SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 5 April 2017

doi: 10.2903/j.efsa.2017.4787

Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Alicja Mortensen, Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright, Piet van den Brandt, Cristina Fortes, Leonardo Merino, Fidel Toldrà, Davide Arcella, Anna Christodoulidou, Federica Barrucci, Ana Garcia, Fabiola Pizzo, Dario Battacchi and Maged Younes

Abstract

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provided a scientific opinion re-evaluating the safety of sodium nitrate (E 251) and potassium nitrate (E 252) when used as food additives. The current acceptable daily intakes (ADIs) for nitrate of 3.7 mg/kg body weight (bw) per day were established by the SCF (1997) and JECFA (2002). The available data did not indicate genotoxic potential for sodium and potassium nitrate. The carcinogenicity studies in mice and rats were negative. The Panel considered the derivation of an ADI for nitrate based on the formation of methaemoglobin, following the conversion of nitrate, excreted in the saliva, to nitrite. However, there were large variations in the data on the nitrate-to-nitrite conversion in the saliva in humans. Therefore, the Panel considered that it was not possible to derive a single value of the ADI from the available data. The Panel noticed that even using the highest nitrate-to-nitrite conversion factor the methaemoglobin levels produced due to nitrite obtained from this conversion would not be clinically significant and would result to a theoretically estimated endogenous *N*-nitroso compounds (ENOC) production at levels which would be of low concern. Hence, and despite the uncertainty associated with the ADI established by the SCF, the Panel concluded that currently there was insufficient evidence to withdraw this ADI. The exposure to nitrate solely from its use as a food additive was estimated to be less than 5% of the overall exposure to nitrate in food based on a *refined estimated exposure scenario*. This exposure did not exceed the current ADI (SCF, 1997). However, if all sources of exposure to dietary nitrate are considered (food additive, natural presence and contamination), the ADI would be exceeded for all age groups at the mean and the highest exposure.

Keywords: sodium nitrate, potassium nitrate, E 251, E 252, food additive, CAS Registry number 7757-79-1, CAS Registry number 7631-99-4

Requestor: European Commission

Question number: EFSA-Q-2011-00462; EFSA-Q-2011-00463

Correspondence: fip@efsa.europa.eu

Panel members: Fernando Aguilar, Ric cardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: The hearing experts from the Scientific Committee: Josef Schlatter and Thorhallur Halldorsson; the hearing experts from the CONTAM Panel: Helle Knutsen and Jan Alexander and the members of the CONTAM Panel: Lars Barregard and the EFSA staff members Andea Bau, Petra Gergelova, Gilles Guillot, Laura Martino and Jose Gomez Ruiz for the support provided to this scientific output. The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambré C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent-Massin D, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, van den Brandt P, Fortes C, Merino L, Toldrà F, Arcella D, Christodoulidou A, Barrucci F, Garcia A, Pizzo F, Battacchi D and Younes M, 2017. Scientific Opinion on the re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. EFSA Journal 2017;15(6):4787, 123 pp. https://doi.org/10.2903/j.efsa.2017.4787

ISSN: 1831-4732

Figure 1: © Stockphoto

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of potassium nitrate (E 251) and sodium nitrate (E 252) when used as food additives.

Sodium (E 251) and potassium (E 252) nitrates are authorised as food additives in the European Union (EU) according to Annex II to Regulation (EC) No 1333/2008 on food additives and they were previously evaluated by the EU Scientific Committee for Food (SCF), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA). The current acceptable daily intakes (ADIs) for sodium and potassium nitrate (expressed as nitrite ion) established by the SCF (1997) and JECFA (2002) are both at 0–3.7 mg/kg body weight (bw) per day.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that came available since then and the data provided following public calls for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Sodium and potassium salts of nitrate, together with those of nitrite, are commonly used in curing mixtures to develop and fix the colour of meat, to inhibit microbial growth and/or to develop characteristics flavours (IARC, 2010; Sindelar and Milkowski, 2012). Specific purity criteria on sodium and potassium nitrites are defined in Commission Regulation (EU) No 231/2012.

Short-term and subchronic toxicity studies in rats showed, overall, that nitrate intake of up to 5% in the diet (equivalent to 4,500 mg sodium nitrate/kg bw per day) did not result in adverse effects in rats. At higher dose levels, animals showed signs of methaemoglobinaemia leading to the death of the animals.

In vitro studies on sodium and potassium nitrate in bacteria and mammalian cells did not provide evidence of a genotoxic potential. In mammals, no reliable indication of genotoxicity was obtained in mice and rats exposed to nitrate by the oral route, both in somatic and in germ cells. Although the database was limited, the Panel concluded that the available experimental data indicated that nitrate salts do not raise concern for genotoxicity.

Chronic toxicity and carcinogenicity studies with sodium and potassium nitrate were available. In studies with mice, sodium nitrate did not show any difference in tumour incidences compared to controls. Four non-standard studies in rats and pigs assessed haematological parameters or effects on thyroid and thyroid-related hormones (Boink et al., 1995; JECFA, 1996; Zaki et al., 2004; Mukhopadhyay et al., 2005; Azeez et al., 2011). Overall, the Panel considered that nitrate did not affect adrenal and thyroid glands function in animals and it was not carcinogenic in animal studies.

No effects were observed in a reproductive/developmental toxicity screening study (OECD TG 422) in rats administered potassium nitrate by gavage at doses up to 1,500 mg/kg bw per day. No developmental toxicity was observed in mice, rats, hamsters or rabbits receiving doses up to 400, 1,980, 280 or 206 mg potassium nitrate/kg bw per day by gavage, respectively. In a reproductive toxicity study in mice given potassium nitrate in drinking water, effects were observed on sperm count and testicular enzymes at the highest dose tested, at which also sperm abnormalities were observed; the no-observed-adverse-effect level (NOAEL) in this study was 122 mg potassium nitrate/kg bw per day. Histopathological changes in testis, epididymis and other sex organs were reported in this study. A conclusion could not be reached since the duration of the dosing in males in this screening study was limited and the number of animals tested was low. Overall, the Panel noted that although some effects were observed in sperm analysis and reproductive organs in this limited study in mice, no indications of reproductive toxicity were observed at higher doses in a rat study conducted according to OECD guideline TG 422.

Human non-cancer effects were observed in the thyroid in several studies, suggesting that nitrate exposure altered human thyroid gland function by competitively inhibiting thyroidal iodide uptake. A large cohort study (n = 21,977 women) showed that increasing intake of nitrate from dietary sources was associated with an increasing occurrence of hypothyroidism (Ward et al., 2010). In addition, in several studies, an enlarged thyroid or even goitre was observed when the intake of nitrate via drinking water was high. Other studies, however, showed no effects. Overall, there was some evidence to relate exposure to nitrate with the development of enlarged thyroid, goitre and hypothyroidism. The exposure levels were in the same range as that within which methaemoglobinaemia was observed.

The methaemoglobin formation reported in animal studies can also be observed in humans. This effect occured both, upon acute exposure as well as after chronic exposure, to nitrate. The effect was a consequence of the endogenous production of nitrite from ingested nitrate.

In epidemiological studies, the summary evidence for an association between nitrate exposure and each type of human cancer was categorised by the Panel as: (i) *there was no evidence* for an association, if studies indicate no association with a specific cancer; (ii) *there was insufficient evidence,* to (unequivocally) link to a cancer (e.g. few studies, contradictory results, etc.); (iii) *there was some evidence* for an association with a specific cancer (e.g. inconsistent results between cohort studies and case–control studies); (iv) *there was evidence,* for an association with a specific cancer (e.g. consistent results from cohort studies and case–control studies).

The Panel concluded that *there was no evidence* for a positive association between: ingested nitrate and oesophageal cancer and its subtypes oesophageal squamous cell carcinomas and oesophageal adenocarcinoma (ESCC and EAC); ingested nitrate and gastric cancer or its subtypes gastric cardia adenocarcinoma (GCA) and gastric non-cardia adenocarcinoma (GNCA); dietary nitrate and colorectal cancer (CRC) or colon or rectum cancer; ingested nitrate and pancreatic cancer; ingested nitrate and lung cancer; dietary nitrate and non-Hodgkin lymphoma (NHL); ingested nitrate and breast cancer; ingested nitrate and renal cell cancer; and ingested nitrate and adult glioma or childhood brain tumours.

There was insufficient evidence for a positive association between: nitrate from processed meat and colorectal cancer (CRC) or its subtypes; drinking water nitrate and CRC or its subtypes; drinking water nitrate and non-Hodgkin lymphoma (NHL); ingested nitrate and leukaemia; ingested nitrate and ovarian cancer; ingested nitrate and bladder cancer; ingested nitrate and prostate cancer; and ingested nitrate and thyroid cancer.

There were insufficient data to draw conclusions on: ingested nitrate and head and neck cancer and ingested nitrate and liver cancer.

The human studies on goitre and the single study on hypothyroidism were considered not sufficient for deriving reference points for a health-based guidance value.

Hence, in the absence of other adverse effects and the unavailability of the original 1958 study used by JECFA and the database currently available, the Panel decided that the most relevant approach for assessing the toxicity of nitrate would be methaemoglobinaemia induced by nitrite formed from nitrate excreted in the saliva, once absorbed. The Panel review the reported information on secretion estimates of nitrate into the mouth in humans and observed that most estimates available varied between 20% and 25% of the dose (Spiegelhalder et al., 1976; Bartholomew and Hill, 1984). Additionally, the Panel noted that these studies were quite old and had limitations (it might be possible to obtain more accurate estimates nowadays). The Panel therefore considered that adequate, well conducted modern studies might decrease the uncertainty associated with this estimate.

The Panel noted that available estimates of the ratio of concentrations of nitrite to nitrate varied within and between individual subjects. The nitrate-to-nitrite conversion in the mouth was estimated to range from 5% to 36% (e.g. Spiegelhalder et al., 1976; Wagner et al., 1983; Bartholomew and Hill, 1984; Bos et al., 1988; Granli et al., 1989; Shapiro et al., 1991; Jin et al., 2013; Bondonno et al., 2015; Hohensin et al., 2016; Montenegro et al., 2016); Woessner et al., 2016. The Panel noted that the available studies were carried out in diverse and differing populations. The Panel considered that to reflect the uncertainties in the underlying data and inter-individual variability in conversion, it was appropriate to use a range of values for the conversion percentage of nitrate to nitrite in the saliva.

The Panel considered that any single estimate of the overall conversion of ingested nitrate to nitrite would not be reliable. The Panel did not consider it was possible to derive a single value as ADI from the data available.

Based on the secretion rates of nitrate (20–25%) into the saliva and the range of conversion rates of nitrate to nitrite (5–36%) in the mouth, a range for the overall conversion percentage between 1% and 9% would be estimated. Using this range and considering the ADI of nitrite (0.07 mg nitrite ion/kg bw per day), the ADI values estimated for nitrate would be between 1.05 and 9.4 mg nitrate ion/kg bw per day. The current ADI established by SCF (1997) (3.7 mg nitrate ion/kg bw per day) falls within those estimates. The Panel considered that, as a point estimate, the current ADI was likely to be as accurate as this range.

The Panel also took into consideration the lack of overt toxicity data reported in the available studies in animals and that there was no cancer concerns overall from epidemiological studies in humans. Despite the uncertainty associated with the ADI set by the SCF (1997) due to the inability to examine its basis thoroughly, the Panel considered that currently there was insufficient evidence to withdraw this ADI.

The Panel recognised that further data from human studies were warranted. These studies could target the secretion of nitrate in the saliva and the conversion of nitrate to nitrite in the mouth, as well

as the subsequent effect of increased nitrite blood concentrations, namely increase in methaemoglobinaemia, or assess the effects of nitrate on thyroid function in a well conducted study. Since these data might also affect evaluations in other areas of EFSA's remit, the Panel recognised that an integrated risk assessment would be required to best define further data requirements.

The Panel noted that nitrates (E 251–E 252) were authorised for use in a wide range of foods and it was therefore not expected that brand-loyalty would result in higher exposure in the general population. The Panel therefore selected the non-brand loyal scenario refined scenario as the most relevant exposure scenario for the safety evaluation of these food additives.

From its *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008, in the *non-brand-loyal scenario*, mean exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) ranged from 0.01 mg/kg bw per day in infants to 0.09 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates (expressed as nitrate ion) ranged from 0.05 mg/kg bw per day in the elderly to 0.22 mg/kg bw per day in toddlers.

Applying these exposure scenarios considered for exposure assessment of nitrates (E 251–E 252) from their use as food additives, the most important contributors to the total mean exposure for all population groups were meat products (preserved meat and sausages) and cheese, whereas fish and fishery products contributed less.

From the exposure scenario considering the exposure to nitrates (expressed as nitrate ion) from all sources (food additives, natural presence and contamination), applying reduction factors for vegetables, mean exposure ranged from 0.97 mg/kg bw per day in the elderly to 4.15 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates ranged from 1.59 mg/kg bw per day in the elderly to 8.73 mg/kg bw per day in children.

The main contributing food categories from the exposure scenario considering all sources (food additives, natural presence and contamination across surveys and population groups, range min–max) were vegetables and vegetable-based foods for all population groups (0–29%). In particular, the main contributing food categories were starchy roots and tubers in infants, toddlers and children (4–35%), whereas the leafy vegetables (0.4–47%) and prepared salads (0–44%) were the main contributors in children, adolescents, adults and the elderly. In infants, also foods for infants and toddlers (4–33%) made an important contribution to the total mean exposure to nitrates from all sources.

The Panel estimated that, when comparing all sources (food additives, natural presence and contamination), including reduction factors for vegetables, using the same refined exposure methodology (non-brand-loyal consumer scenario for general population), the contribution of nitrates (E 251–E 252) from their use as food additives is less than 5% of contribution to the overall exposure to nitrates from all sources in any scenario and for any population group (approximately 2% in average, range 0.4–4.4%).

The Panel noted that exposure to nitrates from their use as food additives was below the ADI of 3.7 mg/kg bw per day using the most appropriate refined exposure scenario (non-brand loyal). The estimates for the high level consumers in all population groups were approximately 15% or less of the current ADI. As such there would not be a safety concern from the current uses and use levels of nitrate as a food additive.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to nitrates (E 251–E 252) as a food additive in European countries for the regulatory maximum level exposure scenario and for the refined scenario considering that it was not possible to include a number of restrictions.

Based on a *refined estimated exposure scenario*, the exposure to nitrate resulting from its use as a food additive does not lead to an exceedance of the ADI of nitrates.

The Panel noted that total dietary exposure to nitrate from all sources exceeded the current ADI in all populations considered. Assessing whether or not the total dietary intake of nitrate was a safety concern was outside the scope of this re-evaluation and beyond the remit of the ANS Panel.

The formation of *N*-nitroso compounds (NOCs) is a key step when considering the carcinogenic risk of nitrites (EFSA ANS Panel, 2017). In order to be able to estimate the amount of nitrosamines formed from nitrate its conversion to nitrite needs to be quantifiable. In view of the large variability observed among the populations on the proportion of nitrite that can be formed from nitrate in the mouth, the Panel considered that even using the highest nitrate-to-nitrite conversion factor of 9% a dose corresponding to the ADI of 3.7 mg/kg bw per day will be converted into 0.25 mg nitrite ion/kg bw per day. With this dose, the methaemoglobin levels will increase above background levels (1–3%) which is measurable, but not clinically significant. Furthermore, ingestion of 0.25 mg/kg bw per day

nitrite ion would lead to an endogenous *N*-nitroso compounds (ENOCs) production of 8.22×10^{-7} mg/kg bw per day calculated using the formula given in the Guideline for Canadian Drinking Water (Health Canada, 2013). Then, the margin of exposure (MoE) between the produced amount of ENOCs and the $BMDL_{10}$ of N-nitrosodimethylamine (NDMA) (0.027 mg/kg bw per day) would be 3.2×10^{4} which is still above the MoE of 10,000 considered by the Scientific Committee as of low concern from a public health point of view (EFSA, 2005; EFSA Scientific Committee, 2012b).

The Panel recommended further human studies with a better control of confounding factors (e.g. radiation exposure dietary iodine intake and other anions that compete with iodide uptake in the thyroid) are needed to confirm the findings in thyroid gland.

The Panel recommended that additional experimental studies in humans measuring the excretion of nitrate into the saliva and its conversion to nitrites and the consequent methaemoglobin formation should be conducted in order to reduce uncertainties.

The Panel recommended that further studies on the levels of nitroso compounds formed in different meat products with known ingoing amounts of nitrates /nitrites added, with appropriate controls and with specified levels of detection (LOD) and levels of quantification (LOQ) for potentially formed nitroso compounds would be necessary.

Table of contents

1. Introduction

The present opinion deals with the re-evaluation of sodium nitrate [E 251(i) and (ii)] and potassium nitrate (E 252) as food additives.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[3] of 2001. The report 'Food additives in Europe 2000[4]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010, the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, especially taking into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.3. Interpretation of Terms of Reference

EFSA has been asked in accordance with the Regulation (EU) No 257/2010 setting up a programme for the re-evaluation of approved food additives, to re-evaluate the safety of nitrates (E 251–252) and nitrites (E 249–250) as food additives. Delivery of the EFSA opinion was initially foreseen by 31 December 2015. During the re-evaluation process, additional activities, listed below, have been initiated:

- Within the frame of M-2010-0374, a call for the continuous collection of data on the occurrence of chemical contaminants in food and feed,[5] the European Commission sent a

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[2] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, pp. 19–27.
[3] COM(2001) 542 final.
[4] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, p. 560.
[5] http://www.efsa.europa.eu/en/data/call/datex101217.htm

request to EFSA (DATA and BIOCONTAM units) on 28 March 2014 for a scientific report on the occurrence of nitrates in leafy vegetables and exposure of the human population, including vulnerable groups. In addition, the European Commission asked for the collection of data from an *ad hoc* study on the use of nitrites by the food industry in different categories of meat products, finalised (in SANCO/2014/E3/029) by January 2016. The Panel considered that the outcome of the exposure assessment considering all food sources and the information on the uses of nitrites by the industry is relevant in the frame of this opinion and the delivery of the opinion was aligned with this activity.

- On 18 May 2015, the European Commission requested EFSA to consider additional information provided by Denmark on the safety of nitrite use during its re-evaluation (EC Reference 1188419).

Therefore, a realistic deadline of 31 December 2016 for finalisation of the scientific opinions on the re-evaluation of nitrates and nitrites has been considered by the ANS Panel.

1.2. Information on existing authorisations and evaluations

Sodium and potassium nitrate are authorised as food additives in the EU under Commission regulation (EU) No 1333/2008 amended by regulation (EU) No 1129/2011 on food additives for use in foodstuffs. Specific purity criteria on sodium and potassium nitrates have been defined in Commission Regulation (EU) No 231/2012.

