05%, pH 4.95, 30 min	Budayová (1985)
Chromosomal aberrations, Chinese hamster fibroblasts <i>in vitro</i>	+	NT	0.25 mg/mL, 24 h	Ishidate <i>et al.</i> (1984)
Chromosomal aberrations, C3H mouse mammary carcinoma cells <i>in vitro</i>	+	NT	3.2 mM, 24, 48 h	Kodama <i>et al.</i> (1976)
Chromosomal aberrations, Syrian hamster embryo cells <i>in vitro</i>	+	NT	20 mM, 24 h	Tsuda & Kato (1977)
Chromosomal aberrations, BS-C-1 (African green monkey fetal liver cells) and HeLa cells <i>in vitro</i>	+	NT	0.265 mg/mL, 24 h ^e	Luca <i>et al.</i> (1987)
Cell transformation, Syrian hamster embryo cells	+	–	50, 100 mM, 24 h ^e	Tsuda <i>et al.</i> (1976)
Cell transformation, mouse BALB/c3T3 cells	+	–	10 mM, 72 h	Tsuda & Hasegawa (1990)
Morphological transformation, hamster embryonic cells <i>in utero</i>	+	–	125, ig × 1	Inui <i>et al.</i> (1979)
Aneuploidy, Syrian hamster embryo cells <i>in vitro</i>	+	–	50, 100 mM, 24 h ^e	Tsuda <i>et al.</i> (1976)

Table 4.2 (contd)

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Gene (8-azaguanine-resistance and ouabain-resistance) mutations, hamster embryonic cells <i>in utero</i>	+		125, ig × 1	Inui <i>et al.</i> (1979)
Micronucleous formation, hamster embryonic cells <i>in utero</i>	–		250, ig × 1	Inui <i>et al.</i> (1979)
Micronucleus formation, male ddY mouse polychromatic erythrocytes	–		200, ip or ig	Hayashi <i>et al.</i> (1988)
Micronucleus formation, male Swiss mouse polychromatic erythrocytes	± ^f		1.72, ig × 2	Luca <i>et al.</i> (1987)
Micronucleus formation, male Fischer 344/N rat polychromatic erythrocytes	–		100, ip × 3	National Toxicology Program (2001)
Micronucleus formation, male B6C3F ₁ mouse, polychromatic erythrocytes	–		125, ip × 3	National Toxicology Program (2001)
Micronucleus formation, male and female B6C3F ₁ mouse, polychromatic erythrocytes	–		5000 mg/L, dw, 14 weeks	National Toxicology Program (2001)
Host-mediated assay, <i>Salmonella typhimurium</i> G46 recovered from the peritoneal cavity of male ICR mice	– ^g		150, ig ^c	Couch & Friedman (1975)
Host-mediated assay, <i>Salmonella typhimurium</i> TA1530 recovered from the livers of female CD-1 mice	– ^h		120, pH 3.4, ig ^c	Edwards <i>et al.</i> (1979)
Host-mediated assay, <i>Salmonella typhimurium</i> G46 recovered from the livers of female CD-1 mice	– ^h		2320 μmol/kg, pH 3.4, ig	Whong <i>et al.</i> (1979)
Host-mediated assay, <i>Salmonella typhimurium</i> G46 recovered from the livers of female CD rat	– ^h		2170 μmol/kg, pH 3.4, ig	Whong <i>et al.</i> (1979)
Host-mediated assay, <i>Escherichia coli</i> K-12 <i>uvrB/recA</i> DNA repair recovered from blood, livers, lungs, kidneys and testicles of male NMRI mice	– ^h		70, 210, ig ^c	Hellmér & Bolcfoldi (1992)
DNA single-strand breaks, male Fischer 344 rat stomach mucosa <i>in vivo</i> , alkaline elution	– ^h		6.9 mg/rat, ig ^c	Ohshima <i>et al.</i> (1989)
Unscheduled DNA synthesis, early-to-mid spermatids <i>in vivo</i>	– ⁱ		60 & 120, 3 days ^c , ig	Alavantić <i>et al.</i> (1988)
Unscheduled DNA synthesis, male Fischer 344 rat stomach mucosa <i>in vivo</i>	– ^h		6.9 mg/rat, ig ^c	Ohshima <i>et al.</i> (1989)
Chromosomal aberrations, male Wistar rat bone-marrow cells <i>in vivo</i>	+		1.72, ig × 2	Luca <i>et al.</i> (1987)
Chromosomal aberrations, male Swiss mouse bone-marrow cells <i>in vivo</i>	+		1.72, ig × 2	Luca <i>et al.</i> (1987)
Chromosomal aberrations, male Chinchilla rabbits bone-marrow cells, <i>in vivo</i>	± ^f		1.72 mg/kg dw, 3 months	Luca <i>et al.</i> (1987)

Table 4.2 (contd)

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Chromosomal aberrations, female albino rat, bone-marrow cells <i>in vivo</i>	+		1.25 g/L dw (210 ⁱ), 13 days ^c	el Nahas <i>et al.</i> (1984)
Chromosomal aberrations, hamster embryonic cells <i>in utero</i>	–		500, ig × 1	Inui <i>et al.</i> (1979)
Chromosomal aberrations, liver cells from embryos after exposure of pregnant albino rats	+		1.25 g/L dw (210 ^j), 13 days (days 5–18 of gestation) ^c	el Nahas <i>et al.</i> (1984)
Chromosomal aberrations, liver cells from embryos after <i>in utero</i> exposure	–		100 mg/mL dw (0.8 mg/mouse/day), 7–18 days of pregnancy	Shimada (1989)
Sperm-head abnormality test	+ ^k		120, 3 days, ig	Alavantić <i>et al.</i> (1988)

^a +, positive; (+), weakly positive; –, negative; ±, positive at certain concentrations only; NT, not tested

^b LED, lowest effective dose; HID, highest ineffective dose; dw, drinking-water; ig, intragastric; ip, intraperitoneal; doses are converted to µg/mL for in-vitro experiments and mg/kg per day for in-vivo experiment unless otherwise specified.

^c Only one dose was examined.

^d Potassium nitrite was used. All other experiments were carried out with sodium nitrite.

^e Only two doses were examined.

^f The highest dose (46.66 mg/kg ig × 2) was negative.

^g 3 h after nitrite dose

^h 2 h after nitrite dose

ⁱ 17, 28 and 34 days after the last dose of nitrite

^j estimated amount consumed per day

^k 11 and 17 days after the last dose of nitrite

transformation. Similarly, incubation of mouse BALB/c3T3 cells with sodium nitrite resulted in the induction of transformed foci (type III foci) in a dose-dependent manner.

An in-vivo intrahepatic host-mediated mutagenicity assay in female CD-1 mice or female CD rats using *S. typhimurium* G46—introduced into the animals as the detecting organism—showed endogenous formation of NDMA from sodium nitrite and dimethylamine, but no increased mutagenicity was found when sodium nitrite was administered alone. Similarly, no increased mutagenicity was observed in the host-mediated assay using *S. typhimurium* G46 or TA1530 in male ICR mice or female CD-1 mice, respectively, given sodium nitrite. Sodium nitrite administered orally to NMRI mice also had no mutagenic effect in the *E. coli* K-12 *uvrB/recA* DNA repair host-mediated assay.

No increases in DNA single-strand breaks or unscheduled DNA synthesis were observed in the pyloric mucosa of rats given sodium nitrite orally. When sodium nitrite was given to mice, no increased unscheduled DNA synthesis in early-to-mid-spermatids was observed, but sperm-head abnormality was detected 11 and 17 days after treatment.

When sodium nitrite was given in the drinking-water to pregnant rats (days 5–18 of gestation) or non-pregnant rats, chromosomal aberrations were induced in the bone marrow of both pregnant and non-pregnant adults and in the embryonic liver. Sodium nitrite was given by gavage to Syrian hamsters on days 11 and 12 of pregnancy, and embryonic cells were obtained 24 h later. A dose-dependent increase in micronucleus formation and in 8-azaguanine- and ouabain-resistant mutations in embryonic fibroblasts was observed, as well as neoplastic transformation of the cells. However, sodium nitrite given to mice in the drinking-water on days 7–18 of gestation did not induce developmental toxicity or chromosomal aberrations in fetal liver cells. No increase in micronucleated polychromatic erythrocytes was found in bone-marrow cells when sodium nitrite was given intraperitoneally or by gavage to mice as single doses.