The current acceptable daily intake (ADI) set by the SCF and the Joint Food and Agriculture Organization/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) for sodium and potassium nitrate (expressed as the nitrate ion) is 0–3.7 mg/kg body weight (bw) per day (SCF, 1997; JECFA, 2003b).

The SCF has reviewed nitrate on two occasions (SCF, 1992, 1997). In its 1990 evaluation, the SCF reviewed the safety of nitrate and nitrite when used as food additive taking into consideration the potential formation of carcinogenic nitrosamines and information on their uses in meat products, cheese milk and fish products (SCF, 1992). The SCF concluded that nitrate *per se* has very low acute toxicity and that any reported adverse effects (formation of methaemoglobin) resulted from its reduction to nitrite either before ingestion or *in vivo*. The SCF report mentioned that 'experimental carcinogenicity studies on nitrate per se had proved negative and that realistic levels of dietary nitrate did not appear to lead to a significant formation of volatile *N*-nitroso compounds, nor of nitroso-amino acids'. On the epidemiological studies available at that time, the SCF considered that 'they have failed to unequivocally demonstrate a link between nitrate exposure and cancer incidence in populations exposed to higher than average nitrate intake either in the diet and drinking water, or occupationally'. Similarly, at the time of SCF evaluation, 'epidemiological studies in populations with high and low cancer incidences have failed to demonstrate a link between cancer risk and nitrate intake'. The SCF derived an ADI of 5 mg/kg bw per day (expressed as sodium nitrate) from a 2-year carcinogenicity study in the rat showing a no-observed-adverse-effect level (NOAEL) of 2,500 mg/kg bw per day (Maekawa et al., 1982). This ADI include human intake from all sources. A safety factor of 500 was applied to this NOAEL to account of interspecies differences between the rat and man in that rat does not secrete nitrate in saliva with subsequent partial reduction to nitrite. Finally, the SCF considered that, because 'infants may be more likely to reduce exogenous nitrate to nitrite and are more sensitive to the acute effects of nitrite, nitrate should not be used as an additive in infant foods.' As regards the formation of nitrosamines and nitrosamides, while *there appeared to be no such clear correlation between added nitrate levels and formation of volatile nitrosamines,* further information was required by the SCF 'about the potential involvement of nitrate in the formation of non-volatile *N*-nitroso compounds'. The SCF considered that exposure, epidemiological and other data relating to dietary nitrosamines provided no direct evidence that the current levels of nitrosamines present in the diet were hazardous to human health. However, the SCF was 'not in a position to make a quantitative assessment of risk from all *N*-nitroso compounds present in foods as eaten or formed by nitrosation in the human gastrointestinal tract' and considered that it was prudent to ensure that exposure to preformed nitrosamines in foods should be minimized by appropriate technological practices (SCF, 1992).

In its 1995 report (SCF, 1997), the SCF updated and extended its previous 1990 evaluation. The SCF updated the dietary exposure to nitrate taking into consideration nitrate levels in vegetables and other foods and reviewed the literature on the toxicology of nitrate, nitrite and *N*-nitroso compounds (NOCs). The SCF concluded that the intake of nitrate from the use of its salts as food additives was

low compared to that resulting from the consumption of vegetables and drinking water, with no likely toxicity arising within the expected daily intake from natural sources and use as food additives. From the available toxicity and epidemiological data, it was concluded that nitrate *per se* was of relatively low toxicity. However, as the toxicity of nitrate is encompassed by its conversion to nitrite and the possible endogenous formation of NOCs and that the toxicokinetics and biotransformation of nitrate in rats is different from humans, the safety evaluation of nitrate was carried out in conjunction with that of nitrite. With regard to the epidemiological studies on gastric cancer risk, the SCF considered them inconsistent (the more reliable case–control and cohort studies not suggesting any association) probably due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer. The SCF evaluation concluded that 'long-term animal studies did not indicate that nitrite or nitrate per se are carcinogenic and that there was no quantitative evidence for the endogenous formation of carcinogenic *N*-nitroso compounds after exposure to realistic levels of nitrate and *N*-nitrosatable precursors. In addition, the Committee concluded that, overall extensive epidemiological studies on nitrate have failed to demonstrate an association with cancer risk in man. The Committee therefore felt it appropriate to derive an ADI for nitrate'. The SCF concluded that the evidence from human metabolism studies on nitrate taken together with the toxicity of nitrite provided confirmation of the ADI for nitrate established previously. The SCF retained thus an ADI of 0–3.7 mg/kg bw per day for nitrate ion (equivalent to 0–5 mg/kg bw per day for sodium nitrate) and confirmed that this ADI was applicable to all sources of dietary exposure to nitrates (SCF, 1997).

Nitrate was reviewed by JECFA on several occasions (JECFA, 1965, 1974, 1995, 1996, 2002, 2003a,b). JECFA (1996), in its evaluation of nitrate, based its ADI on a chronic study in rats, which is referenced as Lehman (1958). However, this reference only summarises unpublished data. The most recent review on nitrate by JECFA (2003a) evaluated new data that had become available since its 1996 evaluation and concluded that the pivotal studies having observed toxic effects of nitrate are consequent on its conversion to nitrite *in vivo*. As the new data on nitrite would not provide a basis for a significant change in the previous ADI for nitrate, the Committee retained the ADI of 0–5 mg/kg bw, expressed as sodium nitrate, or 0–3.7 mg/kg bw, expressed as nitrate ion, during this evaluation. The JECFA evaluation also concluded that 'overall, the epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate. These data, combined with the results of the epidemiological studies considered at a previous meeting, do not provide evidence that nitrate is carcinogenic to humans' (JECFA, 2003a). In addition, in its previous meeting (JECFA, 1995), it was stated that 'since uncertainties still exist with respect to the possible endogenous formation of N-nitroso compounds after nitrate exposure, the most appropriate approach at present is to derive an ADI based on the most sensitive toxicity criteria for nitrite in rats and the toxicokinetics of nitrate in humans, in addition to deriving an ADI directly from toxicity studies with nitrate'.

A risk assessment of the intake of naturally occurring nitrate and its metabolites from vegetables has also been performed by EFSA, with respect to the risks and benefits of consuming nitrate (EFSA CONTAM Panel, 2008). The EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM) concluded that, overall, the estimated exposures to nitrate form vegetables are unlikely to result in appreciable health risks, and therefore the recognised beneficial effects of consumption of vegetables prevail.

In that opinion, the exposure assessment to nitrate from vegetables only was carried out. There was a large variation in median concentrations of nitrate in different vegetables from 1 mg/kg (peas and Brussels sprouts) to 4,800 mg/kg (in rucola). The assessment was not based on real consumption data but was based on several scenarios assuming the consumption of vegetables, excluding potatoes, at a level in line with dietary recommendations (400 g per day).

A further opinion was delivered by the CONTAM Panel on the potential health risks for infants and young children from the presence of naturally occurring nitrate in leafy vegetables (EFSA CONTAM Panel, 2010b). The CONTAM Panel concluded that 'the concentrations of nitrate in spinach have the potential to increase dietary nitrate exposure to levels at which a health concern cannot be excluded. It also observed that inappropriate storage of cooked vegetables can result in *in situ* conversion of nitrate to nitrite, leading to an increased potential for causing methaemoglobinaemia'. The CONTAM Panel noted 'that infants and children with bacterial infections of the gastrointestinal tract are more sensitive to nitrate, and recommended against feeding spinach to such children'.

The scientific opinions delivered by EFSA did not propose revision of the established ADI previously set by the SCF and JECFA (SCF, 1997; JECFA, 2003a).

The International Agency for Research on Cancer (IARC) recently re-evaluated data available on nitrate and nitrite (IARC, 2010) but did not comment on the ADIs set previously by other

organisations. The IARC evaluation includes a review of the effects of ingested nitrate in experimental animals and in humans arising from epidemiological studies. Concerning the animal experiments, the IARC concluded that 'there is inadequate evidence in experimental animals for the carcinogenicity of nitrate'. Concerning the human data, IARC concluded that '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'. Taken into consideration these aspects, the IARC further concluded that 'under conditions that result in endogenous nitrosation ingested nitrate or nitrite, is probably carcinogenic to humans (Group 2A)'.

The WHO produced a background document (WHO, 2007) for the development of WHO guidelines for drinking water quality for nitrate and nitrite. A guideline value for nitrate in water was calculated to be 50 mg/L, with consideration of epidemiological evidence on infant methaemoglobinaemia. A provisional guideline value of 0.2 mg nitrite ion/L of water was calculated based on the JECFA ADI of 0–0.07 mg/kg bw per day, assuming that a 60-kg adult ingests 2 L of drinking water per day, and allocating a 10% contribution of drinking water to the ADI. The provisional status of the guideline was based on the uncertainty with regard to the susceptibility to nitrite toxicity of humans compared to experimental animals. In 2011, the WHO drinking water guideline value for nitrite was set at 3 mg/L based on methaemoglobinaemia, which can be caused at doses as low as 0.4 mg/kg bw in infants. The guideline value was derived by assuming a body weight of 5 kg for infants and consumption of 0.75 L of drinking water per day (WHO, 2011). No guidelines for drinking water have been derived for chronic effects.

The Nordic Council of Ministers (TemaNord) report on sodium and potassium nitrate (TemaNord, 2002) reviewed the results and limits set by the preceding evaluations by the SCF and JECFA (SCF, 1992, 1997, JECFA, 2003a), concluding that the toxicological re-evaluation of these substances was not necessary. However, reference was made to the SCF recommendation that 'exposure to preformed nitrosamines in food should be minimised by appropriate technological practices such as the lowering of levels of nitrate and nitrite added to foods to the minimum required'. Furthermore, it was highlighted that the ADI of 0.06 mg/kg bw set for the nitrite ion was not applicable to infants younger than 3 months of age (TemaNord, 2002). It was also stated that there are several groups of individuals who are expected to be more sensitive to the methaemoglobin-forming potential of the nitrites. These groups include pregnant women, individuals with metabolic disorders and adults with reduced gastric acidity.

Health Canada has published a report on the metabolite *N*-nitrosodimethylamine (NDMA) (Health Canada, 2011), as well as a document on nitrate and nitrite guidelines in drinking water (Health Canada, 2013).

The New Zealand Food Safety Authority has published a risk assessment on dietary nitrate and nitrite (Thomson et al., 2007). The Australian Food Safety Authority has published a report on nitrate and nitrite in 2011 (FSANZ, 2011).

A Screening Information Data Set (SIDS) initial assessment profile is available through the Organisation for Economic Co-operation and Development (OECD) High Production Volume (HPV) Chemicals Program (OECD, 2007) for several nitrates.

2. Data and methodologies

2.1. Data

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier. EFSA launched public calls for data[6,7] to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to 16 January 2017.

[6] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[7] Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however, these were not always available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[8]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of food additives sodium nitrate (E 251) and potassium nitrate (E 252) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety sodium nitrate (E 251) and potassium nitrate (E 252) as food additives in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a) for studies in rodents or, in the case of other animal species, by the JECFA (2000). In these cases, the daily intake is expressed as equivalent. In these cases the daily intake is expressed as equivalent. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a).

Dietary exposure to sodium nitrate (E 251(i) and (ii)) and potassium nitrate (E 252) from their use as food additives was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database, with the maximum permitted levels and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

Specific methodologies, applied to the following parts of the evaluation, are described in detail under the respective chapters:

- Selection of studies evaluating nitrosamines formation in food products authorised to contain nitrites and nitrates in the EU (Section 3.1.5);
- Exposure scenarios (Sections 3.3, 3.4 and 3.5);
- Selection and appraisal of epidemiological studies (Section 3.6.8);
- Derivation of guidance values for nitrate, nitrite and nitrosamines (Section 3.7).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substances

Solid sodium nitrate (E 251(i)) has the molecular formula $NaNO_3$ and a molecular weight of 85.00 g/mol. The CAS Registry number is 7631-99-4 and the European Inventory of Existing Commercial Chemical Substances (EINECS) (or EC) number is 231-554-3.

Solid sodium nitrate is described in Commission Regulation (EU) 231/2012[9] as a white crystalline, slightly hygroscopic powder. The melting point is 306.5°C (Haynes, 2010); the substance decomposes at 380°C (IARC, 2010). At room temperature, sodium nitrate is freely soluble in water (Haynes, 2015).

[8] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

[9] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

According to Commission Regulation (EU) 231/2012, synonyms include Chile saltpetre, cubic or soda nitre.

Liquid sodium nitrate (E 251(ii)) has the molecular formula $NaNO_3$ and a molecular weight of 85.00 g/mol (liquid sodium nitrate). The CAS Registry number is 7631-99-4 and the EINECS (or EC) number is 231-554-3, the same as those of solid sodium nitrate.

Liquid sodium nitrate is defined in Commission Regulation (EU) 231/2012 as 'an aqueous solution of sodium nitrate as the direct result of the chemical reaction between sodium hydroxide and nitric acid in stoichiometric amounts, without subsequent crystallisation. Standardised forms prepared from liquid sodium nitrate meeting these specifications may contain nitric acid in excessive amounts, if clearly stated or labelled' and is described as a clear colourless liquid in which the substance is present from 33.5% to 40.0% (w/w).

Potassium nitrate (E 252) has the molecular formula KNO_3 and a molecular weight of 101.11 g/mol. The CAS Registry number is 7757-79-1 and the EINECS (or EC) number is 231-818-8.

Potassium nitrate is described in Commission Regulation (EU) No 231/2012 as a white crystalline powder or transparent prisms having a cooling, saline, pungent taste. The melting point is 334°C and the substance decomposes at 400°C (Haynes, 2010). At room temperature, potassium nitrate is freely soluble in water (Haynes, 2015).

3.1.2. Specifications

The specifications for solid sodium nitrate (E 251(i)), liquid sodium nitrate (E 251(ii)) and potassium nitrate (E 252) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (JECFA, 2006a,b) are listed in Tables 1–3.

Table 1: Specifications for solid sodium nitrate (E 251(i)) according to Commission Regulation (EU) No 231/2012 and the JECFA (2006a)

	Commission Regulation (EU) No 231/2012	**JECFA (2006a)**
Assay	Content not less than 99% on the anhydrous basis	Not less than 99.0% on the dried basis
Description	White crystalline, slightly hygroscopic powder	Clear, colourless, odourless, transparent crystals, or white granules or powder; deliquescent in moist air
Identification		
Test for nitrate	Passes test	Passes test
Test for sodium	Passes test	Passes test
pH	5.5–8.3 (5% solution)	–
Solubility	–	Freely soluble in water; slightly soluble in ethanol
Purity		
Loss on drying	Not more than 2% (105°C, 4 h)	Not more than 2% (105°C, 4 h)
Nitrites	Not more than 30 mg/kg expressed as $NaNO_2$	Not more than 30 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–

The Panel also noted that the JECFA specifications include the following functional uses for sodium nitrate and potassium nitrate: antimicrobial preservative, colour fixative (JECFA, 2006a,b).

The Panel noted that, according to the EU specifications for sodium nitrate (E 251(i)), liquid sodium nitrate (E 251(ii)) and potassium nitrate (E 252), impurities of the toxic elements arsenic, lead and mercury are accepted up concentrations of 3, 2 and 1 mg/kg, respectively. Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance or benchmark doses (lower confidence limits) values established by the EFSA (EFSA CONTAM Panel, 2009a,b, 2010a, 2012a,b,c, 2014).

Table 2: Specifications[(a)] for liquid sodium nitrate (E 251(ii)) according to Commission Regulation (EU) No 231/2012

	Commission Regulation (EU) No 231/2012
Assay	Content between 33.5% and 40.0% of $NaNO_3$
Description	Clear colourless liquid
Identification	
Test for nitrate	Passes test
Test for sodium	Passes test
pH	1.53.5
Purity	
Free nitric acid	Not more than 0.01%
Nitrites	Not more than 10 mg/kg expressed as $NaNO_2$
Arsenic	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg
Mercury	Not more than 0.3 mg/kg

(a): This specification refers to a 35% aqueous solution.

There is no corresponding JECFA specification for this form of sodium nitrate.

Table 3: Specifications for potassium nitrate (E 252) according to Commission Regulation (EU) No 231/2012 and to (JECFA, 2006b)

	Commission Regulation (EU) No 231/2012	**JECFA (2006b)**
Assay	Content not less than 99% on the anhydrous basis	Not less than 99.0% on the dried basis
Description	White crystalline powder or transparent prisms having a cooling, saline, pungent taste	Colourless, odourless, transparent prisms, or white granular or crystalline powder
Identification		
Test for nitrate	Passes test	Passes test
Test for potassium	Passes test	Passes test
pH	4.5–8.5 (5% solution)	–
Solubility		Soluble in water; slightly soluble in ethanol
Purity		
Loss on drying	Not more than 1% (105°C, 4 h)	Not more than 1% (105°C, 4 h)
Nitrites	Not more than 20 mg/kg expressed as KNO_2	Not more than 20 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–

The Panel noted that in the European Commission specifications for potassium nitrate, information on the taste of the chemical is given and such information requires analysts to taste possibly harmful substances.

3.1.3. Manufacturing process

The majority of the world's production of sodium nitrate is from natural deposits in Chile (Wisniak and Garcés, 2001; Kirk-Othmer, 2006a). Traditionally, sodium nitrate was extracted from the ore by leaching with brine, followed by fractional crystallisation: the Shanks process, which allowed recovery of approximately 60% of the nitrate in the ore, was developed in 1863 and utilised until the late 1970s. The Guggenheim process developed in the late 1920s, which permitted the exploitation of natural reserves having a content of nitrate ore as low as 7%, was similarly based on leaching of caliche, at 30–40°C: a few modifications were introduced over time, but the process in use nowadays is not very different from the original one. Sodium nitrate can also be made synthetically by absorbing

waste gases from nitric acid production in sodium hydroxide or a sodium carbonate solution. The gases contain nitrogen dioxide (NO_2) and nitric oxide (NO). However, the process is not very efficient and tends to produce mainly sodium nitrite with possibly small amounts of sodium nitrate: if some sodium nitrate is formed, it can readily be recovered by differential crystallisation. Sodium nitrate can also be obtained from the nitrite by treatment with nitric acid.

Potassium nitrate can be produced through a double replacement reaction between potassium chloride and sodium nitrate or ammonium nitrate: the by-products of these reactions are sodium chloride and ammonium chloride, respectively. Potassium chloride can also react with nitric acid to yield potassium nitrate and hydrochloric acid: the reaction can be chemically driven so that chlorine is produced as an economically advantageous co-product (Kirk-Othmer, 2006b; Joshi et al., 2015).

Food-grade sodium and potassium nitrate are readily available from the market.

3.1.4. Methods of analysis in food

The measurements of nitrite/nitrate are performed for three main reasons: (a) to assess compliance with the current regulation on additives and contaminants; (b) to determine the concentration of nitrite/nitrate in individual foodstuffs and diet; and (c) to study the fate of nitrite/nitrate in the body. Each of these applications demands specific requirements that the analytical chemist should attended to demonstrate the method is 'fit for purpose'. Thus, the international standardisation bodies (European Committee for Standardization (CEN) and International Organisation for Standardisation (ISO)) have approved five analytical methods for the determination of nitrate in foodstuffs (meat products and milk products).

The CEN describes two methods for the determination of nitrate ion in meat products (CEN, 1997). In the first method (CEN, 2005a), nitrate is extracted from a homogenised sample under alkaline conditions (pH 8–8.5). The extract is clarified with Carrez solution 1 (potassium hexacyanoferrate(II) solution, 150 g/L) and Carrez solution 2 (zinc acetate solution, 230 g/L). Nitrate in the clarified extract is reduced to nitrite by the addition of nitrate reductase. Sulfanilamide is added to the resulting solution, followed by *N*-(1-naphthyl)-ethylenediamine dihydrochloride. This produces a red compound whose concentration is determined spectrometrically at a wavelength of 540 nm. If nitrite is also present in the sample, this is determined in the same way but without the reduction step, and the nitrate content is calculated by subtraction this result from the result including the reduction step. In the second method (CEN, 2005b), nitrate is extracted from the sample in hot water. Acetonitrile is used to remove interfering substances from the extract. Ion exchange chromatography is used to separate nitrate from nitrite, and both are quantified using ultraviolet detection at 205 nm. The limit of detection (LOD) for nitrate was 10 mg/kg.

The ISO describes three alternative implementations of a method related to that described by the CEN (2005a) for the analysis of nitrate (and nitrites) in milk and milk products. In the first (ISO, 2004a), a sample of the milk product is dispersed in warm water. Fat and proteins are precipitated, and the resulting mixture is filtered. A copperised cadmium column is used to reduce the nitrate to nitrite. Sulfanilamide and *N*-1-naphthyl ethylenediamine dihydrochloride are used to produce a coloured derivative, which is determined spectrometrically at 538 nm. When nitrite is present at significant levels (see below) in the sample, derivatisation is carried out on an unreduced sample to determine the nitrite content, and the nitrate content is calculated as the difference. In the second method (ISO, 2004b), the procedure is similar but a flow system is used and a dialysis cell is employed to clean up the dispersion product before reduction (for nitrate), derivatisation and quantification. The nitrate content is calculated as the difference between reduced and unreduced samples as above. The third method (ISO, 2004c) uses a flow injection system, including a dialysis cell. This standard indicates that, if the level of nitrite is above 0.5 mg/kg or exceeds 10% of the nitrate content, then the nitrite content should be subtracted from the total to give the nitrate content. The second and third methods reduce the volume of waste solutions, thus reducing the potential exposure or release of cadmium.

The Nordic Committee of Analysis of Food (NMKL, 2013) specifies a spectrophotometric method for the determination of nitrate/nitrite content in foodstuffs and water after zinc reduction and very sensitive and widely used Griess reaction. The method has been validated in vegetables (lettuce), meat products, baby food, dairy product (milk) and surface water. The LOD of nitrate for surface water and meet products was 0.04 mg/L and 5 mg/kg, respectively.

The Official Methods of Analysis of AOAC INTERNATIONAL present two photometric methods for the determination of nitrate/nitrite in meat and cured meat (AOAC, 2005). In the first method, nitrate ion react with 2,4-xylenol in sulfuric acid, steam-distilled and measured at 450 nm. Nitrite is oxidise to nitrate with

potassium permanganate and determined by difference. The second method is based on diazotisation-coupling principle (Griess reaction). Read at 540 nm. This method was adopted as a Codex Reference method (Type II) for nitrite and potassium and/or sodium salts in canned corned beef and luncheon meat.

IARC (2010) compiled analytical methods for nitrate and nitrite. The majority of the methods are for analysis in water. A number of the methods included are for matrices relevant to the use of sodium and potassium nitrate as additives in foodstuffs: meat and meat products, cured meats, curing preparations, and cheese. No limits of detection or quantification are reported for these methods in the IARC Monograph (IARC, 2010).

Recent examples are provided below of methods for the analysis of nitrate in foodstuffs, including some modifications to the standard methods described above.

Chung et al. (2011) analysed the levels of nitrate and nitrite in vegetables in Hong Kong. Samples were extracted with hot water and the extracts were cooled and filtered. For nitrate, an ion chromatograph with a conductivity detector was used for quantification. The LOD was given as 4 mg/kg and the limit of quantitation (LOQ) was 10 mg/kg.

Leth et al. (2008) analysed a range of meat products for their nitrate and nitrite contents. Samples were extracted with hot water; protein was precipitated by the addition of Carrez solutions I (potassium hexacyanoferrate (II) at 150 g/L) and II (zinc sulfate at 300 g/L). Following filtration, the sample was injected into a flow analysis system. This included a cadmium column to reduce the nitrate to nitrite; this was then reacted in the system with sulfanilamide and *N*-(1-naphthyl)-ethylene diammonium chloride to form a violet azo colour, which was measured spectrophotometrically at 540 nm. The LOD was 5 mg/kg.

Iammarino et al. (2013) reported the results of 5 years of official control and monitoring of nitrate and nitrite in 1,785 samples of fresh meat products, shellfishes, diary product and leafy vegetables. The determinations of nitrate and nitrite were carried out with an in-house validated ion chromatographic method with electrochemical detection. All positive samples were confirmed by a different verified chromatographic method that uses the same procedure for sample preparation and similar chromatographic system but a different ionic exchange mechanism. Acceptable agreement in the comparative performance of both methods was reported. The LOD for nitrate was 3.2 mg/kg.

Croitoru (2012) developed a high-performance liquid chromatography (HPLC)-ultraviolet (UV)/visible (VIS) method for the determination of low concentrations of nitrite and nitrate in vegetables and biological samples. The method combines the simultaneous VIS detection of the nitrite related azo dye (Griess reaction), with the simple UV detection of nitrate. The proposed method provide an alternative to overcome the poor detectability of nitrite due to interference of UV absorbing substances that often masks the nitrite peak and makes unreliable the UV/VIS detection in HPLC methods. The LOD and LOQ in plasma for nitrate ion are 0.06 and 0.2 μg/L, respectively.

The sensitivity and accuracy of the official methods are commonly good enough for enforcing purposes; however, the need to improve the accuracy of the exposure estimate by detecting low levels of nitrite/nitrate in individual foods and diet, as well as the study of their fate in the body, has encouraged the development of new analytical methods using other detection techniques. Thus, in EFSA CONTAM Panel (2009b), several methods were mentioned for the simultaneous quantitative determination of nitrate and nitrite in different food items, including chromatography (Butt et al., 2001; Stalikas et al., 2003; McMullen et al., 2005), amperometry, capillary electrophoresis (Öztekin et al., 2002) and spectrophotometry (Andrade et al., 2003; Ensafi et al., 2004; Casanova et al., 2006). Methods, selectively intended for the quantitative determination of nitrite alone include spectrophotometry with or without flow injection analysis (Ghasemi et al., 2004), chemiluminescence (Gao et al., 2005; He et al., 2007), fluorescence (Gao, 2002; Li et al., 2003), optical sensor technology (Kazemzadeh, 2005) and even dipstick technology (Fang et al., 2005).

3.1.5. Stability of the substance, and reaction and fate in food

Concerning stability in food, no precise information could be identified on nitrate added to food items, although it was reasonable to assume that the nitrate concentration will decrease over time due to the activity of nitrate-reducing bacteria or their own chemical instability and reactivity. Inversely, nitrate concentration can also be increased in some heat-treated meat products from the natural conversion of nitrite to nitrate in a low-acid environment (Honikel, 2008). The formation of nitrate, even in cases when no nitrate has been added, proceeds through the formation of NO_2^- from N_2O_3. Nitrous anhydride (N_2O_3) is formed in food through its formation from nitrite (NO_2^-) in acidic aqueous solution according to the following reaction:

$$2NO_2^- + 2H^+ \leftrightarrows N_2O_3 + H_2O$$

$$N_2O_3 \rightarrow NO + NO_2$$

$$2NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+$$

Furthermore, some NO_2 may be also generated in the presence of air:

$$NO + {}^1/_2\,O_2 \rightarrow NO_2$$

In summary, the overall reaction (Pegg and Honikel, 2015) is:

$$2NO_2^- + {}^1/_2\,O_2 \rightarrow NO_2^- + NO_3^-$$

This means that, from two NO_2^- molecules, one HNO_3 molecule is produced. Part of the nitrate generated is then reduced to nitrite in the meat product through bacterial reduction or might remain as nitrate if the pH is too low and the activity of bacterial nitrate reductase is inhibited. This is why some nitrate may be detected in meat products by laboratory controls where no nitrate was initially added.

It has also been reported that, in some particular cooked meat products with high pH values (within 6.0–6.8), such as liver and blood sausages, the added nitrite may be oxidised to nitrate by the endogenous enzymes or by metal ions (Pegg and Honikel, 2015).

Specific information on the stability of nitrate in vegetables was identified for this re-evaluation. Although the Panel is aware that the information is related to the natural occurrence of nitrate in vegetables and not to their use as food additives, the Panel considered that it is also useful to present this general information on nitrate reaction and fate in foods. Nitrate may naturally occur in vegetables and their levels in raw agricultural commodities can be influenced by a number of factors such as storage time and conditions (i.e. ambient, refrigerated, frozen) and food processing (i.e. washing, peeling, blanching, boiling)[10] (EFSA CONTAM Panel, 2008).

Tamme et al. (2009) monitored the changes in nitrate concentration during the industrial production of vegetable-based infant food. The overall nitrate content decreased as the vegetables were mixed with other ingredients, although the processing did not have any significant effect on the levels. Storage of opened cans of infant food under refrigerated conditions led to an average increase of 15% in the nitrate level over 48 h. Storage over the same duration at 20–22°C led to an increase of 29%. The nitrite content in all analysed samples was below the quantification limit.

The same authors, Tamme et al. (2010), measured the concentrations of nitrate and nitrite in home-made and small-scale industrially produced vegetable juices and the effect of storage. The nitrate contents in the industrially produced juices (carrot, red beetroot and cabbage) decreased with storage at ambient temperatures, by 47%, 39% and 57%, respectively, after 48 h. Lower reductions in nitrate concentrations of 11–30% were seen after 48 h under refrigerated conditions. The greatest decrease in nitrate concentrations from 1,708 to 739 mg/L at 20–22°C was observed in red beetroot juice, and was accompanied by an increase in nitrite concentration from 3.2 to 11.1 mg/L. A similar pattern was seen with the home-made vegetable juices under both ambient and refrigeration conditions, although higher nitrite levels (up to 110 mg/L) were found in the juices stored at ambient temperatures.

Chung et al. (2004) examined nitrate and nitrite levels in four types of vegetables (spinach, crown daisy, organic Chinese spinach and organic non-heading Chinese cabbage) during storage at ambient (22 ± 1°C) and refrigerated (5 ± 1°C) conditions over 7 days. At ambient temperatures, nitrate levels dropped significantly from day 3 onwards; initial levels were ~3,000–5,000 mg/kg, although these were all below the detection limit (not given) after 4 days. Nitrite levels increased from around 5 mg/kg at day 3 to around 4,500 mg/kg in spinach after 5 days, before declining to around 4,000 mg/kg by day 7. A similar pattern was seen in organic Chinese spinach and organic non-heading Chinese cabbage, with nitrite levels peaking on day 4 and declining thereafter, but still at around 2,500 and 1,000 mg/kg, respectively, after 7 days. By contrast, only a slight increase in nitrite levels in crown daisy was seen. No changes in nitrite levels (around 5 mg/kg) or nitrate levels (ranging from 2,830 to 5,270 mg/kg) were seen in the refrigerated samples of all four vegetables.

Some processing conditions can also influence the content in nitrate levels in vegetables. Leszcynska et al. (2009) reported the effect of blanching, boiling, freezing, frozen storage and boiling

[10] Available online: http://www.efsa.europa.eu/en/supporting/pub/514e

on the previous frozen vegetables. The blanching and boiling processes caused a considerable reduction in the nitrate with respect to the raw vegetables, which ranged from 35% in blanched green cauliflower to 73% in boiled curly kale. Other studies have reported a decrease of 52% in cooked carrot (Czarniecka-Skubina and Gołaszewska, 2001) although the losses depend on the cooking method, duration and type of vegetable. During frozen storage of vegetables, increases in nitrite have been observed compared with blanched vegetables due to the fact that nitrate is partly reduced to nitrite after storage periods longer than 4 months (Leszcynska et al., 2009).

Under appropriate conditions (pH and concentration of reactants), nitrites, resulting from the reduction of nitrates, have been shown to form NOCs (nitrosamines and nitrosamides) from constituents in the food. The reactions involved in this process are generally described as nitrosation.

Nitrosamines are formed by the reaction of a nitrosating agent with an amine, and their formation could be due to a chemical and/or microbial reaction. Formation of *N*-nitrosamines is a complex process and their presence in meat products depends on different parameters related to the preparation, thermal processing and storage of meat. The presence of amines and the pH together with the nitrite are the conditions that enhance the formation of nitrosamines (Drabik-Markiewicz et al., 2009).

The basic chemical reaction (nitrosation) that may lead to nitrosamine formation in food has been described in several studies (Lijinsky, 1999; Scanlan, 2003) and, recently, in more detail by Pegg and Honikel (2015). A detailed description of nitrosation has not been included in this opinion as sodium and potassium nitrite are the subjects of a separate opinion by the Panel (EFSA ANS Panel, 2017).

Nitrate, when converted to nitrite, can also react with haem proteins and smoke components (Walters, 1992). The woodwood smoke and liquid smoke used for the production of meat and meat products contain a large number of compounds, of which acids, phenols and carbonyls are the main components (Simko, 2011) together with other compounds such as formaldehyde (Sen et al., 1986). This compound and other aldehydes can condensate with cysteine resulting in the formation of nitrosamines. However, phenols may prevent the nitrosamine formation. Higher levels of the non-volatile *N*-nitroso-thiazolidine-4-carboxylic acid (NTCA) have been reported for smoked meat than for non-smoked meat (Sen et al., 1986; Tricker and Kubacki, 1992). The levels of volatile nitrosamines *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) do not appear to be affected by smoking. Liquid smoke added to a meat product provides an acidic and antioxidant medium that favours the transformation of nitrite in nitrous acid and, consequently, the formation of red nitrosomyoglobin, reduces the nitrite level (Girard, 1991). Theiler et al. (1984) reported that liquid smoke reduced the volatile nitrosamine NPYR in fried minced pork that has been nitrite cured. Another study showed that the use of commercial smoke flavouring to frankfurters, with potassium nitrate (200 mg/kg), resulted in a significant reduction of nitrate to nitrite after 26 days of refrigerated storage (Pérez-Rodríguez et al., 1998).

The pigments responsible for the colour of meat are myoglobin (Mb) and haemoglobin (Ledward, 1984), with Mb being the main component. Many ligands can bind to the iron atom in the haem ring in myoglobin and the resultant bonds are responsible for the various colours observed in meat. The most important Mb forms in fresh meat are red oxymyoglobin. In metmyoglobin (MetMb), the iron is oxidised to Fe^{3+} and this causes a loss of the ability of myoglobin to reversibly bind with oxygen. In addition, nitrosomyoglobin (NOMb) is also an important derivative responsible for the pink colour of uncooked, cured products (Millar et al., 1996).

The colour of cured meat products depends on the reaction of myoglobin with sodium chloride and curing salts (nitrates and/or nitrites). During fermentation in meat products, nitrite forms nitric oxide which reacts with the haem compounds of myoglobin to form NOMb, giving the product its characteristic colour and oxidise Mb into MetMb that also reacts with NO to form nitrosometmyoglobin (NOMetMb). During drying, NOMetMb is reduced to NOMb (Chasco et al., 1996). In cooked cured products, the cooking processing denatures and separates the protein from the non-protein haem and contributes to the visual colour change to the final pink colour of the cooked cured meat due to the presence of nitrosylhemochrome pigment. The system in all cured meats include sodium chloride in different amounts and nitrous acid reacts with the chloride ion to produce nitrosyl chloride (NOCl) which is a more active nitrosylating agent than N_2O_3 (Møller and Skibsted, 2002). This means that chloride ions accelerate colour development in cured meat.

$$HNO_2 + H^+ + Cl^- \rightarrow NOCl + H_2O$$

Nitrous acid can also react with sulfhydryl groups of meat proteins to release nitric oxide an oxidation–reduction reaction that results in a disulfide according to the following reaction (Pegg and Shahidi, 2004):

$$2HNO_2 + 2R\text{-}SH \rightarrow 2R\text{-}S\text{-}S\text{-}R + 2NO + 2H_2O$$

This contributes to meat texture as the crosslinking between proteins could lead to an increase in firmness.

The application of heat during processing, such as frying or baking, to meat products containing nitrates and nitrites affects the levels of nitrosamines in processed meat (Drabik-Markiewicz et al., 2009). In the products heated above 130°C, an increase in nitrosamines can be observed (Honikel, 2008). The presence of NDMA in heated meat products was only influenced by temperatures higher than 120°C and by the nitrite concentration and not by nitrate concentration in the brine. A further increase in the nitrosamines can be observed at even higher temperatures (e.g. the levels of NDMA and NPYR were affected by frying, with temperature ranging from 150 to 200°C being the optimal for their formation) (Drabik-Markiewicz et al., 2009).

The addition of potassium or sodium nitrate in the manufacture of certain types of cheese is a common method for the prevention of blowing and gassy defects (Tompkin, 2005). Nitrate in cheese is reduced to nitrite by xanthine oxidase present in milk or by nitrate reductase produced by microorganisms (Munksgaard and Werner, 1987).

Results of systematic review on the types and levels of nitrosamines and nitrosamides in experimental studies with meat products upon addition of nitrates and nitrites (food additive grade)

The Panel considered important to address the question of which nitrosamines and nitrosamides are produced in food products from the use of nitrates and nitrites as food additives and at which levels they can be found in those food products. Therefore, a systematic review has been performed (see Appendix F) with the objective to select reliable studies performed to identify the type of nitrosamines and nitrosamides and to measure their respective levels in food products found in the European market to which specified amounts of nitrates/nitrites have been added. Based on the search strategy, a total of 33 articles were identified and 10 of them have been evaluated as being of good quality. Out of these 10 articles, there were four studies on cheese with appropriate control samples which were considered by the Panel and they have been summarised in the Appendix F. No relevant studies measuring nitrosamides in cheese products were found. The Panel noted that no studies of good quality were identified in which nitrosamine formation was measured after the addition of nitrates to meat products.

The Panel noted on the results that:

- the compounds measured in dairy products were volatile nitrosamines mostly NDMA, *N*-nitrosodiethylamine (NDEA), *N*-nitroso-di-*n*-propylamine (NDBA) and *N*-nitrosomorpholine (NMOR).
- these studies in which all conditions were held constant and only the amount of nitrates has been changed, did not show a relationship between nitrates added and the formation of the non-volatiles nitrosamines tested in the different cheese.