4.3 Nitrosamines and cancer

4.3.1 Induction of cancer in rodents by nitrosamines

In rodents, nitrosamines principally induce tumours of the liver, oesophagus, nasal and oral mucosa, kidney, pancreas, urinary bladder, lung and thyroid, whereas nitrosamides induce tumours of the lymphatic and nervous systems and (when given orally) of the glandular stomach and duodenum (see Section 3). The site of tumour induction depends on the *N*-nitroso compounds, the rodent species and other factors. The organ specificity probably stems from tissue-specific cytochrome P450 isozymes that activate the nitrosamines which alkylate DNA in the organ where they are activated.

Nitrosamides often induce tumours at the site of administration, e.g. in the glandular stomach when given orally, but can also act at distant sites. Some organ specificity of nitrosamides may arise because, unlike the liver, sensitive tissues such as the brain lack

the alkyltransferase (a DNA-repair enzyme) that regenerates guanine from *O*⁶-alkylguanine (Rajewsky, 1983).

4.3.2 *Activation in organs other than the site of carcinogenesis*

Nitrosamines may become activated in one organ but alkylate DNA in another organ, where they cause cancer. DNA of blood leukocytes, including stem cells in which mutations could produce cancer, may be alkylated by nitrosamine metabolites produced in hepatocytes while blood circulates through the liver. After treatment with methylating carcinogens, including NDMA, the ratio of *N*7-methylguanine in leukocytes to that in hepatocytes was relatively constant at 0.01–0.03 (Bianchini & Wild, 1994).

Dibutylnitrosamine is a bladder carcinogen. It is metabolized in the liver to *N*-4-hydroxybutyl-*N*-butylnitrosamine, which is oxidized to *N*-3-carboxypropyl-*N*-butylnitrosamine (Okada & Ishidate, 1977). The latter is excreted in the urine. After absorption into the bladder mucosa, it could be activated by mitochondrial β -oxidation as was demonstrated in an in-vitro experiment (Janzowski *et al.*, 1994).

4.3.3 *Studies related to the etiology of colon cancer*

The section on formation of *N*-nitroso compounds in the colon presents much of the evidence in support of the view that these compounds may contribute to colon cancer. Both fresh and processed red meat have been associated with colon cancer in epidemiological studies. Processed meat contains added nitrite and fresh and processed meat contain haeme, a component of myoglobin, which may promote the formation of *N*-nitroso compounds. Factors that affect excretion of *N*-nitroso compounds in the 24-h faeces of human subjects kept in a metabolic ward have been studied. Bingham *et al.* (1996) suggested that the association between consumption of red meat and the risk for colon cancer was based on the fact that red meat raises levels of *N*-nitroso compounds in the colon contents.

Faecal excretion of *N*-nitroso compounds in humans increased 3.7-fold when 420 g beef was consumed per day; it did not decline even after this diet was consumed for 40 days; it showed a dose–response relationship with the amount of beef consumed but was not affected when chicken or fish was eaten in place of red meat. Higher faecal levels of *N*-nitroso compounds were associated with longer transit times in the gut (Bingham *et al.*, 1996; Bingham, 1999; Hughes *et al.*, 2001; Bingham *et al.*, 2002).

Faecal water derived from stools of individuals on different diets that contained various amounts of red meat were incubated with human adenocarcinoma cells (HT-29) and examined by the comet assay, which showed the presence of DNA strand breakage. The results were correlated with age, but not with the concentration of apparent *N*-nitroso compounds in the faecal water samples, with the amount of meat in the diet or with the concentration of apparent *N*-nitroso compounds in faecal homogenates (Cross *et al.*, 2006). Possibly, the use of faecal water instead of faecal homogenates prevented

meaningful correlations from being established. Other studies have found that water extracts of faeces are directly mutagenic in the Ames test (Kuhnlein *et al.*, 1981).

Colonic DNA of subjects from the United Kingdom (a high-incidence area for colon cancer) contains *O*⁶-methylguanine. This supports the view that *N*-nitroso compounds in the colon alkylate colonic DNA (Povey *et al.*, 2000). Levels of faecal *N*-nitroso compounds were higher in subjects who ate liver and blood sausages as sources of haeme, but not in subjects who consumed inorganic iron. This suggests that the haeme in myoglobin (the pigment in red meat) increases the formation of *N*-nitroso compounds in the colon and hence explains why red but not white meat is a risk factor for colon cancer (Cross *et al.*, 2003).

In a recent study, humans were fed a red meat diet, exfoliated colonic mucosal cells were collected from the surface of faecal pellets and their DNA was analysed for *O*⁶-carboxymethyl guanine by an immunohistochemical method. Levels of this adduct were higher after a red meat diet than after a control diet (Lewin *et al.*, 2006). This adduct, as well as *O*⁶-methylguanine, were reported earlier to be generated from the reaction of nitrosated glycine derivatives with guanine in DNA (Harrison *et al.*, 1999).

4.3.4 *Studies in rats and mice on total apparent colonic and faecal N-nitroso compounds*

Despite all the research on faecal and colonic *N*-nitroso compounds, it has not been determined whether they are carcinogenic for the colon. The following evidence suggests that colonic *N*-nitroso compounds could cause colon cancer: (a) the high level of *N*-nitroso compounds after consumption of red meat and the low level after consumption of chicken or fish, as reported by Bingham (1999); (b) the finding that the colonic contents of subjects who eat red meat are genotoxic, as determined by the comet assay (Cross *et al.*, 2006); and (c) the finding that *N*-nitroso compound precursors purified from hot dogs and then nitrosated are direct mutagens in the Ames test (Zhou *et al.*, 2006).

In a study to assess the possible chemical transformation by reactive nitrogen–oxygen species of the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), which is formed during the cooking of red meat, various methods to generate these species were investigated in in-vitro systems. Conditions that produced nitric oxide, a nitrosating agent under oxidizing conditions, yielded a product that was identified by mass spectrometry and nuclear magnetic resonance as *N*-nitroso-IQ. The results demonstrate the chemical transformation and activation of IQ by reactive nitrogen–oxygen species and the further activation of its *N*-nitroso product to a DNA-reactive metabolite. The nitrosation of IQ was strongly potentiated by haemin, the ferric porphyrin component of haemoglobin (Lakshmi *et al.*, 2002, 2005).

4.3.5 *Studies related to gastric cancer*

The incidence of gastric cancer is far higher in Japan and China than in the USA. This cancer was extremely common in the USA until the Second World War, but its rate has dropped dramatically since then. This decrease has been associated with the widespread introduction of home refrigerators after the war and the resultant decrease in the use of cured meat and an increase in the consumption of fresh vegetables and fruit. The hypothesis that *N*-nitroso compounds are a cause of gastric cancer was first suggested in 1971 (Mirvish, 1971) and has been reviewed several times (Preston-Martin & Correa, 1989; Boeing, 1991; Eichholzer & Gutzwiller, 1998; Jakszyn & Gonzalez, 2006).

Oral administration of certain nitrosoureas and related nitrosocarbamates, as well as the related compound MNNG, induced glandular stomach adenocarcinomas (similar to human gastric cancer) in rodents and other species (Druckrey *et al.*, 1966; Ogiu *et al.*, 1975; Maekawa *et al.*, 1976; Sugimura & Kawachi, 1978).

Nitrosation of foods associated with gastric cancer produced direct mutagens in the Ames test (Marquardt *et al.*, 1977). Treatment of extracts of mackerel fish with nitrite at pH 3 produced direct mutagens in *S. typhimurium*. When the extracts were administered thrice weekly for 6 months to Wistar rats, eight of the 12 rats developed gastrointestinal tumours 12–18 months later. Five of the rats developed adenomas or adenocarcinomas of the glandular stomach. Precancerous lesions were observed in nearly all treated animals. Feeding extracts that had not been nitrosated did not induce tumours (Weisburger *et al.*, 1980).

(a) *Role of Helicobacter pylori*

Gastric infection with *H. pylori* contributes to the etiology of gastric cancer (Muñoz, 1994; Fischbach *et al.*, 2005). Intestinal metaplasia of the stomach (a precursor lesion for gastric cancer) was associated with *H. pylori* infection, low levels of ascorbic acid and elevated bile acid levels, but not with elevated gastric nitrite or total *N*-nitroso compounds in gastric juice (Sobala *et al.*, 1991). Eradication of *H. pylori* restored gastric ascorbic acid to normal levels (Sobala *et al.*, 1993). In *H. pylori*-positive patients, low gastric levels of ascorbic acid were correlated with the extent of inflammation and with elevated gastric pH (Rood *et al.*, 1994). Gastric adenocarcinomas that resembled human gastric cancer were induced in Mongolian gerbils given *N*-methylnitrosourea (MNU) in the drinking-water and inoculated with *H. pylori* before or after the carcinogen treatment, but not when they were treated with either agent alone (Sugiyama *et al.*, 1998).