3.1.6. Technological function

The technological functions of nitrates have been reviewed by the SCF with respect to their uses in meat products, cheese milk and fish products (SCF, 1992).

Data in the literature indicate that the sodium and potassium salts of nitrate combined with nitrite salts are commonly used in curing mixtures (i.e. sodium chloride solutions) for meats to develop and fix the colour of meat, to inhibit microbial growth and to develop characteristic flavours. Sensory evaluations also indicate that nitrite contributes to cured meat flavour, apparently through its role as antioxidant (Ramarathnam and Rubin, 1994). Nitrite rather than nitrate is the functional constituent the former, also contributing to the characteristic colour development. Preservation of meat is necessary for its extended storage, especially because no raw meat is completely sterile and there is always a potential for the presence and growth of pathogenic microorganisms that might cause infections and intoxications. Meat is an excellent medium for the growth of *Clostridium botulinum* and its toxin production, which is a highly neurotoxic protein. Nitrite production in meat occurs via the bacterial reduction of nitrate. Furthermore, nitrites rather than nitrates inhibit the growth of Clostridia in cured meats. The Panel noted that the use of nitrates in meat products would serve mainly as a necessary reservoir for nitrite production to achieve the foreseen technological functions. Thus, in meat products, nitrates do not appear to serve a direct technological role as opposed to nitrites. The antimicrobial mechanism of nitrite is unknown, although it has been suggested that the acidified nitrite results in the generation of nitric oxide (NO) through the intermediate N_2O_3 as described below, which has potent antibacterial activity against a range of

pathogens, including *Salmonella*, *Yersinia* and *Sheila* species, *Helicobacter pylori* and *Pseudomonas aeruginosa* (Lundberg et al., 2008). The efficacy of nitrite combined with other factors like heat treatment, pH, water activity, salt and redox potential, in inhibiting growth and toxin production by *C. botulinum* is widely recognised in the scientific literature and has an excellent record of safety. However, some traditional products with slow ripening such as traditional dry-fermented sausages or dry-cured ham may need the use of nitrate as a reservoir of nitrite that is generated through the nitrate reductase activity of the microbial flora. Even though nitrate does not directly protect against *C. botulinum*, it is its reduction to nitrite that is active against *C. botulinum* (EFSA, 2003).

As mentioned before, the use of nitrate appears necessary in particular traditional dry meat products, typical of the Mediterranean countries, like long-ripened dry-fermented sausages and dry-cured ham. Traditional dry-fermented sausages experience a long-ripening process (up to several months) with a slow and mild pH drop (usually the pH remains relatively high), where the added nitrate is progressively reduced to nitrite contributing not only to safety, but also to specific and characteristic sensory properties. The rate of reduction is quite variable and mainly depends on the natural microbial flora that is present in the product. Dry-cured ham is an entire piece where curing salt has to be rubbed on the outer surface of the ham and left for several weeks for its diffusion through the entire piece (Toldrá, 2002). This is a very slow process that may take up to 2 months under cold storage, where the progressively diffused nitrate constitutes a reservoir of nitrite. In this way, nitrite effectively reaches the centre of the ham; especially to critical inner locations like the bone joints, where protection by nitrite is essential before the next stages of processing, which increase the temperature for ripening and drying of the product (EFSA, 2003). A recent study on the evolution of nitrate and nitrite inside dry-cured hams formulated with different types of salts showed that residual nitrate in dry-cured ham was < 20 mg/kg (expressed as ion) in the outer muscle semimembranosus and < 40 mg/kg in the inner muscle biceps femoris (Armenteros et al., 2012). In the case of nitrite, the levels were very low, < 1 mg/kg (expressed as ion) in the outer muscle and < 3 mg/kg in the inner muscle (Armenteros et al., 2012). It must be taken into account that, in meat products, the added amount of nitrate is reduced to nitrites with a high variability. So, monitoring residual levels of nitrate in the final product gives poor information on how much nitrate was initially added. This is due to the different rate of nitrate loss in the products, depending on the type of the product, processing conditions and microbiota present (EFSA, 2003).

The situation is different with regard cheese milk in which the addition of nitrate serves a direct role of preservative against bacteria such as *Clostridium tyrobutyricum*, which hampers the manufacturing of certain cheeses. Also, in the case of fish products, the information gathered by the SCF suggested that nitrates were used directly to prevent the growth of microorganisms producing off-flavours during ripening with no apparent preservative function against pathogens (SCF, 1992).

3.2. Authorised uses and use levels

Maximum levels of nitrates (E 251–E 252) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, nitrates (E 251–E 252) are authorised food additives in the EU at MPLs ranging from 10 to 500 mg/kg in 24 food categories (considering different restrictions/exceptions).

Table 4 summarises foods that are permitted to contain nitrates (E 251–E 252) and the corresponding maximum permitted levels (MPLs) as set by Annex II to Regulation (EC) No 1333/2008.

Table 4: Summary of foods that are permitted to contain nitrates (E 251–E 252) and the corresponding maximum permitted levels (MPLs) as set by Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
01.7.2	Ripened cheese	Nitrates (E 251–E 252)	Only hard, semihard and semisoft cheese	150[(a)]
01.7.4	Whey cheese	Nitrates (E 251–E 252)	Only cheese milk of hard, semihard and semisoft cheese	150[(a)]

Food category number	Food category name	E-number/ group	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
01.7.6	Cheese products (excluding products falling in category 16)	Nitrates (E 251–E 252)	Only hard, semihard and semisoft ripened products	150[a]
01.8	Dairy analogues, including beverage whiteners	Nitrates (E 251–E 252)	Only dairy-based cheese analogue	150[a]
08.3.1	Non-heat-treated processed meat	Nitrates (E 251–E 252)		150[b]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *Wiltshire bacon* and similar products: meat is injected with curing solution followed by immersion curing for 3–10 days. The immersion brine solution also includes microbiological starter cultures	250[c,d]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *Wiltshire ham* and similar products: meat is injected with curing solution followed by immersion curing for 3–10 days. The immersion brine solution also includes microbiological starter cultures	250[c,d]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *Entremeada, entrecosto, chispe, orelheira e cabeca (salgados), toucinho fumado* and similar products: Immersion cured for 3–5 days. Product is not heat-treated and has a high water activity	250[c,d]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *cured tongue*: immersion cured for at least 4 days and precooked	10[c,d]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *kylmâsavustettu poronliha/kallrökt renkött*: meat is injected with curing solution followed by immersion curing. Curing time is 14–21 days followed by maturation in cold-smoke for 4–5 weeks	300[b]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *bacon, filet de bacon* and similar products: Immersion cured for 4–5 days at 5–7°C, matured for typically 24–40 h at 22°C, possibly smoked for 24 h at 20–25°C and stored for 3–6 weeks at 12–14°C	250[b,d,e]
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)	Nitrates (E 251–E 252)	Only *rohschinken, nassgepökelt* and similar products: Curing time depends on the shape and weight of meat pieces for approximately 2 days/kg followed by stabilisation/maturation	250[c]
08.3.4.2	Traditional dry cured products. (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation)	Nitrates (E 251–E 252)	Only *dry cured bacon* and similar products: dry curing followed by maturation for at least 4 days	250[c,d]
08.3.4.2	Traditional dry cured products. (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation)	Nitrates (E 251–E 252)	Only *dry cured ham* and similar products: dry curing followed by maturation for at least 4 days	250[c,d]

Food category number	Food category name	E-number/ group	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
08.3.4.2	Traditional dry cured products. (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation)	Nitrates (E 251–E 252)	Only *jamon curado, paleta curada, lomo embuchado y cecina* and similar products: dry curing with a stabilisation period of at least 10 days and a maturation period of more than 45 days	250[c,d]
08.3.4.2	Traditional dry cured products. (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation)	Nitrates (E 251–E 252)	Only *presunto, presunto da pa and paio do lombo* and similar products: dry cured for 10–15 days followed by a 30–45-day stabilisation period and a maturation period of at least 2 months	250[c,d]
08.3.4.2	Traditional dry cured products. (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation)	Nitrates (E 251–E 252)	Only *jambon sec, jambon sel* and other similar dried cured products: dry cured for 3 days + 1 day/kg followed by a 1-week post-salting period and an ageing/ripening period of 45 days to 18 months	250[c,d,e]
08.3.4.2	Traditional dry cured products. (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation)	Nitrates (E 251–E 252)	Only *rohschinken, trockengepökelt* and similar products: curing time depending on the shape and weight of meat pieces for approximately 10–14 days followed by stabilisation/maturation	250[c,d]
08.3.4.3	Other traditionally cured products. (immersion and dry cured processes used in combination or where nitrite and/or nitrate is included in a compound product or where the curing solution is injected into the product prior to cooking)	Nitrates (E 251–E 252)	Only *rohschinken, trocken-/nasgepökelt* and similar products: dry curing and immersion curing used in combination (without injection of curing solution). Curing time depends on the shape and weight of meat pieces for approximately 14–35 days followed by stabilisation/ maturation	250[c,d]
08.3.4.3	Other traditionally cured products. (immersion and dry cured processes used in combination or where nitrite and/or nitrate is included in a compound product or where the curing solution is injected into the product prior to cooking)	Nitrates (E 251–E 252)	Only *jellied veal and brisket*: Injection of curing solution followed, after a minimum of 2 days, by cooking in boiling water for up to 3 h	10[c,d]
08.3.4.3	Other traditionally cured products. (immersion and dry cured processes used in combination or where nitrite and/or nitrate is included in a compound product or where the curing solution is injected into the product prior to cooking)	Nitrates (E 251–E 252)	Only *rohwürste (salami and kantwurst)*: product has a minimum 4-week maturation period and a water/protein ratio of less than 1:7	300[b,e]

Food category number	Food category name	E-number/ group	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
08.3.4.3	Other traditionally cured products. (immersion and dry cured processes used in combination or where nitrite and/or nitrate is included in a compound product or where the curing solution is injected into the product prior to cooking)	Nitrates (E 251–E 252)	Only *Salchichon y chorizo traducionales de larga curacion* and similar products: maturation period of at least 30 days	250(b,d,e)
08.3.4.3	Other traditionally cured products. (immersion and dry cured processes used in combination or where nitrite and/or nitrate is included in a compound product or where the curing solution is injected into the product prior to cooking)	Nitrates (E 251–E 252)	Only *saucissons sec* and similar products: raw fermented dried sausage without added nitrites. Product is fermented at temperatures in the range 18–22°C or lower (10–12°C) and then has a minimum ageing/ripening period of 3 weeks. Product has a water/protein ratio of less than 1:7	250(b,d,e)
09.2	Processed fish and fishery products including molluscs and crustaceans	Nitrates (E 251–E 252)	Only pickled herring and sprat	500

MPL: maximum permitted level.
(a): In the cheese, milk or equivalent level if added after removal of whey and addition of water.
(b): Maximum amount that may be added during the manufacturing, expressed as $NaNO_2$ or $NaNO_3$.
(c): Maximum residual amount, residue level at the end of the production process, expressed as $NaNO_2$ or $NaNO_3$.
(d): Nitrates may be present in some heat-treated meat products resulting from natural conversion of nitrites to nitrates in a low-acid environment.
(e): Without added nitrites.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of nitrates (E 251–E 252)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[11] for occurrence data (usage level and/or concentration data on nitrates (E 251–E 252). In response to these calls, both types of data on nitrates (E 251–E 252) were submitted to EFSA by industry and Member States, respectively.

In addition, analytical data on nitrates were collected through a call for annual collection of chemical contaminant occurrence data in food and feed issued by EFSA in December 2010 with a closing date of 1 October of each year.[12]

Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 6) of nitrates (E 251–E 252) in foods for two out of 24 food categories in which nitrates (E 251–E 252) are authorised. These use levels were reported as potassium nitrate (n = 4) and sodium nitrate (n = 2).

Updated information on the actual use levels of nitrates (E 251–E 252) in foods was made available to EFSA by FoodDrinkEurope for the following food categories of finished products: non-heat-treated processed meat (FCS 08.3.1) and traditionally cured meat products (FCS 08.3.4).

The Panel noted that the reported usage levels were all identical (minimum, typical and maximum levels) and equal to the MPL for all food categories for which data has been reported.

Appendix A.1 provides data on the use levels of nitrates (E 251–E 252) in foods as reported by industry.

[11] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm
[12] Call for continuous collection of chemical contaminants occurrence data in food and feed. Published: 16 December 2010. Available online: http://www.efsa.europa.eu/en/data/call/datex101217

Summarised data on concentration levels in food submitted by Member States

In total, 53,551 analytical results were reported to EFSA by 25 countries: Germany (n = 18,004), Spain (n = 5,303), Slovakia (n = 3,809), Bulgaria (n = 2,778), Austria (n = 2,768), Hungary (n = 2,757), the Netherlands (n = 2,704), Ireland (n = 2,576), Slovenia (n = 2,175), the Czech Republic (n = 2,140), Denmark (n = 1,762), Romania (n = 1,178), Poland (n = 986), France (n = 869), Belgium (n = 790), Finland (n = 764), Luxembourg (n = 475), Cyprus (n = 415), Portugal (n = 406), Estonia (n = 326), Sweden (n = 227), Malta (n = 154), Croatia (n = 102), Latvia (n = 53) and Greece (n = 30). Of these, 12,461 analytical results were related to foods that are permitted to contain nitrates (E 251–E 252) as food additives and 41,090 analytical results were related to nitrates from other foods where nitrates have been considered to be naturally present or due to contamination.

The data on nitrates (E 251–E 252) as food additives were mainly for non-heat-treated processed meat (FCS 08.3.1; n = 4,403), traditionally cured meat products (FCS 08.3.4; n = 5,657) and ripened cheese (FCS 1.7.2; n = 948). Foods were sampled between 2000 and 2014, and the majority of them (99%) were analysed in the year that they were collected. Only three analytical data were available for processed fish and fishery products (FCS 09.2).

The analytical data on nitrates from other sources (natural presence and contamination) were mainly for vegetables and vegetable products (n = 25,603), drinking water (n = 4,365), food for infants and small children (n = 3,548), meat and meat products (n = 3,154) and starchy roots and tubers (n = 2,197). Foods were sampled between 2000 and 2015, and the majority of them (99%) were analysed in the year that they were collected. These analytical data were used only for the exposure scenario considering all sources (food additives, natural presence and contaminants) as described in Section 3.5.

Considering data on nitrates (E 251–E 252), 11.7% of the analytical results were left-censored: either not quantified (< LOQ) in 1,081 samples or not detected (< LOD) in 374 samples. Considering data on nitrates from other sources (natural presence and contamination), 13.4% of the analytical results were left-censored: either not quantified (< LOQ) in 4,875 samples or not detected (< LOD) in 617 samples. Therefore, it should be noted that the use of middle-bound (MB) LOD/LOQ values (half of LOD or LOQ) in the exposure assessment (Section 3.5), may have resulted in either an overestimation, where nitrates (E 251–E 252) or nitrates from other sources were not present, or an underestimation, where the concentration was between the MB and LOQ/LOD value, although the analytical method was not able to detect or quantify it.

Regarding the sampling strategy, 1,174 data on nitrates (E 251–E 252) and 4,223 data on nitrates from other sources (natural presence and contamination) were excluded due to an inappropriate sampling strategy (e.g. suspect sampling).

Occurrence data sampled from 2000 to 2006 (8,724 records from Germany, Denmark and Slovakia) were considered as obsolete and were removed from the assessment. Of these, 5,817 results were on nitrates (E 251–E 252) and 2,907 results were on nitrates from other sources (natural presence and contamination).

Four results on nitrates from other sources (natural presence and contamination) were removed because neither the concentration nor the LOD or LOQ values were reported. Complete information on the methods of analysis (e.g. validation) was not made available to EFSA, although the majority (95%) of samples were derived from accredited laboratories.

Overall, 50 results were removed because they were considered as outliers.

The Panel considered only exposure resulting from authorised uses with occurrence levels not exceeding the MPLs due to the fact that results over MPL is part of risk management measures (e.g. non-compliance purpose). For this reason, such analytical results over the MPLs (n = 168) were not used in the present exposure assessment.

Overall, 39,208 analytical results, 5,717 results reported for nitrates (E 251–E 252) and 33,491 results reported for nitrates from other sources (natural presence and contamination), were considered by the Panel in the present exposure assessment. For the scenario considering only nitrates (E 251–E 252) as food additives, data were available for 21 food categories out of 24 in which nitrates (E 251–E 252) are authorised as food additives (Annex II to Regulation No 1333/2008).

Because a part of the analytical data was reported on salts, the following conversion factors were applied to convert those data into nitrate ion: for potassium nitrate and sodium nitrate, the analytical results were divided by 1.62 and by 1.37, respectively.

Appendix A.2 shows the analytical results of nitrates (E 251–E 252) and nitrates from other sources (natural presence or contamination) in foods as reported by Member States.

3.3.2. Summarised data extracted from the Mintel GNPD database

The Mintel's Global New Products Database (GNPD) is an online database that monitors product introductions in consumer packaged goods markets worldwide. It contains information for over 2 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering the EU food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the GNPD.[13]

For the purpose of this Scientific Opinion, the GNPD[14] was used for checking the labelling of products containing nitrates (E 251–E 252) within EU food products because the GNPD shows the compulsory ingredient information presented in the labelling of products.

In the 20 EU countries, nitrates (E 251–E 252) are labelled on products (n = 6568) mainly of 'Processed Fish, Meat & Egg Products', 'Meals &Meal Centers', 'Dairy', 'Snacks', 'Side Dishes' and 'Savoury Spreads'.

Appendix B presents the percentage of the food products labelled with nitrates (E 251–E 252) within the last 5 years, out of the total number of food products per food sub-categories according to the Mintel food classification.

3.3.3. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys recently[15] added in the Comprehensive database were also taken into account in this assessment.[8]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' under-reporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 5).

Table 5: Population groups considered for the exposure estimates of nitrates (E 251–252)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK

[13] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[14] Available online: http://www.gnpd.com/sinatra/home/
[15] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

Population	Age range	Countries with food consumption surveys covering more than 1 day
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Categorisation System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

Food categories considered for the exposure assessment of nitrates (E 251–E 252)

The food categories in which the use of nitrates (E 251–E 252) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx Classification System), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This may have resulted in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 01.7.6 Cheese products (excluding products falling in category 16), only hard, semihard and semisoft ripened products;
- 01.8 Dairy analogues, including beverage whiteners, only dairy-based cheese analogue.

For the following food categories, the restrictions/exceptions which apply to the use of nitrates (E 251–E 252) could not be taken into account, and therefore specific assumptions were applied and the whole food category was considered in the exposure assessment. This may have resulted in an overestimation of the exposure:

- 08.3.1 Non-heat-treated processed meat;
- 08.3.4.1 Traditional immersion cured products, only *Wiltshire bacon* and similar products;
- 08.3.4.1 Traditional immersion cured products, only *Wiltshire ham* and similar products;
- 08.3.4.1 Traditional immersion cured products, only *Entremeada, entrecosto, chispe, orelheira e cabeca (salgados), toucinho fumado* and similar products;
- 08.3.4.1 Traditional immersion cured products, only *cured tongue*;
- 08.3.4.1 Traditional immersion cured products, only *kylmâsavustettu poronliha/kallrökt renkött*;
- 08.3.4.1 Traditional immersion cured products, only *bacon, filet de bacon* and similar products;
- 08.3.4.1 Traditional immersion cured products, only *rohschinken, nassgepökelt* and similar products;
- 08.3.4.2 Traditional dry cured products, only *dry cured bacon* and similar products;
- 08.3.4.2 Traditional dry cured products, only *dry cured ham* and similar products;
- 08.3.4.2 Traditional dry cured products, only *jamon curado, paleta curada, lomo embuchado y cecina* and similar products;
- 08.3.4.2 Traditional dry cured products, only *presunto, presunto da pa and paio do lombo* and similar products; only *jambon sec, jambon sel* and other similar dried cured products;
- 08.3.4.2 Traditional dry cured products, only *jambon sec, jambon sel* and other similar dried cured products;
- 08.3.4.2 Traditional dry cured products, only *rohschinken, trockengepökelt* and similar products;
- 08.3.4.3 Other traditionally cured products, only *rohschinken, trocken-/nasgepökelt* and similar products;
- 08.3.4.3 Other traditionally cured products, only *jellied veal and brisket*;
- 08.3.4.3 Other traditionally cured products, only *rohwürste (salami and kantwurst)*;
- 08.3.4.3 Other traditionally cured products, only *Salchichon y chorizo traducionales de larga curacion* and similar products;
- 08.3.4.3 Other traditionally cured products, only *saucissons sec* and similar products;
- 09.2 Processed fish and fishery products including molluscs and crustaceans, only pickled herring and sprat.

In particular, the following assumptions were applied:

- the food category 'non heat-treated processed meat' (FCS 08.3.1) was linked to the FoodEx categories 'uncooked smoked sausage' and 'fresh and lightly cooked sausage';
- the food categories within 'traditionally cured meat products' (FCS 08.3.4) were linked to the FoodEx categories 'semi-dry sausage' and 'dry sausage' and 'preserved meat';
- the food category 'processed fish and fishery products including molluscs and crustaceans' was linked to the FoodEx categories 'herring' and 'sprat'.

For the scenarios considering nitrates (E 251–E 252) as food additives, 20 food categories were included in the exposure assessment without considering the restrictions/exceptions as set in Annex II to Regulation (EC) No 1333/2008. Two food categories were not taken into account in the exposure assessment because they or their specific restrictions/exceptions are not referenced in the EFSA Comprehensive Database. For the refined scenario, the food category FCS 01.7.4 was not taken into account because no concentration data were provided for this food category to EFSA. For the food category FCS 01.7.2, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied. Overall, for the regulatory maximum level exposure scenario, 22 food categories were included in the present exposure assessment to nitrates (E 251–E 252) (Appendix C.1).

For the scenario considering all sources (food additives, natural presence and contamination), besides the food categories in which nitrates (E 251–E 252) are authorised as food additives (Annex II to Regulation No 1333/2008), additional food commodities, in which nitrates occur naturally or as contaminants, were considered to perform exposure estimates (Section 3.5).

Also, in this case, some food commodities of the EFSA Comprehensive Database were not taken into account because no concentration data (or very few) were provided to EFSA, including animal and vegetable fats and oils (two analytical results), eggs and egg products (no data), products for special nutritional use (two analytical results) and sugar and confectionary (12 analytical results).

In most of the cases, the FoodEx level 2 category was used to link occurrence and consumption data. Only in the case of alcoholic beverages, drinking-water and fruit and fruit products was FoodEx level 1 category used. FoodEx level 2 category for which limited or no occurrence data were available have been excluded from the assessment or the concentration level for the corresponding FoodEx level 1 category was used.

For the following FoodEx level 3 category, relatively high levels of nitrates were identified and therefore this FoodEx level was used to link occurrence and consumption data:

- herring, salmon and trout;
- potatoes and potato products, potatoes and potatoes products, new potatoes, main-crop potatoes and French fries;
- livestock meat, preserved meat and sausages;
- *Brassica* vegetables, bulb vegetables, fruiting vegetables, leaf vegetables, root vegetables, stem vegetables and vegetables products.

Similarly, for the FoodEx level 3 category having limited or no occurrence data available, they were excluded from the assessment or the concentration value for the corresponding FoodEx level 2 category was used.

For specific FoodEx categories, ad hoc assumptions have been considered in order to overcome lack or poorness of data:

- for the FoodEx level 1 category 'Composite food', the concentration level assigned to food categories at FoodEx level 2 was based on the levels related to the main ingredients (e.g. for bean-based meals, the concentration level of nitrates in legumes, beans, green with pods was used);
- limited and contradictory information were available for infant formulae and follow-on formula (liquid infant formula: n = 2; liquid follow-on formula: n = 14; powder infant formula: n = 7; powder follow-on formula: n = 8), and therefore, the occurrence levels from literature (Hord et al., 2011; Jones et al., 2014) were used for these food categories. In particular:
  - for infant formulae and follow-on formula, both liquid, the nitrates concentration of 2.5 mg/kg was considered;
  - for infant formulae and follow-on formula, both powder, the nitrates concentration of 20 mg/kg was considered.

The addition of nitrate (E 251–E 252) is not authorised in fresh meat and fresh meat preparations; nevertheless, different studies have reported not-negligible concentrations of nitrates in these products (Bernini et al., 2001; Tanzi and Saccani, 2005; Hsu et al., 2009). These residues seem to derive from their natural presence and not from additives addition. Iammarino and Di Taranto (2012) conducted an investigation on 200 samples of beef, pork, equine and chicken fresh meats from local markets of Foggia (Italy) to trace quantifiable concentrations of nitrate. The analyses were carried out by a validated ion chromatography with conductometric detection method. In beef, pork and equine fresh meats, quantifiable concentrations of nitrate were found at a concentration in the range 10.2–36.5 mg/kg. This nitrate development appeared more marked in equine fresh meats compared to beef and pork. In particular, the nitrate mean concentration was 20.6 mg/kg in equine fresh meats, 13.2 mg/kg in beef and 11.6 mg/kg in pork fresh meats. Nitrates resulted absent in chicken fresh meats.

In the present assessment, the reported results on mean concentration for fresh meat ranged from 0.11 mg/kg for generic livestock meat to 61.34 mg/kg for mutton/lamb livestock meat. In particular, based on the reported analytical results, the nitrate mean concentration level was 44.7 mg/kg for pork fresh meat (n = 144) and 23.0 mg/kg for poultry (n = 32). These values are higher than the mean values reported in literature and are likely to be an overestimation of the nitrate concentration in fresh meat. This overestimation could be explained by a possible misclassification of some of the food samples reported as fresh meat as, despite careful checking of the data, it cannot be excluded that some of them may refer to processed meat, in particular for samples of pork meat and poultry. Indeed, initially a wrong classification was identified for 447 analytical results of fresh meat that, on the basis of the information reported with respect to the food description and processing description, were reclassified as 'meat products' (FCS 08.3). The levels of nitrate in fresh meat, and consequently, the exposure to nitrate from all sources, could therefore be overestimated.

3.4. Exposure to nitrates (E 251–E 252) from their use as food additives

The Panel estimated chronic exposure to nitrates (E 251–E 252) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to nitrates (E 251–E 252) was calculated by multiplying nitrate (E 251–E 252) concentrations for each food category (Appendix C.1) with their respective consumption amount per kilogram body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded because they are considered to be adequate for assessing repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 5). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy, as well as for toddlers from Belgium, Italy and Spain, was not included.

Two exposure scenarios were defined and carried out by the ANS Panel regarding the concentration data of nitrates (E 251–E 252) used: (1) MPLs as set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the reported analytical data (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below.

3.4.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 4.

Food categories of traditionally cured meat products (FCS 08.3.4), were included in the exposure assessment without considering the restrictions/exceptions (as described in Section 3.2) as set in Annex II to Regulation (EC) No 1333/2008, and the MPL of 250 mg/kg was considered for each subcategory in the exposure assessment. This is the prescripted value for 14 out of 18 restrictions/exceptions.

The Panel considers the exposure estimates derived following this scenario as the most conservative because it is assumed that the population group will be exposed to nitrates (E 251–E 252) present in food at the MPL over a longer period of time.

3.4.2. Refined exposure assessment scenario

The refined exposure assessment scenario was only based on analytical results reported by Member States. The Panel decided not to further consider use levels reported by the industry because, for each food category, they were all identical and equal to the MPL. This exposure scenario can consider only food categories for which the above data were available to the Panel.

Appendix C.1 summarises the concentration levels of nitrates (E 251–E 252) used in the refined exposure assessment scenario considering their use as a food additive. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to nitrates (E 251–E 252) present at the 95th percentile of reported analytical level for one food category. This exposure estimate was calculated as follows:
  - Combining food consumption with the 95th percentile of the analytical results for the main contributing food category at the individual level.
  - Using the mean of analytical results for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to nitrates (E 251–E 252) present at the mean reported analytical levels in food. This exposure estimate was calculated using the mean of analytical results for all food categories.

In the two refined exposure assessment scenarios, the concentration levels considered by the Panel were extracted from the whole data set (i.e. analytical results). To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ), the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 2009) and the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010a) was used. In the present opinion, analytical data below LOD or LOQ were assigned half of LOD or LOQ, respectively (MB). Subsequently, per food category, the mean or median, whichever is highest, MB concentration was calculated.

3.4.3. Dietary exposure to nitrates (E 251 and E 252) from their use as food additives

Table 6 summarises the estimated exposure to nitrates (E 251–E 252) from their use as food additives in six population groups (Table 5) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix C.2.

Table 6: Summary of dietary exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
Maximum level exposure assessment scenario												
Mean	0.07	0.22	0.26	0.65	0.14	0.49	0.15	0.38	0.13	0.28	0.11	0.19
95th percentile	0.36	0.71	0.8	1.19	0.46	1.31	0.43	0.91	0.35	0.76	0.31	0.54
Refined estimated exposure assessment scenario												
Brand-loyal scenario												
Mean	0.03	0.08	0.12	0.24	0.05	0.20	0.06	0.14	0.05	0.10	0.05	0.08
95th percentile	0.16	0.30	0.35	0.56	0.17	0.51	0.15	0.43	0.12	0.29	0.11	0.22

	Infants (12 weeks–11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Non-brand-loyal scenario												
Mean	0.01	0.03	0.04	0.10	0.02	0.08	0.02	0.07	0.02	0.05	0.02	0.04
95th percentile	0.06	0.14	0.12	0.25	0.07	0.23	0.07	0.19	0.06	0.14	0.05	0.10

min: minimum; max: maximum.

From the *regulatory maximum level exposure assessment scenario*, mean exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) ranged from 0.07 mg/kg bw per day in infants to 0.65 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates (E 251–E 252) ranged from 0.31 mg/kg bw per day in the elderly to 1.31 mg/kg bw per day in children.

From the *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008, in the *brand-loyal scenario*, mean exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) ranged from 0.03 mg/kg bw per day in infants to 0.24 mg/kg bw per day in toddlers. The high exposure to nitrates ranged from 0.11 mg/kg bw per day in the elderly to 0.56 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) ranged from 0.01 mg/kg bw per day in infants to 0.10 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates (expressed as nitrate ion) ranged from 0.05 mg/kg bw per day in the elderly to 0.25 mg/kg bw per day in toddlers.

From all exposure scenarios considered for exposure assessment of nitrates (E 251–E 252) from their use as food additives, the most important contributors to the total mean exposure for all population groups were meat products (preserved meat and sausages) and cheese, whereas fish and fishery products contributed less. The food categories and their contribution to the exposure to nitrates (E 251–E 252) are presented in Appendices C.3 and C.4.

3.4.4. Uncertainty analysis

Uncertainties in the exposure assessment of nitrates (E 251–E 252) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 7.

Table 7: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 2/24 food categories)	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 20 for the maximum permitted level (MPL) and refined scenarios out of 24 food categories)	+
Food categories included in the exposure assessment: data not available for certain food categories which were excluded from the exposure estimates (n = 1 only for the refined scenarios out of 24 food categories, considering different restrictions/exceptions)	–
Concentration data: • levels considered applicable for all items within the entire food category	+/–

Sources of uncertainties	Direction[a]
Regulatory maximum level exposure assessment scenario:	
• food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008	+
Refined exposure assessment scenarios:	
• use of middle-bound (MB) for left-censored data in the exposure assessment	+/–
Uncertainty in possible national differences in use levels of food categories	+/–

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to nitrates (E 251–E 252) as food additives in European countries for the regulatory maximum level exposure scenario and for the refined scenario considering that it was not possible to include a number of restrictions.

3.5. Exposure to nitrates from all sources (food additives, natural presence and contamination)

The analytical levels provided by the Member States reflect the levels of nitrates in foods, whatever their origin (food additives, natural presence and contamination). Numerous analytical data were reported on food categories in which nitrates (E 251–E 252) are not authorised as a food additive (Annex II to Regulation (EC) No 1333/2008).

The exposure estimated using all analytical data should reflect more closely what is ingested through the diet via all sources for which data are available and thus should represent the exposure coming from all dietary sources. Therefore, the Panel calculated the exposure to nitrates from all sources in a separate scenario (defined as *general population scenario all sources*; Section 3.5), considering, besides the food categories in which nitrates (E 251–E 252) are authorised as a food additive (Annex II to Regulation (EC) No 1333/2008), additional food commodities, in which nitrates occur naturally or due to a contamination.

In 2012, EFSA outsourced a study[10] on the effect of processing on nitrate content in vegetables. The study included nine different vegetables: lettuce, spinach, white cabbage, Chinese cabbage, green bean, courgette, potato, carrot and red beet. The processing techniques considered are washing, peeling, slicing, boiling, microwave, steaming, blanching, sauté, deep-frying, grill, baking, stir-frying, purée and fermentation, differently selected and combined for each specific type of vegetable. Nitrate content in vegetable samples was determined by two methods, in both cases by ion exchange chromatography, and the results for each group of vegetables were compared using non-parametric statistical test to analyse the change in nitrate content.

The report identified a statistically significant ($p < 0.001$) average reduction, based on fresh weight, for washing lettuce (−19%), Chinese cabbage (–26%) and spinach (−27%). Boiling significantly ($p < 0.001$) reduced the nitrate content for courgette (−35%), green bean (−15%) and potatoes (−43%). In the case of spinach, both washing and boiling statistically significantly decreased nitrate levels on average by 56%.

Grilling and deep-frying showed a significant ($p < 0.001$) increase in nitrate content for courgette (grilling +49%) and potatoes (deep-frying +97%), although this could be mainly explained by the loss of weight due to these types of processing.

In response to this study, an additional scenario with correction factors for loss of nitrates during the manufacturing process applied to fresh vegetables (defined as *general population scenario all sources, including reduction factors for vegetables*) was also estimated, based on the following assumptions:

- spinaches are always consumed boiled and a factor of 0.47 was applied to the concentration levels;
- potatoes and potatoes products, new potatoes, main-crop potatoes are always consumed boiled and a factor of 0.57 was applied to the concentration levels;
- legumes and beans are always consumed boiled and a factor of 0.85 was applied to the concentration levels;

- *Brassica* vegetables and leaf vegetables are always washed before consumption and a factor 0.75 of was applied to the nitrate concentration level.

Appendix D.1 summarises the levels of nitrates used in the exposure assessment scenario considering all sources.

Table 8 summarises the estimated exposure to nitrates from all sources (food additives, natural presence and contamination) in six population groups (Table 5). The estimates were calculated both without and with considering the losses of nitrates during the manufacturing process. Detailed results per population group and survey are presented in Appendix D.2.

Table 8: Summary of dietary exposure to nitrates (expressed as nitrate ion) from all sources (food additives, natural presence and contamination) based on all analytical data using the refined exposure scenario (non-brand-loyal approach for general population) in six population groups (min–max across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
General population scenario all sources												
Mean	2.31	3.67	2.45	4.62	2.27	4.26	1.27	2.47	1.07	2.35	1.13	2.47
95th percentile	4.67	8.90	3.85	9.17	3.99	9.26	2.36	5.30	2.16	5.12	1.93	5.25
General population scenario all sources, including reduction factors for processed vegetables												
Mean	1.87	3.30	2.28	4.15	2.18	3.83	1.21	2.22	1.00	2.09	0.97	2.08
95th percentile	3.75	6.93	3.54	7.82	3.82	8.73	2.17	5.28	1.88	4.68	1.59	4.57

min: minimum; max: maximum.

From the exposure scenario considering the exposure to nitrates (expressed as nitrate ion) from all sources (food additives, natural presence and contamination), mean exposure ranged from 1.07 mg/kg bw per day in adults to 4.62 mg/kg bw per day in toddlers. The high exposure to nitrates ranged from 1.93 mg/kg bw per day in the elderly to 9.26 mg/kg bw per day in children.

In the scenario applying reduction factors for vegetables, mean exposure to nitrates (expressed as nitrate ion) from all sources (food additives, natural presence and contamination) ranged from 0.97 mg/kg bw per day in the elderly to 4.15 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates ranged from 1.59 mg/kg bw per day in the elderly to 8.73 mg/kg bw per day in children.

In addition, the Panel calculated the contribution of the mean exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) to overall exposure to nitrates from all sources (food additives, natural presence and contamination) (Table 9).

The same uncertainties presented above (Section 3.4) for the refined exposure to nitrates (E 251–E 252) from their use as food additives are also valid for the refined exposure estimates to nitrates from all sources (food additives, natural presence and contamination). In this case, an additional uncertainty leading to an overestimation of exposure to nitrates is related to food processing. To evaluate this uncertainty, the Panel also considered a scenario including all dietary sources and reduction factors for processed vegetables. However, for the highest 95th percentile, a reduction in the order of 5% was noted. Nevertheless, it is not possible to exclude a reduction effect related to the processing of foods other than vegetables for which no data are currently available.

Table 9: The percentage of contribution of the mean exposure to nitrates (E 251–E 252) from their use as food additive to overall exposure to nitrates from all sources (food additives, natural presence and contamination)

	Infants (12 weeks to 11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	%		%		%		%		%		%	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
General population scenario all sources												
Contribution from their use as food additives	0.4	1.2	1.0	4.1	0.6	3.2	1.2	3.9	1.1	3.6	0.9	2.6
General population all sources, including reduction factors for vegetables												
Contribution from their use as food additives	0.4	1.4	1.1	4.4	0.6	3.7	1.4	4.4	1.3	3.9	1.1	2.8

min: minimum; max: maximum.