(b) *O⁶-Methylguanine in leukocyte DNA*

Samples of peripheral blood were collected from randomly selected subjects from 17 populations with different incidences of gastric cancer. Leukocyte DNA was analysed for *O⁶*-methylguanine by a competitive repair assay that can detect 0.05 fmol/μg DNA. Adducts were detected in 21 of 407 samples, including 16 of 102 samples from Portugal and Japan, where the incidence of gastric cancer is high. The presence of adducts was

significantly correlated with the incidence of gastric cancer and with a low (< 25 ng/mL) serum level of pepsinogen A, a marker of severe chronic atrophic gastritis (The EUROGAST Study Group, 1994). This suggests that the achlorhydric stomach produces methylating agents, which could be *N*-nitroso compounds or a compound formed by the nitrosation of methionine (Chen *et al.*, 1996).

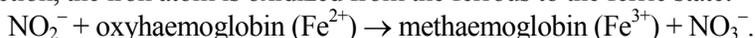
(c) *Mutations in the P53 gene*

Mutations in the *P53* gene were studied in 105 cases of gastric and colon cancer from Florence, Italy, to determine whether environmental factors contributed to the induction of gastric cancer in cases who had different types of *P53* mutation. The 33 cases who showed *P53* mutations ate a more traditional diet, including corn meal and meat soup, ate less fresh fruit and vegetables and had a higher nitrite intake than cases of gastric cancer who had no *P53* mutations. Of the 33 cases with *P53* mutations, 19 had G:C→A:T mutations at CpG sites. The 14 cases with mutations at non-CpG sites may have had higher exposure to dietary alkylating agents, whereas patients with *P53* mutations at CpG sites were more closely related to *H. pylori* infection (Palli *et al.*, 1997).

4.4 Other relevant toxic effects

4.4.1 *Methaemoglobinaemia*

The most widely recognized effect of excess nitrate on health is methaemoglobinaemia, a condition whereby methaemoglobin is formed in the blood due to the interaction of haemoglobin with nitrite, the reduction product of nitrate. During this reaction, the iron atom is oxidized from the ferrous to the ferric state:



As a consequence, oxygen is less efficiently transported by the red blood cell.

The condition has been observed primarily in infants under 6 months of age, and particularly in those under 3 months of age (Comly, 1945; Bosch *et al.*, 1950). Infantile methaemoglobinaemia is also commonly known as the blue baby syndrome. Infants are particularly susceptible because (a) fetal haemoglobin is more easily oxidized to methaemoglobin and a large proportion of fetal haemoglobin is still present in the infant's blood (60–80% at less than 3 months, 20–30% by 3 months of age) (National Research Council, 1981; WHO, 1996a); (b) infants have a transient deficiency in nicotinamide adenine dinucleotide phosphate-dependent cytochrome *b*₅ methaemoglobin reductase, which is responsible for the reduction of methaemoglobin back to haemoglobin; (c) lower production of gastric acid (i.e. a higher gastric pH) in infants leads to greater reduction of nitrate to nitrite by gastric bacteria; (d) on a weight basis, infants have a higher intake of nitrate (in the diet and because of greater fluid intake relative to body weight); and (e) the occurrence of infantile gastroenteritis with acidosis and gastrointestinal infections increases the yield of nitrite and thus the formation of methaemoglobin.

In addition to methaemoglobin being incapable of binding oxygen, the oxidation of one or more of the haeme iron atoms in the haeme tetramer distorts the tetramer structure, so that the remaining non-oxidized haeme subunits bind oxygen avidly but release it less efficiently, which shifts the oxygen dissociation curve to the left. Therefore, methaemoglobinaemia becomes manifest as tissue hypoxia (Bradberry *et al.*, 2001).

As the level of methaemoglobin increases, cyanosis (with a change in skin colour to bluish grey or brownish grey), anoxaemia and even death can result. Low concentrations of 0.5–3% methaemoglobin occur in normal people and concentrations up to 10% can occur without clinical signs (National Research Council, 1981). The signs and symptoms associated with increasing levels of methaemoglobin are: 10–20%, central cyanosis of the limbs and/or trunk; 20–45%, central nervous system depression (headache, dizziness, fatigue, lethargy) and dyspnoea; 45–55%, coma, arrhythmias, shock and convulsions; and > 60%, high risk of mortality (Fewtrell, 2004). Methylene blue is used as an antidote and ascorbic acid has been shown to have protective effects against nitrite-induced formation of methaemoglobin.

The current WHO (2003) drinking-water guideline (50 mg/L) and the Environmental Protection Agency (2003) drinking-water standard for the USA (45 mg/L or 10 mg/L nitrate-N) for nitrate were established to prevent infantile methaemoglobinemia (see Section 1). The level was based principally on the report of a worldwide survey in which no cases of methaemoglobinaemia were observed with concentrations of nitrate below 45 mg/L (Walton, 1951). Reported cases of methaemoglobinaemia have generally occurred in infants under 3 months of age who had consumed water that contained levels of nitrate higher than 22 mg/L nitrate-N (Craun *et al.*, 1981). The absence of cases with levels of nitrate below 45 mg/L (< 10 mg/L nitrate-N) was confirmed in more recent reviews (Fan & Steinberg, 1996; WHO, 1996b, 2003). It was noted that gastrointestinal illness that results from exposure to bacterially contaminated water containing high levels of nitrate may also play an important role in methaemoglobinaemia (Craun *et al.*, 1981).

Methaemoglobinaemia has been reported in children who were fed powdered milk or infant formula diluted with well-water that contained nitrate. Cases have also been reported in babies fed vegetables, particularly carrot juice and spinach, in which nitrate had been converted to nitrite through bacterial action during storage or transportation (Simon, 1966; Phillips, 1968; Keating *et al.*, 1973).

Clinical cases of adult methaemoglobinaemia due to consumption of water contaminated by nitrate have not been reported, but cases in adults who consumed high doses of nitrate or nitrite by accident or as a medical treatment have been described (Cornblath & Hartmann, 1948).

In a recent literature survey for WHO, it was found that reports of nitrate concentrations in drinking-water higher than 50 mg/L were rarely paralleled by reports of methaemoglobinaemia (Hoering & Chapman, 2004). Another literature-based investigation for WHO (Fewtrell, 2004) did not identify exposure–response relationships that relate the level of nitrate in the drinking-water to methaemoglobinaemia.

Individuals may vary in susceptibility to the formation of methaemoglobin (WHO, 2002). The nitrate:nitrite conversion rate ranges from 5 to 7% for normal individuals to 20% for individuals with a high rate of conversion. Susceptible groups other than infants include people deficient in glucose-6-phosphate dehydrogenase (Foltz *et al.*, 2006) or methaemoglobin reductase (Percy *et al.*, 2005). In healthy individuals, low gastric pH suppresses the growth of nitrate-reducing bacteria and hence gastric levels of nitrite are low. Although the gastric pH in infants may be higher than that in adults, it is possible that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors that contribute to infantile methaemoglobinaemia (WHO, 2002).

4.4.2 *Reproductive and developmental effects*

Some epidemiological studies have suggested an association between exposure to nitrates in the drinking-water and spontaneous abortions, intrauterine growth restrictions and various birth defects. These studies have uncertainties or limitations such as the lack of individual exposure assessment, the inability to rule out confounding factors or the small size of the study populations. Currently, there is insufficient evidence for an exposure–response or causal relationship between exposure to nitrate in the drinking-water and adverse reproductive or developmental effects (Manassaram *et al.*, 2006), although some studies suggested an association.

Scragg *et al.* (1982) conducted a study among pregnant women in South Australia and reported an excess of malformations among newborns (mainly neural tube defects and defects that affected multiple systems) in association with drinking-water consumption from specific sources that differed in nitrate content. Women who used groundwater sources that contained levels of nitrate above 5 mg/L had a statistically significantly increased risk for delivering a child with congenital malformations than women who drank rainwater. The risk was higher for central nervous system defects.

In a follow-up study, Dorsch *et al.* (1984) found that women who consumed principally groundwater had a statistically significant increase in risk for giving birth to a malformed child compared with women who drank only rainwater during their pregnancy. Water from private bores mostly had nitrate levels in excess of 15 mg/L, and municipal water supplies derived from government bores had nitrate levels below or equal to 1 mg/L. The increase occurred specifically for malformations of the central nervous system and musculoskeletal system. There was a nearly threefold increase in risk for women who drank water that contained nitrate at 5–15 mg/L, and a fourfold increase for those who consumed > 15 mg/L nitrate. A seasonal gradient in risk was observed among groundwater consumers, ranging from 0.9 for babies who were conceived in winter, to 3.0 in autumn, to 7.0 in spring and to 6.3 in summer.