The Panel estimated that, when comparing all sources (food additives, natural presence and contamination), including reduction factors for vegetables, using the same refined exposure methodology (non-brand-loyal consumer scenario for general population), the contribution of nitrates (E 251–E 252) from their use as food additives may represent approximately 2% (range 0.4–4.4%) of the overall exposure to nitrates.

The main contributing food categories from the exposure scenario considering all sources (food additives, natural presence and contamination), for both without and with reduction factor, were vegetables and vegetable-based foods for all population groups. In particular, the main contributing food categories were starchy roots and tubers in infants, toddlers and children, whereas the leafy vegetables and prepared salads were the main contributors in children, adolescents, adults and the elderly. In infants, also foods for infants and toddlers made an important contribution to the total mean exposure to nitrates from all sources.

The Panel noted that none of food categories considered for the exposure assessment to nitrates (E 251–E 252) from their use as food additives exceeded 5% of contribution to the overall exposure to nitrates from all sources in any scenario and for any population group.

The Panel noted that its exposure estimates from all sources were in line with results published in a French study (Menard et al., 2008). Also in this study, major food contributors to exposure to nitrates were vegetables other than potatoes and potatoes, processed meats products and other food categories that could contain nitrates from their use as food additives (E 251–E 252) did not appear among main food contributors.

The food categories and their contribution to the exposure to nitrates from all sources (food additives, natural presence and contamination) are presented in detail in Appendices D.3 and D.4.

3.6. Biological and Toxicological data

There were no new toxicokinetic or toxicological studies submitted to EFSA following a public call for data. A literature search was conducted on the most commonly available online databases for toxicological and biological information (Pubmed, Toxnet and Chemical Abstracts). The search was restricted to reveal information published from 2002 onwards, which is the year before the last major review was conducted by JECFA (2003a,b,c).

The literature search revealed relevant toxicokinetic studies that are included in the appropriate sections below, together with a summary of the current understanding with regard to the absorption, distribution, metabolism and excretion of these two food additives. The opinion reports, in brief, key data discussed in previous major reviews by the SCF and JECFA (SCF, 1992, 1997; JECFA, 2003a) but does not describe them in detail as these data have already been extensively reviewed by these bodies, and their findings with regard to toxicology are comparable. Previously unavailable data that have been published after 2002 have been described in detail, and the results discussed in relation to the existing findings from the major reviews.

The sodium and potassium ions are expected to enter normal homoeostatic processes, and are not expected to impact on the toxicity of the salts, which is determined by the nitrate ion, at the levels of intended uses. Thus, the properties of the cations are not discussed further.

As summarised in the IARC report, during recent years, the biochemistry of nitrate has been shown to be involved in biological pathways implicating several important compounds such as nitrite, nitric oxide, carbon dioxide, superoxide anion and hydrogen peroxide. These compounds have been shown to be part of normal and abnormal physiological conditions related to various pathways such as neural signalling, blood pressure, inflammation and ischaemia. Nitrate is thus becoming to be considered as a compound playing roles in metabolic cycles in which nitrite and nitric oxide are interconverted depending on physiological needs and not only as an undesirable contaminant arising from food intake or as a hazardous by-product formed endogenously (IARC, 2010).

3.6.1. Absorption, distribution, metabolism and excretion (ADME)

3.6.1.1. Animal studies

Several experimental studies on the toxicokinetic behaviour of the nitrate ion in animals have been carried out and many of these have been discussed in previous evaluations (SCF, 1997; JECFA, 2003a; IARC, 2010). It is generally agreed that nitrate is rapidly and almost completely absorbed from the proximal and small intestine subsequent to ingestion in most animals, with little if any absorption from the stomach and lower intestine (JECFA, 1996, 2003a). Nitrate can also be formed endogenously and nitrate/nitrite occurring in many tissues and sites of inflammation has been described as a 'steady-state baseline level in the body, which is then episodically supplemented by ingested nitrate/nitrite' (IARC, 2010).

It is generally accepted that, in most laboratory animal species excepted rats, about 25% of the oral dose of nitrate is recirculated into the saliva in a dose-dependent manner via an active transport mechanism of which around 20% is reduced to nitrite by the oral bacteria; this amount would be equivalent to about 5% of the ingested nitrate but these estimates show great interindividual and day-time variations (JECFA, 2003a; IARC, 2010; Health Canada, 2013).

By contrast to humans, rats show low salivary nitrate secretion leading to a low production of nitrite in the mouth. Despite the absence of active salivary secretion in the rats, nitrate undergoes enterosystemic circulation by the secretion of nitrate into other gastric and intestinal fluids via an active transport mechanism (JECFA, 1996, 2003a). The absorbed nitrate, not converted to nitrite, is distributed throughout the body, including the stomach, intestines, urinary bladder, kidney and other organs and, after equilibrium in body fluids (milk, gastric fluid, endotracheal secretion), it is rapidly excreted via urine (JECFA, 2003a; IARC, 2010; Health Canada, 2013). Some studies on germ-free and conventional-flora Sprague–Dawley rats exposed to radiolabelled nitrate show a rapid distribution of radioactivity throughout the body within 1 h after dosing (IARC, 2010). Nitrate can be reduced to nitrite by enteric bacteria but its reduction depends on gastric pH conditions. The gastric pH and concurrent bacterial populations are low in the stomachs of rabbits and ferrets, whereas rats and dogs are known to have higher gastric pH and therefore also higher reduction of nitrate to nitrite in the stomach (Mirvish, 1975).

Germ-free and conventional-flora Sprague–Dawley rats (five to seven rats per group, 60–90 days of age, sex not stated) were administered 1,000 μg/mL sodium nitrate in their drinking water for 48 h (to 0.05 mg/kg bw per day) and sections of their intestinal tract assessed for nitrate and nitrite (Witter and Balish, 1979). The germ-free rats had detectable levels of nitrate only in the stomach, small intestine, caecum and colon in response to being fed sodium nitrate. The rats with conventional flora had both nitrate and nitrite in the stomach, although only nitrate in the small intestine and colon in response to being fed sodium nitrate. When fed sodium nitrite, the germ-free rats had both nitrate and nitrite in the entire gastrointestinal tract, whereas conventional flora rats fed sodium nitrite had both ions in the stomach and small intestine but only nitrate in the large intestine. Control animals (germ-free or conventional flora) that were not administered sodium nitrate had only trace amounts of nitrate in their stomachs and bladders. The *in vivo* study was complemented with *in vitro* studies assessing intestinal contents. The overall conclusion of the study was that nitrate to nitrite conversion is a function of normal flora, whereas nitrite oxidation depends on the host.

Overall, according to JECFA (2003a) the 'major site of conversion of nitrate to nitrite varies by species and depends on the sites of microbial colonization and absorption of nitrate'.

Germ-free and conventional rats were used to investigate nitrate-dependent nitrite formation by measuring methaemoglobin levels (Ward et al., 1986). Germ-free rats were housed in plastic isolators

and fed a sterilised, commercial diet and autoclaved water. Conventional rats received the same diet and unsterilised tap water. In the case of the germ-free rats, faecal samples were collected to monitor whether microbial growth could be detected. The first experiment, assessing *in vivo* nitrate reduction used gnotobiotic BDIX rats (two males, four females per group; 5 months old), monocontaminated with an unspecified yeast. Conventional rats were used as comparison. Both groups of rats were administered 2% (w/v) solution of potassium nitrate *ad libitum* for 6 days in drinking water (equivalent to 1,000 mg/kg bw per day). Blood samples were collected on days 2 and 6 and analysed for haemoglobin and methaemoglobin content; thereafter, distilled water was provided to rats instead of the potassium nitrate solution for 13 days. In a second experiment, with germ-free and conventional Wistar rats (four males, 11 weeks old per group) were administered a 2% (w/v) solution of potassium nitrate *ad libitum* for 9 days in distilled drinking water (equivalent to 2,400 mg/kg bw per day). Blood samples were collected at 2, 6 and 9 days after dosing began and analysed for haemoglobin and methaemoglobin content (Ward et al., 1986). The methaemoglobin levels were higher in the germ-free rats than in the conventional rats ($p < 0.01$). No difference was observed between germ-free and these specific gnotobiotic rats. The gastrointestinal pH of the germ-free rats was also measured to be generally higher than that of the conventional rats, an attribute which was concluded to influence the higher rate of nitrosation in the conventional rats compared to their germ-free counterparts.

Reduction of nitrate was shown to occur in tissues of rats and mice (Jansson et al., 2008; Huang et al., 2010), possibly through the action of xanthine oxidoreductase. JECFA (2003a) reported comparative studies with germ-free and conventional rats that showed that nitrate reduction activity in mammals affects the rate of conversion of nitrate to nitrite in these animals to a large extent. It has been reported that, in rats, 90% of total nitrate reduction activity in mammalian tissue was present in the liver (JECFA, 1996). It is likely that, also in the latter cases, enzymes such as xanthine oxidoreductase are involved.

Additionally, nitrite can be oxidised to nitrate via reactions with oxyhaemoglobin showing that nitrite and nitrate are in dynamical equilibrium (Health Canada, 2013; see Section 3.1.5).

3.6.1.2. Studies in humans

Absorption

In humans, dietary nitrate is rapidly and extensively absorbed through the gastrointestinal tract (Iijima et al., 2002). Nitrate is found in high concentrations in the saliva due to its secretion by salivary glands. In the mouth, bacterial metabolism converts the secreted nitrate into nitrite. Some nitrite is also formed in the stomach. In the gastrointestinal tract, nitrite is absorbed and thereafter enters the general circulation where it can be oxidised by haemoglobin to form nitrate and methaemoglobin. Nitrate is metabolised to a minor extent. Its biotransformation comprises nitrate reduction, nitrite formation, nitrite reoxidation to nitrate, and formation of methaemoglobin or NO, in a dynamic equilibrium (Gladwin et al., 2005; Lundberg et al., 2008). Due to the very low gastric pH, very little further reduction of nitrate to nitrite occurs in man (Mirvish, 1975). Between 70% (Wagner et al., 1983) and 100% (Pannala et al., 2003) of a nitrate dose is excreted by the kidneys.

In a study by RIVM (Lambers et al., 2000; published van Velzen et al., 2008), absorption of nitrate was measured in 12 healthy subjects (six males and six females). The oral doses were sodium nitrate contained either in 300 mg of spinach, 300 mg of lettuce or 300 mg of beetroot given in the fasting state. The content of nitrate was 1,880 mg/kg spinach, 3,017 mg/kg lettuce and 2,144 mg/kg beetroot. The intravenous dose was 500 mg of sodium nitrate. The systemic availability was calculated to be roughly 100% (70–116%) from all food sources based on the comparison of AUCs in plasma after oral doses to the AUC after intravenous administration. It should be noted that in this study the nitrite concentration was in most of the plasma samples below the limit of detection.

Distribution

The volume of distribution was calculated to be between 18 and 32 L, or 0.24 and 0.44 L/kg bw (mean 0.32 L/kg bw) (Lambers et al., 2000). The volume of distribution is smaller than the body water and higher than the blood volume, indicating that nitrate is distributed throughout the body.

The Panel calculated the volume of distribution from the data reported in Hunault et al. (2009) and confirmed the value of Lambers et al. (2000). Nitrate is also found in the milk of lactating women where the concentration is similar to that in the plasma (Green et al., 1982).

Metabolism (see Figure 1)

Nitrate is mainly converted to nitrite in a specific mechanism. Nitrate is secreted into the saliva by an active mechanism most probably involving Na^+/I^- symporter (NIS). This symporter seems to mediate the active transport of nitrate from plasma to a wide variety of organs, including the thyroid, gastric mucosa, salivary glands and mammary glands (Jhiang et al., 1998; Spitzweg et al., 1998, Spitzweg et al., 1999; Lacroix et al., 2001; Riedel et al., 2001).

The amount secreted into the saliva is estimated to vary between 20% and 25% of the dose (Spiegelhalder et al., 1976; Bartholomew and Hill, 1984). In mouth, nitrate is metabolised to nitrite whereby bacteria in the mouth play an important role (Gangolli et al., 1994). The ratio of the nitrite concentration in saliva to nitrate concentration in saliva varies widely between the different authors and even in one individual. From this ratio, it can be calculated that 5–36% of the nitrate secreted into the saliva is converted to nitrite by bacterial metabolism in the mouth (e.g. Spiegelhalder et al., 1976; Wagner et al., 1983; Bartholomew and Hill, 1984; Bos et al., 1988; Granli et al., 1989; Shapiro et al., 1991; Jin et al., 2013; Bondonno et al., 2015; Hohensin et al., 2016; Montenegro et al., 2016; Woessner et al., 2016). Also, in the upper part of stomach, some ingested nitrate is transformed to nitrite. In infants, the higher stomach pH (2–5) may lead to the growth of nitrate-reducing bacteria (Zeman et al., 2002). Mouth washing with antibacterial fluids reduced the amount of nitrate converted to nitrite (Govoni et al., 2008). Several models have been proposed for nitric oxide formation and further nitrosylation of proteins from nitrite (Vitturi and Patel, 2011). More details on the nitrate–nitrite cycle are provided in a separate opinion by the Panel on sodium and potassium nitrite (EFSA ANS Panel, 2017 and on Section 3.1.5).

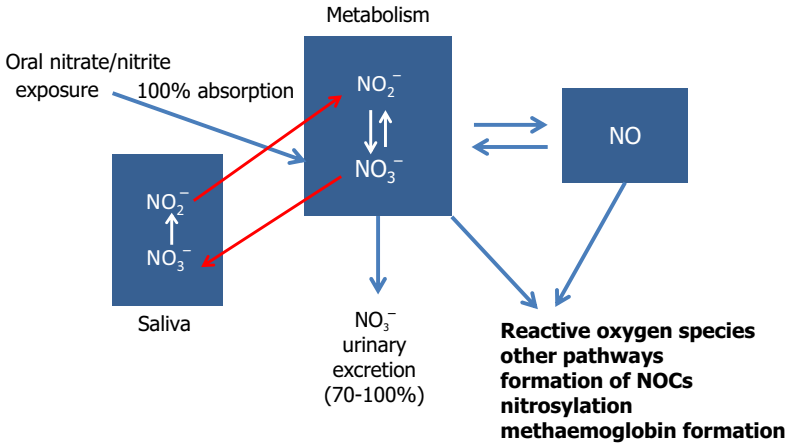


Figure 1: Fate of nitrate and nitrite in the body (NOCs comprise *N*-nitrosamines and *N*-nitrosamides)

Excretion

Urinary excretion was measured in several studies. In the study by Pannala et al. (2003), urinary excretion of nitrate after three high nitrate containing meals (mean 221 ± 10.8 mg of content) was about 100%. Other studies (Bartholomew and Hill, 1984) could only recover 50–73% of the dose in the urine and Wagner et al. (1983) reported that the urinary excretion of a dose of N^{15}-labelled nitrate was 60%. Nitrite excretion was measured in a study in which meals with high nitrate contents were given and the amount was roughly 0.02% of the dose (as calculated by the Panel) (Pannala et al., 2003). This indicates that renal excretion is an only minor pathway of excretion for nitrite.

Clearance of nitrate can be calculated by using the information on dose and AUC from two studies (van Velzen et al., 2008; Hunault et al., 2009). In the study by van Velzen et al. (2008), the dose given intravenously was nitrate, whereas, in the study by Hunault et al. (2009), the dose given was nitrite, which was converted 100% into nitrate. When recalculating the AUC into mmol × h/L instead of mg × h/L as given in the two publications, the AUCs resulted in 2.93 mmol × h/L after 6.7 mmol and 1.81 mmol × h/L after 5.88 mmol, which shows good agreement. The Panel calculated that the total clearance (dose/AUC) was 38 and 54 mL/min, respectively. Cortas and Wakid (1991) calculated a renal nitrate clearance of 28 mL/min, normalised to 1.73 m^2 body surface. The renal clearance of less than the glomerular filtration rate (120 mL/min in a healthy young subject) indicates that reabsorption

from the renal tubulus must occur and explains the longer half-life of nitrate (5–13 h; van Velzen et al., 2008; Hunault et al., 2009) compared to nitrite (30 min) (Hunault et al., 2009).

In summary, in humans, nitrate is systemically available to 100%. The volume of distribution is smaller than the body water and higher than the blood volume. Nitrate is secreted into saliva (20–25% of the dose) and converted to nitrite by bacteria in the mouth (5–36%). Nitrite is absorbed and further metabolised to nitrate. Small amounts of nitrite are metabolised to NO and reactive oxygen species and 0.02% of a dose of nitrite is found in the urine. Most of nitrate is excreted into the urine, with the amounts varying between 50% and 100%. The Panel noted that the most reliable studies reported excretion of nitrate in urine of nearly 100%.

3.6.2. Acute toxicity

Acute oral values for sodium nitrate were reported to range between 2,480 and 6,250 mg sodium nitrate/kg bw for mice, and between 4,860 and 9,000 mg sodium nitrate/kg bw for rats. For rabbits, a LD_{50} value of 1,600 mg/kg bw was reported (RIVM, 1989).

3.6.3. Short-term and subchronic toxicity

The literature search for data produced from 2002 onwards identified two new sub-chronic studies (Bako et al., 2009; Azeez et al., 2011).

3.6.3.1. Rats

In a 4-week study in rats (10 per sex; age and strain not specified), animals were administered 0%, 1%, 2%, 3%, 4% or 6% potassium nitrate (KNO_3) or 5% sodium nitrate ($NaNO_3$) in feed (equivalent to 0, 900, 1,800, 2,700, 3,600 or 5,400 mg potassium nitrate/kg bw per day and 4,500 mg sodium nitrate/kg bw per day, respectively) (Til et al., 1985a,b). Both references tested the same conditions of exposure the only difference being that in Til et al. (1985a) the tested compounds were added to a semipurified, basal diet whereas in Til et al. (1985b) they were added to a cereal basal diet. The same experimental protocols were tested and results obtained were similar in both studies. Mean body weights were decreased in a dose-related manner only at 3% KNO_3 and above in both sexes. No differences were reported in body weights between animals of the KNO_3 and $NaNO_3$ groups. Mean water intake was increase in a dose-related manner at 2% KNO_3 and above in males and at 4% KNO_3 and above in females. Animals placed in the 5% $NaNO_3$ drunk more water that the corresponding rats on the 6% KNO_3 group. At doses of 3% KNO_3 and above, the methaemoglobin levels in female rats increased in a dose-related manner. Frequent slight changes in red blood cell parameters were reported and in the 4% KNO_3 group haemoglobin levels in males and packed cell volume in females were increased. Absolute weight of the hearts, livers and lungs were decreased in the 6% KNO_3 and the 5% $NaNO_3$ group. The kidney relative weights were increased in a dose-related manner in males of 2% and in females of the 3% KNO_3 groups. No effects related to nitrate exposure were observed upon gross and microscopic examination of heart, kidneys, liver, spleen and lungs tissues.