Ericson *et al.* (1988) examined all deliveries in Sweden in a case–control study of neural tube defects and did not report any difference in levels of nitrate.

In a Swedish study, a weak association was noted between nitrate in water at a level ≥ 2 mg/L and cardiac malformations (Cedergren *et al.*, 2002).

Brender *et al.* (2004) examined exposure to nitrosatable drugs and pregnancies affected by neural tubal defects in relation to dietary nitrite and total nitrite intake (dietary nitrite plus 5% dietary nitrate) in a case-control study of Mexican-American women in Texas, USA. Interviews were conducted with 184 women who had had pregnancies affected by neural tubal defects and 225 women who had had normal livebirths. In addition, nitrate was measured in the usual source of drinking-water for 110 study participants. Nitrosatable drugs increased the probability (2.7-fold; 95% CI, 1.4–5.3) of a pregnancy affected by neural tubal defects. Women with nitrite intake of more than 10.5 mg per day had a 7.5-fold (95% CI, 1.8–45) probability of a pregnancy affected by neural tubal defects if they took nitrosatable drugs. A stronger association (14-fold) was also reported between exposure to nitrosatable drugs and neural tubal defects in women who used drinking-water containing ≥ 3.5 mg/L nitrate.

4.4.3 *Cardiovascular effects*

As discussed above, exposure to nitrate can result in methaemoglobinaemia after conversion to nitrite in the body. Nitrite, but not nitrate, is a smooth muscle relaxant and can produce hypotension especially in the vascular system at doses that overlap those that cause methaemoglobinaemia (National Research Council, 1995). High concentrations of methaemoglobin are associated with hypotension, rapid pulse and rapid breathing, as a result of the vasodilatory effects of nitrite.

A hospital-based study in Colorado, USA, reported on cardiovascular effects in nine communities in Weld County where community water supplies were evaluated as nitrate-free and nine areas where the drinking-water contained 19–125 mg/L nitrate. Among 487 cases of hypertension, there was no evidence of an increased risk for hypertension with increased nitrate consumption. Among residents in the nitrate-exposed communities, an earlier onset of hypertension was observed, which peaked at 50–59 years of age versus 70–79 years in the unexposed (Malberg *et al.*, 1978).

4.4.4 *Effects on the thyroid*

(a) *Nitrate*

van Maanen *et al.* (1994) studied the effect of nitrate on the volume and function of the thyroid in humans exposed to nitrate in the drinking-water. The test populations consisted of healthy women volunteers who had no disease, did not use medication, were not pregnant and had no outdoor jobs. The study included two groups exposed to low (0.02 mg/L, $n = 24$) and medium (17.5 mg/L, $n = 27$) levels of nitrate in tap-water and two groups exposed to medium (≤ 50 mg/L, $n = 19$) and high (> 50 mg/L, $n = 12$) levels of nitrate in well-water. No iodine deficiency was observed in any of the groups. Concentrations of urinary and salivary nitrate were related in a dose-dependent manner to the consumption of water that contained nitrate. A dose-dependent difference in thyroid volume was observed between low and medium versus high exposure to nitrate, and this

thyroid hypertrophy was significant at nitrate levels exceeding 50 mg/L. Linear regression analysis showed a significant inverse relationship between thyroid volume and the concentration of thyroid-stimulating hormone in serum.

An epidemiological survey was conducted on the incidence of goiter among 181 children 6–14 years of age in the village of Karadzhalovo, a district of Plovdiv, Bulgaria, where the concentration of nitrates in drinking-water was constantly above the tolerable level (range: 78–112 mg/L during 1990–94). Population morbidity was assessed according to ICD-9 and recorded over 5 years (1990–94). The data were compared with those from 178 children of a control village in the same district, where nitrate levels in drinking-water were 28–48 mg/L during the same period. Non-parametrical analysis was used to make a comparative analysis of incidence of disease in both communities during the observation period. A statistically significant difference in the incidence of goiter in children in Karadzhalovo was found (40.9%) compared with that in children in the control village (Gatseva & Dimitrov, 1997; Gatseva *et al.*, 1998).

Tajtáková *et al.* (2006) conducted an investigation to assess whether long-term intake of water that contained high levels of nitrate and meals from local agricultural products in eastern Slovakia influence the volume and function of the thyroid in school children. Thyroid volume, serum thyrotropin and anti-thyroperoxidase antibodies were measured in 324 children (10–13 years of age) from high-nitrate areas (51–264 mg/L) who received drinking-water from shallow wells and compared with those of 168 children of the same age from neighbouring low-nitrate areas (< 2 mg/L) and 596 children from the city of Kosice located in the vicinity of the low-nitrate area and also supplied by low-nitrate water. Thyroid volume in the children from high-nitrate areas was significantly larger compared with that in the two groups from the low-nitrate areas. The frequency of serum thyrotropin levels in the range of subclinical hypothyroidism (> 4.0 mU/L) in pooled age groups from the high-nitrate area was 13/324 (4.0%) and the frequency of anti-thyroperoxidase antibodies was 8/324 (2.5%), while the 109 children from the low-nitrate area did not show any of these changes. There were no differences in concentrations of total thyroxine or free triiodothyronine.

4.4.5 *Diabetes mellitus*

A prospective case–control study was conducted in Sweden on dietary factors and the risk for diabetes mellitus. The study population included 339 children aged 0–14 years who had recently developed type I diabetes mellitus and 528 control children matched for age, sex and county of residence. Foods were identified as rich in various nutrients and additives and ‘nitrate or nitrite’ but no quantitative data were given relating to the levels of nitrate or nitrite intake. A significant but non-linear trend was found for dietary intake of nitrates or nitrites in relation to type I diabetes mellitus. The trend was not affected when the results were standardized for possible confounders (Dahlquist *et al.*, 1990).

In a nationwide Finnish case–control study on the epidemiology of type I diabetes, dietary intake of nitrite of children and mothers was positively associated with the risk for

type I diabetes independently from duration of mother's education, child's or mother's age, place of residence or mother's smoking status. The study population consisted of 684 case and 595 control children, 548 case-control pairs of fathers and 620 case-control pairs of mothers. Food consumption frequencies were obtained by structured questionnaire and data on nitrate and nitrite in household water were provided by the Finnish waterworks. The positive association between nitrite intake of the child and mother and risk for type I diabetes in children was seen in all age groups (0–4, 5–9 and 10–14 years) and in both boys and girls. The nitrate intake of the mother was associated with a decreased risk for type I diabetes in children (Virtanen *et al.*, 1994).

The possible relationship between the incidence of type I diabetes and nitrate levels in drinking-water in the Netherlands was studied in an ecological study (van Maanen *et al.*, 2000). Among a total of 2 829 020 children, 1104 cases of type I diabetes were diagnosed in children aged 0–14 years during 1993–95, and 1064 of these cases were included in the analysis. Two exposure categories were used: one was based on an equal number of children exposed to various levels of nitrate (0.25–2.08, 2.10–6.42 and 6.44–41.9 mg/L), and the other was based on cut-off values of 10 and 25 mg/L nitrate. An effect was observed with increasing age of the children on the incidence of type I diabetes but no significant effects were found relating to sex or concentration of nitrate in the drinking-water. The 1.46-fold increase in the incidence rate ratio (95% CI, 0.88–2.43) at nitrate exposures > 25 mg/L was not significant, probably due to small numbers (15 observed, 10 expected).

Zhao *et al.* (2001) conducted a study in Cornwall and the former Plymouth Health Authority Regions in the United Kingdom to examine the relationships between nitrate, zinc and magnesium in the drinking-water and risk for childhood onset of type I diabetes mellitus. Children aged 0–15 years who had been diagnosed with type I diabetes mellitus between 1975 and 1996 (total, 517 subjects) were included. The mean concentration (\pm standard deviation) of nitrate was 6.62 \pm 5.02 mg/L (range, 0.49–31.9). The study found no association between nitrate in the drinking-water and risk for childhood onset of type I diabetes mellitus.

4.5 Mechanistic considerations

4.5.1 Possible carcinogenic pathways involving nitrite

Under acidic conditions, nitrite is converted to nitrous acid, which can then be converted to nitrosating agents such as nitrous anhydride, dinitrogen tetroxide and the nitrous acidium ion. Similar nitrosating agents can also be formed from nitrogen oxides in the presence of oxygen. Nitrogen oxide may be formed by synthases that are expressed in many cell types and by bacterial reduction of nitrite/nitrate. Once formed, nitrous anhydride or dinitrogen tetroxide can react with unprotonated amino groups at neutral pH, resulting in the formation of *N*-nitroso compounds.

Nitrosating agents react with secondary amines to form dialkyl or cyclic nitrosamines, which can cause a variety of types of damage to DNA as well as DNA mutations. Many of these compounds induce tumours experimentally at various sites of all species (more than 40, including primates) that have been tested to date (Searle, 1976; Lijinsky, 1979; Brown, 1999).

Nitrosamines can be oxidized *in vivo* by cytochrome P450 enzymes (Yang *et al.*, 1990) to form α -hydroxynitrosamines that rapidly rearrange and decompose to form aldehydes and electrophilic diazohydroxides. The diazohydroxides react with nucleophilic sites in DNA to form premutagenic lesions (Park *et al.*, 1977; Gold & Linder, 1979; Lown *et al.*, 1984; Poulsen *et al.*, 1987; Ukawa-Ishikawa *et al.*, 1998). The mutagenicity of nitrosamines has been investigated extensively (Guttenplan, 1987). Nitrosation of *N*-alkylamides yields *N*-nitrosamides, which can form analogous alkylating species that do not require enzymatic oxidation, especially under basic conditions. For example, a group of over 30 nitrosamides with varying structures were all found to be direct-acting mutagens (Lee *et al.*, 1977; Lijinsky & Andrews, 1979) and several of these have been shown to be direct-acting carcinogens in animals (Lijinsky, 1977; Sugimura & Kawachi, 1978; Yamashita *et al.*, 1988; Wakabayashi *et al.*, 1989). Hence, nitrosamines are probably activated mostly in the organs in which tumours develop.

Nitrosation of primary amines can lead directly to alkyl diazohydroxides or alkyl diazonium ions that, if formed in close proximity to DNA, can lead to alkylation of DNA bases. This reaction can also lead to deamination of DNA bases by nitrosating agents through the nitrosation of exocyclic amino groups. Examples include the deamination of 5-methylcytosine to thymine, of cytosine to uracil, of adenine to hypoxanthine and of guanine to xanthine. Nguyen *et al.* (1992) summarized the types of mutation induced by these deaminations. Deamination of 5-methylcytosine results in the formation of thymine which is a normal base and is not easily repaired. This base change leads to G:C→A:T transition mutations at CpG sites, which are frequently detected in many types of human cancer.

Early studies demonstrated that *N*-nitroso compounds form adducts at several sites in bases. The *O*⁶-alkyl adducts of guanine are promutagenic and can induce G:C→A:T transitions. MNU-induced rat mammary tumours had a high incidence of c-H-*ras* oncogene mutations with G:C→A:T transitions at codon 12 (Zarbl *et al.*, 1985; Inui *et al.*, 1994). Similarly, NDMA-induced kidney tumours and MNU-induced oesophageal tumours in rats had a high incidence of mutated *p53* genes with G:C→A:T transition at codons 204 and 213 (Ohgaki *et al.*, 1991). G:C→A:T transitions at both CpG and non-CpG sites are among the most common types of mutation found in *P53* genes from human tumours (Olivier *et al.*, 2004).

Thus, either ingested or endogenously formed *N*-nitroso compounds are potentially carcinogenic through several related pathways. Examples of these in the context of the epidemiological findings presented earlier with regard to specific tumours are summarized below.

4.5.2 *Colon cancer*

The mechanism proposed for the association between consumption of nitrite-preserved meat and colon cancer is that *N*-nitroso compounds present in the meat pass down the gut to the colon and act there to induce cancer, or that *N*-nitroso precursors present in processed meat are converted to *N*-nitroso compounds in the stomach or intestines and that these *N*-nitroso compounds act as carcinogens in the colon. Evidence for this hypothesis is (a) that total *N*-nitroso compounds in the faeces increased nearly fourfold when volunteers consumed beef compared with those who did not (Hughes *et al.*, 2001); (b) that total *N*-nitroso compound precursors from hot dogs, when partially purified and then nitrosated *in vitro*, were directly mutagenic in the *Salmonella* mutagenicity assay (Ames test); (c) that *O*⁶-carboxymethylguanine was identified in the DNA of exfoliated colonic cells after human volunteers had consumed red meat (Lewin *et al.*, 2006), which is the adduct expected from the reaction of nitrosated glycine derivatives with DNA (Harrison *et al.*, 1999); and (d) that IQ, one of the heterocyclic amine carcinogens produced when meat is cooked at high temperatures (Sugimura *et al.*, 1990; Nagao & Sugimura, 1993), reacts readily with nitrogen oxide in the presence of haemin and hydrogen peroxide to produce *N*-nitroso-IQ, which is a potent mutagen (Wolz *et al.*, 1995).

4.5.3 *Oesophageal cancer*

The evidence that nitrosamines are a cause of oesophageal cancer can be summarized as follows (Mirvish, 1995): (a) in rats, oesophageal tumours are induced by many unsymmetrical and cyclic nitrosamines; (b) urinary excretion of NPRO was correlated in ecological studies with the incidence of oesophageal cancer (see Section 4.1.4); (c) *O*⁶-methylguanine was detected in the DNA of normal oesophageal tissue of oesophageal cancer patients in China, which suggests the action of a methylating nitrosamine, such as NDMA (Umbenhauer *et al.*, 1985); (d) tobacco-specific nitrosamines, especially *N*'-nitrosornicotine and 4-(nitrosomethylamino)-1-(3-quinolyl)-1-butanone, induce oesophageal cancer in rats and are very probably the cause of human oesophageal cancer induced by tobacco smoking, which acts synergistically with alcohol.

4.5.4 *Brain tumours*

A large number of epidemiological studies have investigated a possible relationship between intake of nitrate/nitrite and tumours of the brain, and suggestive associations were apparent in several of these (see Section 2.3). However, the association is somewhat less straightforward than that for the colon and stomach, since the route from the digestive system to the site of action is less apparent. There is nevertheless reliable evidence that nitrosoureas can act at sites remote from the site of administration. For example, after an intraperitoneal injection of radiolabelled ENU into rats, Goth and Rajewsky (1974) found *O*⁶-ethylguanine in the brain. The label was also found in non-target tissues (e.g. liver),

but the rate of clearance was lower in the brain, which led the authors to suggest that the longer persistence might allow more DNA replication in the brain and thus present a greater possibility of mutation induction and subsequent tumour formation.

4.6 References

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5. Summary of Data Reported

5.1 Exposure data

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle and are ubiquitous in the environment. Since the early 1900s, nitrate and other nitrogen compounds have been used extensively as fertilizers in agriculture. Moreover, nitrate and nitrite are added to meat and fish for preservation, as colour fixatives and as flavouring agents.

The natural background level of nitrate in groundwater is generally below 10 mg/L. Both groundwater and surface water can be contaminated by nitrate as a result of agricultural activities. Levels of contamination are usually significantly higher in groundwater, particularly in shallow wells. Concentrations that exceed the current WHO guideline of 50 mg/L, and exceptionally exceed 500 mg/L, have been reported in intensive agricultural regions. Nitrite is not frequently detected in drinking-water; when it is present, its concentrations rarely exceed 3 mg/L.

Human exposure to nitrate is principally from exogenous sources, i.e. through the ingestion of food and water, whereas exposure to nitrite is primarily endogenous (see Section 5.4 on the endogenous formation of nitrite).

The main potential contributors to ingested nitrate are vegetables, especially leafy vegetables. Other food sources include bakery goods, cereal products and cured meat. Water is generally a minor source of nitrate, but may become a major contributor to overall nitrate intake at concentrations above 50 mg/L. For an average adult consumer who lives in an area with low drinking-water contamination, total exposure to nitrate from food and water is estimated to be about 60–90 mg per person per day. For high consumers of vegetables, the intake of nitrate may reach 200 mg per person per day. Similar intakes could result from high consumption of water contaminated with more than 50 mg/L nitrate. The impact of contaminated water was confirmed by studies of total internal exposure to nitrate that was measured through 24-h urinary excretion.

The main sources of exogenous human exposure to nitrite are food (see Section 5.4 on the endogenous formation of nitrite). Over the past 30 years, the relative contribution of nitrite-cured meat to dietary exposure to nitrite for an average consumer has decreased substantially. Other sources of nitrite include cereal products and vegetables that are rich in nitrate. The total intake of exogenous nitrite is estimated to be about 0.75–2.2 mg per day for an adult with average food consumption and drinking patterns. Nitrite may react with amines and amides to form *N*-nitroso compounds during the storage of food.