In a 6-week study, F-344 rats (10 per sex per group, 5 weeks old) were exposed to sodium nitrate *ad libitum* in the diet at doses of 0%, 1.25%, 2.5%, 5%, 10% or 20% (equivalent to 0, 1,125, 2,250, 4,500, 9,000 or 18,000 mg/kg bw per day, respectively) (Maekawa et al., 1982). All females and seven males in the high-dose group (20%) died. At autopsy, the abnormal colour of the blood and the spleen due to methaemoglobin was marked in these animals. With the exception of males in the highest dose group and females at the 10% dose group, all other groups did not differ by more than 10% in their body weight gain. The maximum tolerated dose for sodium nitrate was established to be 5% in the diet (equivalent to 4,500 mg/kg bw per day; Maekawa et al., 1982).

A 6-week study in male Wistar rats (6–8 per group) assessed the effects of nitrate exposure in feed on various biochemical parameters in a non-standard study. Group 1 served as the control group, group 2 was treated with 2% potassium nitrate in feed and group 3 was co-administered 2% potassium nitrate and 1% ascorbic acid (equivalent to 1,800 mg potassium nitrate/kg bw per day and 900 mg ascorbic acid/kg bw per day, respectively). Blood samples were collected weekly (time of day not specified) and serum glucose, cholesterol, alanine aminotransferase and aspartate aminotransferase levels were analysed. A significant increase ($p < 0.05$) in all measured parameters was detected from weeks 3–6, and this effect was reversed by the addition of 1% ascorbic acid to the diet (Azeez et al., 2011). The Panel noted that no necropsy was carried out.

As part of a study investigating the antioxidant effects of ethanolic seed extract of *Hibiscus sabdariffa linn*, two groups of five Wistar rats (sex not specified) were administered either 25 mg sodium nitrate/kg (it was not clear whether dose refers to body weight of the animals) per day alone or the same amount of sodium nitrate together with 10 mg/kg per day vitamin C or 100 and 200 mg/kg per day ethanolic extract for 60 days (Bako et al., 2009). The method of administration of the test substance is not stated. At the end of the study period, the animals were sacrificed and blood samples taken for the analysis of haematological parameters and total protein. A significant reduction in ($p < 0.05$) total serum protein and measured haematological parameters (white blood cells, haemoglobin) in response to sodium nitrate administration was evident in comparison with the control group. Vitamin C and ethanolic extracts treatments did not show statistically significant differences on measured parameters compared to control group (Bako et al., 2009).

3.6.3.2. Rabbits

In a limited described 4-week study, rabbits (six males per group) were administered 0, 200, 400 or 600 mg/kg bw per day potassium nitrate by a pulse dose via gelatin capsules (Nighat et al., 1981). In all treatment groups, signs of effects were present within 2 weeks after administration. Clinical signs included significant weight reduction, tachycardia, weakness and polyuria.

3.6.4. Genotoxicity

The genotoxicity of nitrate has been evaluated in various *in vitro* and *in vivo* test systems, and also has been reviewed in the IARC monograph No 94 (IARC, 2010); in this section, genotoxicity data reviewed in the IARC publication are briefly summarised and discussed together with findings from studies published after the preparation of IARC Monograph or that were not included in that document.

3.6.4.1. *In vitro*

Tests in bacteria

In a screening of food additives, potassium and sodium nitrate were tested in bacterial reversion assays (Ames test) using the *Salmonella* Typhimurium tester strains TA92, TA94, TA100, TA1535 and TA1537 with and without rat liver microsome fraction (S9) (Ishidate et al., 1984). Maximum tested doses were 20 mg/plate for potassium nitrate and 5 mg/plate for sodium nitrate. The results were evaluated as negative by the study authors, based on the 'twofold' rule (i.e. no increase in revertant colonies above twice the control was observed in treated plates). The Panel noted that no raw experimental data are shown in this publication.

Tests in mammalian cells

In the same study quoted above, potassium and sodium nitrate were tested in an *in vitro* chromosomal aberration assay in Chinese hamster lung fibroblasts (Ishidate et al., 1984). Cells were treated for 24 and 48 h with potassium and sodium nitrate at maximum concentrations of 1 and 6 mg/mL, respectively. All experiments were carried out without exogenous metabolic activation. According to the study authors, the spontaneous incidence of chromosomal aberrations in this cell line was usually below 3.0%; as in-house evaluation criteria, a substance was evaluated as positive when the aberration frequency was 10% or above, equivocal at between 5% and 9.9%, and negative below 5%. Accordingly, potassium nitrate was evaluated as negative (maximum aberration frequency was 3.0%), whereas sodium nitrate was considered positive as it induced 24% of aberrations at the maximum tested dose of 6.0 mg/mL (at harvest time of 48 h) and 10% of aberrations at the lower dose of 4.0 mg/mL at both 24 and 48 h. The Panel noted that the doses of sodium nitrate evaluated as positive in this study are above the highest doses recommended by current guidelines (OECD, 2014) and correspond to 70 and 47 mM, respectively, which is well above the limit of 10 mM set to avoid artefacts due to osmolality. Moreover, the Panel noted that no concurrent measurement of cytotoxicity was performed in the assays, and that gaps were included in the calculation of chromosome aberration frequencies. Based on the above considerations, the Panel concluded that the positive findings reported with sodium nitrate in this study have questionable biological significance and that the overall result of the study is negative.

Potassium nitrate was tested in an *in vitro* sister chromatid exchange assay in human lymphocytes, only performed without metabolic activation (Mpountoukas et al., 2008). Blood samples were collected from six healthy individuals, and lymphocytes cultured in the presence of 0, 0.02, 0.2, 2, 4 and 8 mM

potassium nitrate for 72 h. No treatment-related increase of sister chromatic exchange (SCE), as well as no effect on cell proliferation, was observed in treated cultures.

In another published study, no induction of DNA single-strand breaks, as measured by the alkaline elution assay, was observed in Chinese hamster V79 cells treated with 0.5–1.0 mM sodium nitrate (Gorsdorf et al., 1990).

3.6.4.2. *In vivo*

Drosophila

Sodium and potassium nitrate, alone or in combination, were tested in the somatic mutation and recombination test (SMART) in *Drosophila melanogaster* (Sarikaya and Cakir, 2005). This test measures the loss of heterozygosity in somatic cells of larvae fed with the test agent, which are visible in flies after metamorphosis as spots on their wings. A significant and dose-related increase in small and large spots was observed in adults emerging from larvae fed with medium containing sodium or potassium nitrate (50, 75 or 100 mM). The lowest tested dose (25 mM) was ineffective, whereas both salts tested in combination at 25 mM each elicited a positive response. The Panel noted that the SMART test in *Drosophila* has not been validated for risk assessment, nor it is recommended in current testing strategies.

Mammals

Inui et al. (1979) examined the transplacental effect of sodium nitrate on Syrian hamster embryo cells. To this aim, pregnant females were treated per os with 500 mg sodium nitrate/kg bw on days 11 and 12 of pregnancy and embryos were isolated 24 h later. Primary cultures of embryo cells were set up for the detection of induced gene mutations (8-azaguanine and ouabain resistance), cytogenetic abnormalities (chromosomal aberrations and micronuclei) and morphological transformation. For the induction of resistant mutants, cells were grown 72 h before selection; chromosomal aberrations were evaluated in metaphases harvested after 24 h of culture, and micronuclei in interphase nuclei after 30 h of culture; cell transformation was evaluated after 3–5 days of culture. No induction of micronuclei, chromosomal aberrations, gene mutations and transformation was observed in embryonic cells exposed *in utero*. A clear-cut positive response was elicited by the positive control dimethylnitrosamine. The Panel noted that the methods applied in this study have not been validated further and therefore their results cannot be used for risk assessment.

Luca et al. (1985) evaluated the cytogenetic effects of sodium nitrate in male Wistar rats and Swiss mice (four per group) treated per os twice 24 h apart with 2,120, 706.6, 235.5 and 78.5 mg/kg bw (corresponding to 1/3, 1/9, 1/27 and 1/81 of LD_{50}); the same daily doses were given by gavage to rats (six per group) for 2 weeks. Twenty-four hours after last treatment, chromosomal aberrations were evaluated in bone marrow cells of mice and rats (receiving acute or subacute treatment). Micronuclei were scored in mice only (sacrificed 6 h after last administration). No consistent increase in the frequency of chromosomal aberrations was observed in either mice or rats after acute treatments with sodium nitrate. In mice, a twofold increase in the percentage of micronucleated polychromatic erythrocytes was only observed at the two lowest doses. On the other hand, a significant increase in the percentage of aberrant metaphases (excluding gaps) was reported in bone marrow of rats after subacute treatment with the two highest doses of sodium nitrate (7.95% and 7.33% vs 2.33% in control). The Panel noted the limited protocol of this study, with less animals and cells scored than recommended, the lack of a metaphase-arresting agent in chromosomal aberration assays, as well as the lack of positive controls and historical control data. Overall, the Panel concluded that this study cannot be considered for risk assessment.

The effect of oral administration of sodium nitrate (600 and 1,200 mg/kg) for 14 days on mouse germ cells was investigated by Alavantičet al. (1988a). No induction of heritable translocations, as well as no sperm abnormalities and morphological changes, were observed in the progeny of treated males. In the latter, a significant increase in sex-chromosomal univalency at diakinesis in spermatocytes, as well as sperm head abnormalities, was observed after treatment of differentiating spermatogonia. The Panel noted that sex-chromosomal univalency and sperm abnormalities are not considered as reliable indicators of genotoxicity.

The same research group evaluated the effect of oral administration of sodium nitrate on unscheduled DNA synthesis (UDS) and sperm abnormalities in mice after treatment of spermatids (Alavantičet al., 1988b). To this aim, sodium nitrate was given for 3 days at 600 and 1,200 mg/kg, and UDS and morphological head abnormalities evaluated in sperm 17 days after the last treatment. No

induction of UDS and sperm-head abnormalities was observed in nitrate treated animals, whereas the positive control elicited a strong response.

Finally, in a study cited in the IARC Monograph (2010), increased cytogenetic changes were observed in the bone marrow of mice injected intraperitoneally with 50 or 100 mg sodium nitrate/kg bw (Rasheva et al., 1990). However, the Panel noted that the intraperitoneal route of administration is not considered relevant for the evaluation of the *in vivo* genotoxic hazard related to oral exposure.

Studies in humans

The genotoxic effects associated with oral exposure to nitrate in drinking water were evaluated in a study in children aged 12–15 years resident in areas with high (56–88 mg/L, 17 subjects) and low (0.7 mg/L, 20 subjects) nitrate concentrations in drinking water. SCEs and chromosomal aberrations were measured in peripheral blood lymphocytes as markers of genotoxicity. The number of mean chromatid or chromosomal breaks was not found to be higher in children from areas with nitrate concentrations > 55 mg/L; however, the frequency was increased in the subgroup with intake of > 70 mg/L. No significant increase was detected in the mean number of sister chromatid exchanges per cell (Tsezou et al., 1996). The Panel noted that the design of the study did not allow establishment of a causal role for nitrate in the effects observed, nor the possible involvement of other uncontrolled study variables, and it was concluded that this study cannot be considered for risk assessment.

In summary, *in vitro* studies on sodium and potassium nitrate in bacteria and mammalian cells did not show evidence of a genotoxic potential. In *in vivo* studies, no reliable indication of genotoxicity was obtained in mice and rats exposed to nitrate by the oral route, both in somatic and in germ cells. Similarly, no transplacental genotoxic effect was observed in Syrian hamster. Although the database is limited, the Panel concluded, based on the available experimental data, that nitrate salts did not raise concern for genotoxicity.

3.6.5. Chronic toxicity and carcinogenicity

The EFSA call for data did not reveal any chronic toxicity or carcinogenicity data for sodium nitrate or potassium nitrate. A literature search (conducted to retrieve new data produced from 2002 onwards) revealed one new publication on carcinogenicity (Del Negro et al., 2008). The key studies discussed in previous major reviews are also summarised.

3.6.5.1. Animal studies

Mice

Mice (10 per sex per group) receiving for more than 1 year diets containing 0, 25,000 or 50,000 mg sodium nitrate/kg of feed (equivalent to 0, 3,750 and 7,500 mg/kg bw per day). No differences in tumour incidences were reported in the animals (Sugiyama et al., 1979 and as referred to in JECFA, 1995). IARC (2010) described the same study as 'testing groups of 10 male and 10 female ICR mice, 8 weeks of age, 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 incidences were observed between untreated control and treated animals'. However, IARC noted the small number of animals per group.

In a study by Greenblatt and Mirvish (1973), the effect of sodium nitrate was tested in strain A mice (40 males per group; 7 weeks old at the start of the study) at a concentration of 12.3 g/L (equivalent to 1,267 mg/kg bw per day of drinking water for 20–25 weeks, followed by a recovery period of 13 weeks. Untreated controls received food and tap water only. Treatments did not affect the survival rates (> 90%). The presence of adenomas at the surface of the lungs was counted and histopathological observations (one of five mice) served as confirmation. Body weights at the end of the study did not differ significantly compared to controls. Sodium nitrate did not increase lung adenomas yield compared to controls.

In an 18-month study, female mice (100 per group) weighing 27–31 g, received 0, 100 or 1,000 mg nitrate/L of drinking water. These concentrations were calculated to be equivalent to 0, 30 or 300 mg calcium nitrate/kg bw by IARC (2010). According to the authors, the parameters studied were liver function, kidney function, total iron, ammonium, total protein and electrophoresis of the various serum proteins, body weight, and *N*-glycolyl-neuraminic acid as a tumour marker. The exposed mice lost weight and died prematurely. This was observed for the mice exposed to 100 mg nitrate/L (equivalent to 30 mg/kg bw per day) drinking water, and not at the highest dose tested (Mascher and

Marth, 1993). IARC reports that survival of the high-dose group was lower than that of the controls and also that no increase in tumour incidence was observed in the nitrate-treated groups.

Rats

In a 14-month study, male rats (10 per group, strain not identified) received a dose of 0 or 4,000 mg/L (equivalent to 200 mg/kg bw per day) of sodium nitrate in drinking water (Chow et al., 1980). There were no major differences in the methaemoglobin levels among both groups. Animals receiving nitrate showed methaemoglobin average values from 0% to 2% as compared to less than 1% in controls. The average glutathione (GSH) levels in plasma were only 0.7% lower as compared to controls. High incidence of chronic pneumonitis was reported in the nitrate group but of mild severity as well as lower liver weights.

F344 rats (50 animals per sex per group, 8 weeks old) were given 0%, 2.5% and 5% sodium nitrate in the diet *ad libitum* (equivalent to 0, 1,250 and 2,500 mg/kg bw per day) in a 2-year feeding study (Maekawa et al., 1982). Rats in the control group were given a basic diet without nitrate *ad libitum*. Treatment was stopped at week 104 and the rats were given a basic diet until week 123 to account for the number of survivors in at least one group of either sex that was less than 20%. Diet and water consumption was constant in all groups throughout the study. The growth curves showed that the mean body weight of male fats did not differ more than 10%, whereas female rats differed by more than 10% in the high-dose group after week 60. However, no statistically significant differences were reported for this parameter. Treatment with sodium nitrate did not have statistically significant effect on survival rates, although male rats in the 5% group (equivalent to 2,500 mg/kg bw per day) had 10% higher cumulative mortality compared to the 2.5% group (equivalent to 1,250 mg/kg bw per day). At the end of the study (2 years), both male and female control groups showed a statistically significant lower number of survivors compared to both treatment groups. However, the mean survival time did not differ significantly among all groups. The incidence of tumours in all groups, including controls, was high. For example, the percentage of animals showing tumours in the control groups was reported to reach 94% and 92% for male and female rats, respectively. In male rats from the 2.5% sodium nitrate group (equivalent to 1,250 mg/kg bw per day), tumour incidence was reported to be 100%, whereas, in male rats from the 5% dose group (equivalent to 2,500 mg/kg bw per day), the incidence was 96%. All other groups showed lower absolute tumour incidences than controls. The most commonly observed tumour in males was in the testes (interstitial cell) followed by the mammary gland, adrenal gland and liver. Tumours of the mammary gland, pituitary gland, uterus and adrenal gland were the most commonly observed tumours in females. Overall, there was no statistically significant difference of any specific tumour. The time of appearance of the first background tumour was not affected in any treatment group. Only the incidences of tumours of the haematopoietic organs were reported to be statistically significantly lower in the treated groups compared to controls, especially concerning mononuclear cell leukaemia. The authors concluded that sodium nitrate did not have carcinogenic activity in F344 rats when administered continuously for 2 years.

The Panel noted that this study (Maekawa et al., 1982) was the basis for the derivation by the SCF of the ADI of 0–3.7 mg/kg bw per day for the nitrate ion (equivalent to 0–5 mg/kg bw per day for sodium nitrate) (SCF, 1997). However, the Panel noted the high incidence of tumours in all groups, which renders the study not sensitive enough to detect any treatment-related cancer effects.

JECFA (1996) briefly describes three long term studies in rats as follows:

In a 2-year study, rats (20/sex/group) were fed a diet containing 0, 0.1, 1, 5 or 10% sodium nitrate. At the 5% dose level a slight growth inhibition was observed, whereas inanition was noticed at the 10% dose level. Complete histopathological examination, including tumour incidences, was performed. No abnormalities or increased tumour incidences were found. The NOEL in this study was 1%, equivalent to 500 mg sodium nitrate/kg bw per day, or 370 mg/kg bw per day expressed as nitrate ion (Lehman, 1958; based on unpublished data). The Panel noted that the actual data for this study (Fitzhugh and Nelson, no date) considered in JECFA (1996) are unpublished and only a summary was available to the Panel for review.

In a carcinogenicity study, rats (15/sex per group) received 0 or 5% sodium nitrate/l of drinking-water for 84 weeks and were killed 20 weeks later. Histopathological examination did not reveal any increase in tumour incidence (Lijinsky et al., 1973).

In a 2-year carcinogenicity study, F344 rats (50/sex per group) received diets containing 0, 2.5 or 5.0% sodium nitrate, equivalent to 0, 1,250 or 2,500 mg/kg bw per day, or 0, 910, or 1,820 mg/kg bw per day expressed as nitrate ion. No carcinogenic effects were detected. This strain of rats is

known to have a high incidence of mononuclear leukemia which was higher in controls than in the experimental groups (Maekawa et al., 1982).