5.2 Human carcinogenicity data

Ingested nitrate and nitrite have been studied in relation to the occurrence of many cancers; however, with the exception of stomach and brain cancers, few case-control or cohort studies are available for any given cancer site. Ingestion of nitrate and nitrite can result in the endogenous formation of *N*-nitroso compounds, particularly in the presence of nitrosatable precursors and in the absence of inhibitors of nitrosation such as vitamin C. Some of the epidemiological studies evaluated nitrite intake from cured meats or animal sources separately. Others evaluated risk among persons who had a higher nitrite and lower vitamin C intake, a dietary pattern that may result in increased endogenous formation of *N*-nitroso compounds. The Working Group gave these latter studies more weight in the evaluation of the literature.

Vegetables are the primary source of nitrate when levels of nitrate in the drinking-water are low. Because many vegetables contain vitamin C or other inhibitors of endogenous nitrosation, nitrate from these sources may result in less endogenous formation of *N*-nitroso compounds than nitrate in the drinking-water. The Working Group therefore considered the evaluation of nitrate ingested in the diet and in drinking-water separately.

5.2.1 *Gastric and oesophageal tumours*

(a) *Ingested nitrate*

The relationship between stomach cancer and nitrate in the drinking-water has been addressed in 15 ecological studies, two case-control studies and one cohort study. The two case-control studies (one in the USA and the other in Taiwan, China) used deceased cases and reported no association. A cohort study in the Netherlands addressed the contribution of nitrate from water as part of total nitrate intake separately from dietary sources; no association was observed. No clear evidence emerged from the ecological studies.

The association between the intake of nitrate from foods and stomach cancer was analysed in seven case-control and two cohort studies. Three case-control studies reported no association while four found a lower risk for stomach cancer with higher levels of dietary nitrate; no association between dietary nitrate and risk for stomach cancer was seen in the two cohort studies in the Netherlands and Finland. Most of these studies were carried out in populations who had relatively low levels of nitrate in the drinking-water (populations who used public water supplies); thus the majority of the ingested nitrate was from foods, of which vegetables were the main source. The inverse association found in some studies may be attributed to a high correlation between dietary levels of nitrate and high vegetable consumption.

Only one case-control study that was conducted in the USA assessed the relationship between oesophageal cancer and dietary intake of nitrate, for which an inverse association was found. In ecological studies of nitrate in the drinking-water, the pattern for

oesophageal tumours was similar to that described for stomach cancer, although the number of studies is substantially smaller. The few ecological studies provided no clear evidence for an association between nitrate in the drinking-water and oesophageal cancer.

(b) *Ingested nitrite*

The evidence for an association between dietary intake of nitrite and cancer of the stomach is based on seven well designed case-control studies and two cohort studies, all of which were carried out in Europe and North America.

Six of seven case-control studies found a positive association, which was significant in four. Two studies considered the potential interaction between dietary intake of nitrite and potential inhibitors of nitrosation: a large case-control study conducted in several areas of Italy reported a fivefold increase in risk for subjects who ingested high levels of nitrite and proteins and low levels of antioxidant micronutrients (vitamin C and α -tocopherol) compared with those who had a low intake of nitrite and proteins and a high intake of antioxidants. A multicentric study in the USA reported a significant increase in risk for stomach tumours located in both the cardia and non-cardia regions among subjects who had a high intake of nitrite and a low intake of vitamin C compared with those who had a low intake of nitrite but high vitamin C consumption. Four of the seven case-control studies also assessed the association between stomach cancer and preformed *N*-nitrosodimethylamine: one study in Canada and a study in Italy found no association, while one study in Spain and another in France found that a high intake of *N*-nitrosodimethylamine was positively associated with risk for stomach cancer. None of the studies reported effect estimates for nitrite adjusted for *N*-nitrosodimethylamine.

Two cohort studies were reviewed, one of which was conducted in the Netherlands and the other in Finland. In the Finnish study, no association was found between the risk for stomach cancer and dietary intake of nitrites or *N*-nitrosodimethylamine. The Dutch cohort reported a significant increase in risk for nitrite that was limited to the highest level of intake and became non-significant after adjustment for potential confounders, including vitamin C and β -carotene. However, effect modification of nitrite by vitamin C was not assessed in this study. Overall, the results were consistent for case-control studies, but none of the prospective studies found a clear positive association. Furthermore, none of the studies that were reviewed had taken into account potential confounding or effect modification by *Helicobacter pylori*, an important risk factor for stomach cancer, when assessing the effect of nitrite.

Only two case-control studies of oesophageal cancer, both of which were conducted in the USA, assessed the association with nitrite intake. Both were well designed and adjustment was made for the main risk factors for oesophageal cancer. Both studies reported a positive but non-significant association. When intake of vitamin C was taken into account, both studies observed a significant increase in risk for subjects who had a high intake of nitrite and a low intake of vitamin C compared with those who had low nitrite and high vitamin C consumption. The potential confounding effect of preformed *N*-nitroso compounds was not investigated.

5.2.2 *Brain tumours*

(a) *Ingested nitrate*

Overall, the Working Group evaluated 10 case-control studies of brain tumours, six of which were conducted in adults and four in children. In one of the four case-control studies of childhood brain tumours, current levels of nitrate in the tap-water in homes in which the pregnancies had occurred were estimated using non-validated measurements from semiquantitative water test strips. No significant association was observed, although the risk for astroglial tumours was non-significantly increased twofold for the highest category of exposure (≥ 50 mg/L as ion).

Two case-control studies of adult brain tumours estimated levels of nitrate in the drinking-water by linking information on residence to public water quality monitoring data from regions that had mainly low to moderate levels of nitrate contamination. One of them also measured nitrate levels in a current tap-water sample from users of private wells. Nitrate in drinking-water was not associated with risk for brain tumours in either of these studies.

None of the studies that focused on dietary sources of nitrate observed a positive association with brain tumours among adults or children. Some observed a decreased risk for brain tumours in relation to dietary nitrate. However, in studies that further adjusted for vitamin C intake, this association was attenuated or disappeared.

(b) *Ingested nitrite*

The Working Group evaluated 12 case-control studies that focused on nitrite in the diet or in drinking-water, five of which investigated brain tumours in children and four of which examined maternal diet during pregnancy as a possible risk factor for the development of brain tumours in the offspring. The largest case-control study that was conducted in western USA observed no association between estimated dietary intake of nitrite and the incidence of childhood brain tumours. However, when the source of dietary nitrite was considered, children born to mothers who had the highest category of intake of nitrite specifically from cured meat (> 1.28 mg per day) had an almost twofold increased risk for brain tumours; nitrite intake from vegetable sources was not associated with the occurrence of brain tumours. A re-analysis of these data using estimated nitrite levels in cured meats during the year of the pregnancy suggested a stronger association (a threefold increase in the highest category of intake of nitrite from cured meat; ≥ 3.0 mg per day). No increased risk in relation to overall dietary nitrite was observed in studies in Israel or France or in a study in North America that focused on children $<$ under 6 years of age who had either astrocytic gliomas or primitive neuroectodermal tumours. These studies did not quantify nitrite intake from cured meat specifically.

Nitrite in the drinking-water was investigated in a study of the children who were included in the studies from western USA and France together with children from Spain. Current levels of nitrite in the tap-water in homes in which the pregnancies had occurred were estimated using non-validated measurements from semiquantitative water test strips.

This study reported a twofold increase in risk for brain tumours in the offspring for both categories of detectable nitrite in the drinking-water. This association was stronger among women who did not rely on bottled water and was confined to astroglial tumours.

Seven studies of dietary intake of nitrite and adult brain tumours were conducted; five were restricted to glioma and another study in Germany provided risk estimates for glioma only. No significant associations were reported for dietary nitrite intake overall. The largest study in California, USA, observed a twofold increase in risk among men who consumed levels of nitrite above the median and levels of vitamin C below the median; this pattern did not occur among women. Two small studies in the USA, one in Ohio and one among women in California, observed a positive association with intake of nitrite from cured meat; a larger case-control study in Nebraska, USA, observed no association with nitrite from animal sources but a threefold increase in risk for glioma among persons who had high consumption of nitrite from plant sources. The study from Nebraska observed no interaction between dietary intake of nitrite and vitamin C. No study reported risk estimates for meningioma or other tumour types.

5.2.3 *Tumours of the urinary tract*

(a) *Ingested nitrate*

Five ecological studies, one cohort and one case-control study of tumours of the urinary tract and nitrate intake in the diet and drinking-water were reviewed. These included investigations of cancers of the urinary bladder, kidney or all tumours of the lower urinary tract combined, of which bladder cancer predominated. Both analytical studies of intake of nitrate in the drinking-water were conducted in the USA and estimated exposure to nitrate among people who used public water systems; thus average levels were generally below 50 mg/L. In the cohort study of women from Iowa, risk for cancer of the urinary bladder was positively associated with average concentrations of nitrate in the water. No association was observed with renal cancer. This study did not evaluate any potential interactions between nitrate and inhibitors of nitrosation such as vitamin C. A largely non-overlapping case-control study of bladder cancer from Iowa examined nitrate intake from food. No association with estimates of nitrate intake from the diet or drinking-water was detected, and no interaction was observed between intake of vitamin C and ingestion of nitrate in water. Five ecological studies that were conducted in Europe were uninformative.

(b) *Ingested nitrite*

Two well-designed case-control studies of tumours of the urinary tract assessed dietary intake of nitrite; both used a food-frequency questionnaire to ascertain dietary history and both considered potentially confounding factors. A case-control study of cancers of the lower urinary tract from Oahu, Hawaii, USA, found an increased risk for cancer of the urinary bladder with greater dietary intake of nitrite among Japanese men. There was no association among Japanese women or among Caucasian men or women.

In a study of cancer of the urinary bladder from Iowa (largely Caucasian), dietary intake of nitrite was not associated with risk. Neither of the studies evaluated interactions with nitrosating agents or their inhibitors, such as vitamin C.

5.2.4 *Non-Hodgkin lymphoma*

(a) *Ingested nitrate*

One cohort study and two case-control studies, all of which were conducted in agricultural regions of the USA, evaluated ingestion of nitrate in the diet and drinking-water and risk for non-Hodgkin lymphoma. Another case-control study in the USA evaluated intake of nitrate in drinking-water only. The four studies that evaluated nitrate in drinking-water linked historical data on levels of nitrate in public water supplies to a residential history of water source. Increasing quartiles of the average nitrate level in public supplies were associated with an increased risk for non-Hodgkin lymphoma in one study in Nebraska where the highest average nitrate quartile was > 4.0 mg/L nitrate-N. The case-control study and cohort study in Iowa had slightly lower average exposures to nitrate (highest quartiles, > 2.5 and ≥ 2.9 mg/L nitrate-N); increasing quartiles of exposure were not associated with risk. The case-control study in Minnesota had the lowest exposure levels (highest quartile, > 1.5 mg/L nitrate-N) and observed an inverse association with risk for exposure at this level. The case-control studies in Nebraska and Iowa evaluated risk by comparing high and low strata of nitrate intake in water with strata of vitamin C intake. In the study in Nebraska, there was a statistically significant threefold increased risk among persons who had a higher nitrate intake from water and lower vitamin C intake compared with those who had a lower nitrate intake from water and high vitamin C intake. There was no evidence of such a pattern in risk in the case-control study in Iowa that used similar exposure categories. Five ecological studies evaluated nitrate levels in public water supplies and the risk for non-Hodgkin lymphoma. The Working Group considered that these studies provided little information for the evaluation because levels of nitrate were mainly below 10 mg/L and because of the limitations of the ecological study design.

Dietary intake of nitrate was associated with non-significant inverse risks in the Iowa cohort study and Nebraska case-control study; a significantly inverse association with higher intake of dietary nitrate was reported in the Iowa case-control study.

(b) *Ingested nitrite*

The relationship between ingested nitrite and non-Hodgkin lymphoma was evaluated in two case-control studies in the USA. Dietary nitrite was not associated with risk for non-Hodgkin lymphoma in one study but there was an increase in risk with increasing quartiles of nitrite intake in the second study. When plant and animal sources of dietary nitrite were evaluated separately, the positive association was observed only for plant sources.

5.2.5 *Colon and rectal tumours*

(a) *Ingested nitrate*

One case-control study and two cohort studies evaluated the intake of nitrate from drinking-water and dietary sources in relation to risk for cancers of the colon and rectum. The case-control study found no overall association between average levels of nitrate in drinking-water in public water supplies and risk for either type of cancer. However, for cancer of the colon, a significant twofold elevated risk was observed among persons who had a higher intake of nitrate from water and low vitamin C intake. A twofold significantly elevated risk was also observed with higher intake of nitrate from water and high meat intake. Average nitrate levels in public supplies were not associated with an increase in risk for cancer of the colon or rectum in the cohort study; however, elevated risks were observed in the middle exposure categories for colon cancer.

Dietary nitrate intake was not associated with the risk for colorectal cancer in the cohort studies. Higher dietary intake of nitrate was associated with a decreased risk for colon cancer in the Iowa case-control study, whereas there was no association with rectal cancer.

(b) *Ingested nitrite*

One case-control study in the USA and one cohort study in Finland evaluated dietary nitrite intake and risk for cancers of the colon and rectum. The case-control study found a 50% increased risk for colon cancer and a 70% increased risk for rectal cancer. Dietary intake of nitrite was not associated with risk in the cohort study.

5.2.6 *Other cancers*

(a) *Ingested nitrate*

Levels of nitrate in the drinking-water were investigated in analytical case-control studies of cancers of the pancreas, lung, ovary, corpus uteri, breast and testis.

Dietary intake of nitrate has been studied in case-control or cohort studies of pancreatic, oral, laryngeal, lung, ovarian, corpus uteri, endometrial and breast cancer. Three case-control studies of pancreatic cancer and two or fewer studies of cancer at other sites were available. There was no association or inverse association between cancers at these sites and dietary intake of nitrate.

(b) *Ingested nitrite*

Dietary nitrite intake was evaluated in case-control or cohort studies in relation to oral, laryngeal, nasopharyngeal, pancreatic and lung cancers. The number of studies of any given cancer site were few: three case-control studies of pancreatic cancer and two or fewer studies of cancers at other sites were available.

5.3 Animal carcinogenicity data

5.3.1 Nitrate

In three studies in mice, no evidence of carcinogenic activity of nitrate alone was observed whether it was administered in the drinking-water or in the diet at high concentrations. One study showed that less than 2.5% of the administered nitrate was reduced to nitrite in mice.

In four studies in rats, no increased incidence of tumours was observed when sodium nitrate alone was administered in the drinking-water or in the diet. One study showed that nitrate promoted urinary bladder carcinogenesis induced by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine.

5.3.2 Nitrite

In most of the studies, mice or rats that were exposed to nitrite alone in the diet, by gavage or in the drinking-water did not have higher incidences of tumours compared with untreated controls. It was noted that the negative findings may be due to the low doses of nitrite used, the short duration of exposure or the instability of nitrite. In some instances, when a carcinogenic effect of nitrite was observed, the investigators noted the formation of *N*-nitroso compounds in the diet mix or in the stomach contents.

One study in female mice treated with nitrite in the drinking-water showed an increased trend in the incidence of forestomach papillomas and carcinomas combined. In one study in male mice exposed to nitrite in the drinking-water *in utero* (exposure of dams) and throughout life (until natural death), an increased incidence of lymphoma and lung tumours (benign and malignant combined) was observed.

In two studies, female rats that were fed nitrite in the diet had an increased incidence of hepatocellular carcinomas or adenomas and carcinomas combined. In one study in which rats were exposed to dietary nitrite *in utero* (exposure of dams) and throughout life, an increased incidence of lymphoreticular tumours was observed. In one study, rats that received nitrite in the drinking-water had an increased incidence of forestomach papillomas.

In many studies in mice that were exposed to nitrite by gavage, in the drinking-water or in the diet in combination with specific secondary or tertiary amines or amides (e.g. butylurea, *N*-methylaniline, piperazine, morpholine, methylurea, diethylamine hydrochloride, ethylurea, ethylthiourea, carbendazim or dibutylamine), the incidence of tumours, including benign and malignant lung tumours, malignant lymphomas, benign and malignant forestomach tumours, urinary bladder papillomas, benign and malignant liver tumours, or uterine adenocarcinomas was increased. In one of these studies, when piperazine was administered at a constant level in the diet with varying levels of sodium nitrite in the drinking-water, the increase in the incidence of lung adenomas was directly proportional to the levels of nitrite intake.

In many studies in rats, when sodium nitrite and specific secondary or tertiary amines or amides (e.g. morpholine, butylurea, disulfiram, aminopyrine, diphenhydramine, chlorpheniramine maleate, heptamethyleneimine hydrochloride, *N,N*-dimethyldodecylamine-*N*-oxide or bis(2-hydroxypropyl)amine) were mixed in the diet or given in the drinking-water or by gastric intubation, an increased incidence of tumours, including benign and malignant oesophageal tumours, haemangiosarcomas, hepatocellular adenomas and carcinomas, lung squamous-cell carcinomas or benign and malignant nasal cavity tumours was observed. In some of these studies, at a constant level of sodium nitrite, the tumour incidence induced was directly related to the levels of amine. When the level of amine was kept constant, tumour yield was also directly related to the level of sodium nitrite. When pregnant rats were given ethylurea and sodium nitrite in the drinking-water, neurogenic tumours developed in the offspring.

A dose-related increase in the incidence of renal-cell carcinoma was observed when rats were administered nitrite in the drinking-water in combination with varying amounts of fishmeal in the diet. Levels of *N*-nitrosodimethylamine in the stomach contents also showed a dose-related increase.

In one study, nitrite in the drinking-water marginally enhanced the carcinogenic effects of two different leukaemia viruses in infected mice.

Studies with antioxidants in rats showed that sodium ascorbate enhanced the incidence of forestomach tumours produced by concurrent administration of morpholine and sodium nitrite and that produced by initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and concurrent administration of sodium nitrite, while the concurrent administration of sodium ascorbate delayed the induction of liver tumours produced by morpholine and sodium nitrite. Ascorbic acid or soya bean prevented nitrite and dibutylamine from producing liver and urinary bladder tumours in mice and ascorbic acid protected against the induction of liver tumours by nitrite and aminopyrine in rats. Thioproline, a nitrate scavenger, decreased the incidence of forestomach carcinomas produced by nitrite and *N*-benzylmethylamine in rats. α -Tocopherol, *tert*-butylhydroquinone and propyl gallate enhanced the incidence of forestomach papillomas induced by concurrent administration with sodium nitrite after initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats. Concurrent administration of the antioxidant 1-*O*-hexyl-2,3,5-trimethylhydroquinone enhanced the incidence of forestomach tumours induced by aminopyrine and sodium nitrite in a rat multiorgan carcinogenesis model.

In rats, concurrent administration of sodium nitrite and 3-methoxycatechol produced forestomach papillomas, and catechol and 3-methoxycatechol increased the incidence of forestomach tumours produced by concurrent administration of sodium nitrite in a two-stage gastric carcinogenesis model.

In one study in hamsters, the incidence of liver-cell carcinomas was increased when sodium nitrite was fed together with morpholine.

5.4 Other relevant data

5.4.1 *Absorption, distribution, metabolism and excretion*

In humans, nitrate and nitrite participate in a dynamic interchange—the human nitrogen cycle—that involves ingestion, endogenous synthesis and ultimate excretion mainly as nitrate in the urine. Exposure by ingestion is primarily to nitrate, approximately 5% of which is reduced by oral bacteria to nitrite. The nitrate/nitrite mixture enters the gastrointestinal system in swallowed saliva. Nitrate and nitrite are absorbed from the upper intestine into the general circulation where the nitrite is oxidized by haemoglobin to nitrate that then re-enters the cycle. Endogenous synthesis is mainly via the arginine-to-nitric oxide pathway followed by ultimate conversion of nitric oxide to nitrate/nitrite. Nitrate and nitrite are widely distributed in the body, where nitrate predominates. Under some physiological conditions, e.g. hypoxia, nitrite can be converted in the reverse direction to nitric oxide; this pathway is suggestive of an important biological role for this anion.

5.4.2 *Endogenous nitrosation*

Nitrosation of proline to form the non-carcinogenic nitrosamine, *N*-nitrosoproline, has been used as a test of endogenous nitrosation, and a large number of studies have now demonstrated the nitrosation of proline in humans. In a typical experiment, proline and nitrate are administered and the nitrite that arises from oral reduction of the nitrate can then nitrosate proline in the stomach. Gastric nitrosation of proline in humans can be inhibited by vitamin C, as seen in animal and human experiments. Examples of endogenously formed carcinogenic nitrosamines are *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine and tobacco-specific nitrosamines in humans. Nitrite-preserved meat contains substantial amounts of nitrosatable compounds (*N*-nitroso compound precursors). Other pathways for endogenous nitrosation include bacterially mediated and nitric oxide-related nitrosation.

5.4.3 *Genotoxic effects*

High intake of nitrate was associated with an increased frequency of hypoxanthine-guanine phosphoribosyl transferase gene mutants in peripheral blood lymphocytes in human populations who had been exposed to various levels of nitrate in their drinking-water. Another study showed an increase in the number of chromosomal aberrations, but not of sister chromatid exchange, in lymphocytes of children exposed to concentrations of nitrate in the drinking-water that exceeded 70.5 mg/mL.

Sodium nitrate gave negative results in the *Salmonella typhimurium* reverse mutation assay. Potassium nitrate caused chromosomal aberrations in Chinese hamster ovary cells *in vitro* in one study. In two *in-vivo* studies, sodium nitrate increased the frequency of chromosomal aberrations in rats and mice. Sodium nitrate did not cause chromosomal

aberrations, micronuclei, 8-azaguanine-resistant mutations, ouabain-resistant mutations or morphological transformation in the cells of hamster embryos after transplacental exposure. It was also negative in the mouse sperm-head abnormality test.

Sodium nitrite gave generally positive results in the *Salmonella* mutagenicity assay, but was negative in the SOS chromotest. It did not induce mutations in bacteria recovered in the host-mediated assay from rats or mice of various strains. In a single study, sodium nitrite induced somatic mutations in the wing-spot test in *Drosophila melanogaster*. It gave a positive response in several assays for chromosomal aberrations and micronucleus formation, both *in vitro* and *in vivo*. In several *in vitro* studies, sodium nitrite was consistently positive in inducing aneuploidy, cell transformation, 8-azaguanine-resistant mutations, 6-thioguanine-resistant mutations and ouabain-resistant mutations. Similarly, sodium nitrite induced 8-azaguanine-resistant mutations, ouabain-resistant mutations and morphological transformation in cells of hamster embryos after transplacental exposure, but did not induce chromosomal aberrations or micronuclei in this assay. It was also positive in the mouse sperm-head abnormality test.

5.4.4 *Other toxic effects*

Nitrate, via reduction to nitrite as noted above, causes methaemoglobinaemia, especially in infants. High concentrations of methaemoglobin are associated with hypotension, as a result of the vasodilatory effects of nitrite. Epidemiological evidence suggests a possible association between nitrate in the drinking-water and spontaneous abortions, intrauterine growth restrictions, birth defects, childhood onset of diabetes mellitus, thyroid hypertrophy, hypertension and recurrent diseases (respiratory tract infection, diarrhoea, stomatitis) in children. No teratogenic effects were observed in tests with nitrate and nitrite.

5.4.5 *Mechanistic considerations*

Nitrosating agents—e.g. nitrous acid and nitrous anhydride—that arise from nitrite under acidic gastric conditions react with amines or amides to form nitrosamines or nitrosamides, and the induction of tumours in animals via endogenous synthesis of *N*-nitroso compounds has been demonstrated. Nitrosamines need to be activated metabolically by cytochrome P450 enzymes to electrophilic intermediates to exert a carcinogenic effect, while nitrosamides are direct-acting carcinogens. Nitrosation of primary amines produces electrophiles that can alkylate nucleophilic sites in DNA. Nitrosation of primary exocyclic amino groups on DNA, followed by deamination, may lead directly to mutations.

Ascorbic acid, a known inhibitor of nitrosation reactions, lowers the incidence of tumours in these experiments. The effect of ascorbic acid in the reduction of the risk for cancer that is associated with ingested nitrite has also been shown in epidemiological

studies (see Section 5.2). These observations support the role of endogenous nitrosation in tumorigenesis.

6. Evaluation and Rationale

There is *inadequate evidence* in humans for the carcinogenicity of nitrate in food.

There is *inadequate evidence* in humans for the carcinogenicity of nitrate in drinking-water.

There is *limited evidence* in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with an increased incidence of stomach cancer.

There is *inadequate evidence* in experimental animals for the carcinogenicity of nitrate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of nitrite in combination with amines or amides.

There is *limited evidence* in experimental animals for the carcinogenicity of nitrite *per se*.

Overall evaluation

Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)*.

There is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite, which are interconvertible *in vivo*. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary amines and amides, to generate *N*-nitroso compounds. These nitrosating conditions are enhanced following ingestion of additional nitrate, nitrite or nitrosatable compounds. Some of the *N*-nitroso compounds that could be formed in humans under these conditions are known carcinogens.