In its evaluation, the JECFA commented that, in the two long-term feeding studies in rats, doses of 370 and 1,820 mg/kg bw per day, expressed as nitrate ion, failed to produce any effects (JECFA, 1996). JECFA considered, however, that the study in which a dose of 1,820 mg/kg bw per day was reported was solely a carcinogenicity study, and therefore, this dose could not be considered as a no-observed-effect level (NOEL). Overall, JECFA considered that 'on the basis of the NOEL of 370 mg of nitrate ion/kg bw per day in the long-term study in rats and a safety factor of 100, an ADI of 0–5 mg/kg bw per day, expressed as sodium nitrate, or 0–3.7 mg/kg bw per day expressed as nitrate ion, could be allocated' (JECFA, 1996). JECFA further considered that 'because nitrate may be converted to nitrite in significant amounts and infants below the age of 3 months are more vulnerable to the toxicity of nitrite than adults, the ADI does not apply to such infants'.

In its more recent evaluation, JECFA retained the ADI of 0–5 mg/kg bw per day expressed as sodium nitrate, or 0–3.7 mg/kg bw per day, expressed as nitrate ion, established in its 2002 evaluation (JECFA, 2003a).

3.6.5.2. Endogenous formation of *N*-nitroso compounds upon nitrate intake

It has generally been considered that the toxicity of nitrate is encompassed by its conversion to nitrite and the possible endogenous formation of NOCs (SCF, 1997). Many NOCs, but not all, are carcinogens (IARC, 1998; NTP, 2011). Under the appropriate conditions (pH, concentration of reactants), nitrites have been shown to form NOCs, nitrosamines and nitrosamides from constituents in the food. The reactions involved in this process are generally described as nitrosation. As mentioned before, a detailed description of nitrosation and its relevant risk to humans has not been included in this opinion as sodium and potassium nitrite are the subjects of a separate opinion by the Panel (EFSA ANS Panel, 2017).

3.6.6. Reproductive and developmental toxicity

The literature search conducted (as described in Section 3.6) identified two new reports on reproductive and developmental effects (OECD 422 study referred to OECD, 2007; Pant and Srivastava, 2002) are summarised below. Previously reviewed toxicity studies in existing evaluations that has been considered by the Panel to be 'key studies' are also discussed below.

3.6.6.1. Reproductive toxicity studies

In an OECD TG 422 reproductive/developmental toxicity screening study performed under good laboratory practice (GLP), rats were exposed to 0, 250, 750 and 1,500 mg potassium nitrate mg/kg bw per day by gavage (referred to OECD, 2007). The NOAEL for reproduction and developmental toxicity was 1,500 mg/kg bw/day based on the absence of adverse effects.

3.6.6.2. Developmental studies

JECFA (2003a) referred to several prenatal developmental toxicity studies with sodium nitrate and potassium nitrate were conducted in CD1 mice, Wistar rats, golden hamsters and Dutch-belted rabbits (FDRL, 1972a,b). Animals were administered different doses of sodium nitrate by gavage; the control groups were vehicle treated (vehicle not specified). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, one-third underwent detailed visceral examinations and two-thirds were stained and examined for skeletal defects.

Mice

Groups of 20–24 pregnant albino CD-1 mice were dosed via gavage with 0, 4, 20, 100 or 400 mg sodium nitrate/kg bw per day from gestational days (GD) 6–15 (FDRL, 1972a). Body weights were recorded on GD 0, 6, 11, 15 and at necropsy on GD 17. For both dams and fetuses, no adverse effects were noted at doses of up to 400 mg/kg bw per day.

Groups of 20–25 pregnant albino CD-1 mice were dosed via gavage with 0, 4, 20, 100 or 400 mg potassium nitrate/kg bw per day from gestational GD 6–15 (FDRL, 1972b). Body weights were recorded on GD 0, 6, 11, 15 and at necropsy on GD 17. For both dams and fetuses, no adverse effects were noted at doses of up to 400 mg/kg bw per day.

Rats

Groups of 22–24 pregnant albino Wistar rats were dosed via gavage with 0, 2.5, 12, 54 or 250 mg sodium nitrate/kg bw per day from GD 6–15 (FDRL, 1972a). Body weights were recorded on days 0, 6, 11, 15 and at necropsy on GD 20. Dams and fetuses were examined as described in the above study with mice. No adverse effects for both dams and fetuses were noted at doses of up to 250 mg/kg bw per day.

Groups of 19–21 pregnant albino Wistar rats were dosed via gavage with 0, 2, 9, 40 or 180 mg potassium nitrate/kg bw per day from GD 6–15 (FDRL, 1972b). Body weights were recorded on days 0, 6, 11, 15 and at necropsy on GD 20. Dams and fetuses were examined as described in the above study with mice. No adverse effects for both dams and fetuses were noted at doses of up to 180 mg/kg bw per day.

Hamster

Groups of 22–26 pregnant golden hamsters were dosed via gavage with 0, 4, 20, 100 or 400 mg sodium nitrate/kg bw per day from GD 6–10 (FDRL, 1972a). Body weights were recorded on days 0, 8, 10 and at necropsy on GD 14. Dams and fetuses were examined as described in the above study with mice. No adverse effects for both dams and fetuses were noted at doses of up to 400 mg/kg bw per day.

Groups of 21–25 pregnant Golden hamsters were dosed via gavage with 0, 3, 20, 70 or 280 mg potassium/kg bw per day from GD 6–10 (FDRL, 1972b). Body weights were recorded on days 0, 8, 10 and at necropsy on GD 14. Dams and fetuses were examined as described in the above study with mice. No adverse effects for both dams and fetuses were noted at doses of up to 280 mg/kg bw per day.

Rabbits

Groups of 15 Dutch-belted rabbits were dosed once daily via gavage with 0, 2.5, 12, 90 or 250 mg sodium nitrate/kg bw per day from GD 6–18 (FDRL, 1972a). A Caesarean section was performed on GD 29. Live litters were observed in 9, 8, 9, 3 or 5 dams of the 0, 2.5, 12, 90 or 250 mg sodium nitrate/kg bw per day groups, respectively. The incidences of corpora lutea, implantations, live and dead fetuses, and resorptions in dams were within the normal range. In fetuses, there were no increased incidences of external, visceral and skeletal abnormalities and also fetal weights were not affected. Due to the low number of dams with live litters, the Panel considered this study not relevant for risk assessment.

Groups of 15 Dutch-belted rabbits were dosed once daily via gavage with 0, 2, 10, 50 or 206 mg potassium nitrate/kg bw per day from GD 6–18 (FDRL, 1972b). A caesarean section was performed on GD 29. From the 0, 2, 10, 50 or 206 mg potassium nitrate/kg bw per day groups 1, 1, 1, 4 or 3 animals (pregnant or non-pregnant) died. Therefore, at necropsy, only 12, 13, 9, 6 or 10 litters were available in the 0, 2, 10, 50 or 206 mg potassium nitrate/kg bw per day groups. The incidences of corpora lutea, implantations, live and dead fetuses, and resorptions in dams were within the normal range. In fetuses, there were no increased incidences of external, visceral and skeletal abnormalities and also fetal weights were not affected. Due to the low number of dams with live litters and high mortality, the Panel considered this study not relevant for risk assessment.

3.6.6.3. Other studies on reproductive endpoints

Sexually mature white Swiss mice (5 males/group; 7 weeks old) were administered with 0, 90, 200, 500, 700 or 900 mg potassium nitrate/L drinking water (equivalent to 0, 15.7, 34.8, 87, 121.8 or 156.6 mg potassium nitrate/kg bw) for 35 days to assess the testicular and spermatotoxic effects of nitrate (Pant and Srivastava, 2002). A decrease in sperm count and motility, and an increase in total percentage on sperm abnormalities were reported in the males of the highest dose group. Histopathological changes in the highest dose group included congestion and moderate atrophy in testis with prominent interstitial spaces, although it was not mentioned if these changes were statistical significant different to controls. In the highest dose group, the level of 17-β-hydroxysteroid dehydrogenase (17-βHSD) was decreased and γ-glutamyl transpeptidase (γ-GT) increased ($p < 0.05$) in a homogenate of a portion of the testis. No other overt signs of toxicity were recorded during the study and body weight gain, testicular, epididymal and accessory sex organ weight were similar to controls at all doses tested. The Panel noted the small number of animals tested and the short duration of exposure in the study. The authors concluded that a long-term reproduction toxicity study is necessary to examine this effects detected. The Panel agreed with this conclusion.

Overall, the Panel noted that no effects were observed in a reproductive/developmental toxicity screening study (OECD TG 422) in rats by gavage at a dose up to 1,500 mg potassium nitrate/kg bw per day (the highest dose tested) (referred to OECD, 2007). No developmental toxicity was observed in mice, rats or hamsters receiving by gavage doses up to 400, 250 or 400 mg sodium nitrate/kg bw per day, respectively (FDRL, 1972a). In prenatal developmental studies with potassium nitrate by gavage in mice, rat or hamsters, no developmental toxicity was observed up to 400, 180 or 280 mg/kg bw per day (FDRL, 1972b). In a study in mice given potassium nitrate in drinking water effects were observed on sperm count, sperm abnormalities, histological changes of the testis and testicular enzymes at the highest dose tested; the NOAEL in this study was 122 mg potassium nitrate/kg bw per day (Pant and Srivastava, 2002). A definitive conclusion cannot be reached as the duration of the dosing in males in the screening study is limited as well as the number of animals tested.

The Panel noted that although some effects were observed in sperm analysis and reproductive organs in a limited study in mice, no indications of reproductive toxicity were observed at higher doses in a rat study conducted according to OECD guideline TG 422.

3.6.7. Other studies

3.6.7.1. Methaemoglobinaemia

Ingested nitrate is reduced to nitrite in humans by the nitrite reductase of bacteria in the oral cavity. In infants, the higher stomach pH (2–5), as compared to the adult (pH 1–3), may lead to the growth of nitrate-reducing bacteria (Zeman et al.,2002). This might increase the risk of methaemoglobin formation in infants. Nitrite can convert Fe^{2+} in the haemoglobin into Fe^{3+}, leading to methaemoglobinaemia. Methaemoglobinaemia is the most reported side effect in humans correlated with the exposure towards nitrate. Fe^{3+} can be reconverted into Fe^{2+} by cytochrome b5 reductase.

Individuals genetically deficient in cytochrome b5 reductase, fetus and infants where concentrations of the cytochrome b5 reductase enzyme were found to be only about 60% of the concentration found in adult red blood cells (Ross, 1963; Bartos and Desforges, 1966).

The specific sensitivity of infants below 16 weeks of age to methaemoglobinaemia can be explained by differences in the activity of cytochrome b5 reductase (which converts methaemoglobin back to haemoglobin), which is 40–50% lower than in the adults (Bartos and Desforges, 1966, Wright et al., 1999). In addition, these infants have a large proportion of fetal haemoglobin still present in their blood, which has been shown to form twice as much methaemoglobin than adult haemoglobin under *in vitro* conditions (Wind and Stern, 1977; WHO, 2007).

From 151 publications found in a literature search between 1951 and 2015, there were case reports on methaemoglobinaemia including cases of suicidal attempts. A wide range of doses were reported to cause methaemoglobinaemia. Human oral lethal doses range from 4 to 50 g (Mirvish, 1991) and from 67 to 833 mg/kg bw (Boink et al., 1999). However, the data were not suitable for an analysis of a dose–response relationship. Experimental data on methaemoglobin formation have been evaluated under the section toxicokinetics (e.g. Lambers et al., 2000; published van Velzen et al., 2008). However, the data were not suitable for a BMD modelling.

3.6.7.2. Animal study on laryngopharyngeal mucosa

A new study by Del Negro et al. (2008) was identified in the literature search, in which the carcinogenic potential of hydrochloric acid associated with pepsin, with and without the addition of sodium nitrate on rat oropharyngeal mucosa simulating the reflux of gastric contents, was investigated. Wistar rats (12 males per group; weighing between 300 and 400 g) were divided into seven groups and administered various combinations of test solutions directly on the laryngopharyngeal mucosa. Groups V and VI received treatment with sodium nitrate: Group V received a mixture of 400 mg of sodium nitrate (diluted in 300 mL of water equal to 1,333 mg sodium nitrate/kg bw per day), with a solution of 0.1 N hydrochloric acid applied directly on the laryngopharyngeal mucosa three times per week. Group VI was treated similar to group V but twice weekly. Group VII served as the control group and was treated twice weekly with filtered water. The rats were treated for a period of 6 months, after which specimens for study were taken out by dissecting the risk mucosa and removing the parts that were fixed in formalin and stored in a 70% alcohol solution. Inflammatory histological changes were present in several animals, including the presence of lymphocytes and mast cells. The presence of mast cells was more pronounced in groups V and VI ($p = 0.006$) compared to the control group. No epithelial changes indicating carcinogenicity (dysplasia, intra-epithelial neoplasia or invasive carcinoma) were detected in any of the animals.

In previous evaluations, nitrate safety has been evaluated in conjunction with that of nitrite by considering conversion values of nitrate to nitrite (JECFA, 1996). JECFA, (2003a) mentioned a combined physiologically based pharmacokinetic/toxicodynamic model allowing quantification of the kinetics of nitrate and nitrite in humans. The model was published later as Supplementary Information to the publication Zeilmaker et al. (2010). The model included values for the absorption of nitrate from drinking water and vegetables, secretion of nitrate from blood into saliva, conversion of nitrate to nitrite, and absorption of nitrite and its interaction with haemoglobin, yielding methaemoglobin, as obtained by applying rate constants of reaction to available experimental data sets. According to the report by JECFA, a dose of 44 mg/kg bw per day of nitrate would not induce clinical methaemoglobinaemia in adults, doses of 88–270 mg/kg bw per day would progressively induce methaemoglobinaemia and clinical hypoxia, and doses higher than 440 mg/kg bw per day would induce lethal toxicity. According to JECFA's report on unpublished authors conclusions 'the intake by healthy adults of nitrate from food and/or drinking-water has negligible effects on methaemoglobin formation but may significantly affect methaemoglobin formation in neonates with inflammatory disease' (JECFA, 2003a).

3.6.7.3. Effects on adrenal and thyroid glands

As nitrate shares a common transport mechanism with iodine, the influence of nitrate intake on thyroid function had been studied previously in risk evaluations of nitrate as a food additive.

In vitro studies

In a study by Tonacchera et al. (2004), the relative inhibitory activity of several anions on the NIS was measured in an *in vitro* model (CHO cell line transfected with human NIS). Inhibition of NIS by NO_3^- was 249 times lower than that by CLO_4^-, and the affinity of NO_3^- to bind to NIS was eightfold lower than the binding affinity of iodine. Inhibition of NIS by other anions leads to a decrease in iodine ion uptake by the thyroid and, consequently, has a negative effect on the production of thyroid hormones. From the *in vitro* study, it can be deduced that nitrate, by reducing thyroid hormone production, may lead to enlarged thyroid and hypothyroidism depending on the intake.

In vivo studies

Animal studies with nitrate exposure and effects on the thyroid (summarised in Appendix E, Table E.2).

Rat

JECFA (1996) described briefly two studies addressing this potential effect. 'Rats dosed with 40 to 4000 mg nitrate/l in drinking water (equivalent to 3.6 to 360 mg/kg bw per day) had no effect on the serum iodine level or protein-bound iodine. Minor changes were reported in ^{131}I uptake in thyroid, thyroid weight and the histology of the thyroid. These effects were seen at all dose levels, but there was no dose–response relation' (Höring, 1985; Höring et al., 1988; Seffner, 1985; as referred to in JECFA, 1996). In the second described study, 'potassium nitrate was administered to 56-day old pigs at a dietary concentration of 3% for 2 days or 6 weeks (equivalent to 730 mg/kg bw per day expressed as nitrate ion). Levels of methaemoglobin, serum T_4, T_3, nitrate and somatomedin were determined. Sufficient iodine uptake by mothers prevented a decrease in T_4 levels after administration of potassium nitrate for 2 days. After 6 weeks of treatment, however, the decrease in T_4 levels could not be prevented by supplementing the diet with 0.5 mg iodine/kg bw. A decrease in serum somatomedin activity due to nitrate administration was also observed which correlated with a decreased body-weight gain in pigs' (Jahreis et al., 1987; as referred in JECFA, 1996).

JECFA (2003a) reported a study on groups of male Wistar rats (10 per group) administered potassium nitrate at a concentration of 36 mmol/L in drinking water (equivalent to approximately 3,636 mg/L) for 90 days, aiming to evaluate potential effects on the zona glomerulosa of the adrenal glands of these animals (Boink et al., 1995, 1996, as referred to in JECFA, 2005). The control group was treated with the same amount of potassium chloride in drinking water. 'No differences in food intake per kg bw were noted although body-weight gain was lower in the treated animals. Blood nitrate concentrations were higher in the treated group. Treatment has no consistent effect on the concentrations of thyroxin, free thyroxin, thyroid simulating hormones, adrenocorticotrophic hormone, corticosterone or aldosterone in blood. Microscopic examination of the tissues showed minimal hypertrophy in 20% of animals treated with potassium nitrate'. Although no indication is given on statistical differences on this effect compared to controls, it is reported that the minimal hypertrophy of the adrenal zona glomerulosa occasionally observed in rats was barely detectable by morphometric

analysis 90 days after treatment and that the fractional zona glomerulosa surface area of the adrenal glands did not differ from the control group. Absolute and relative weights of the adrenal glands did not differ statistically significantly from those of controls. It was concluded by JECFA that nitrate does not play a role in the aetiology of hypertrophy of the zona glomerulosa of the adrenal glands in rats.

A study in Wistar rats (10 per group per sex not stated, age not specified) investigated the effects of potassium nitrate intake on thyroid function (Mukhopadhyay et al., 2005). One test group was fed a diet containing 3% potassium nitrate (equivalent to 1500 mg/kg bw per day) and one control group was fed standardised normal diet, both for 28 days. Blood samples and thyroid glands were collected for analysis of thyroid hormones and to assess thyroid peroxidase (TPO) activity. No effect was noted on body weights at the end of the study. Statistically significant increases were reported in thyroid gland weight and urinary iodine concentration, and TPO activity and serum thyroid-stimulating hormone (TSH) were also reported to be higher in the treated group. In those animals, T4 and T3 levels were reported to be decreased.

In another study with Wistar rats (12 males per group, age not specified), four groups received drinking water containing 50, 100, 150 or 500 mg/L of potassium nitrate (equivalent to 4.5, 9, 13.5 and 45 mg/kg bw per day, respectively) (Zaki et al., 2004). Controls received drinking water containing approximately 14 mg/L nitrate (equivalent to 1.3 mg/kg bw per day). It is indicated that all rats were fed a diet containing 'iodine nitrate' at a concentration of approximately 22 mg/kg diet (equivalent to 1 mg/kg bw per day). Animals were sacrificed after 5 months of treatment. A significant dose-dependent decrease in body weight gain was reported ($p < 0.001$). At the two highest doses group, the decrease was reported to be 16% and 25%, respectively. Total plasma proteins were decreased in all treated groups, whereas the plasma urea concentration was increased. Thyroid weight was increased at all doses, becoming statistically significant for the 13.5 mg/kg bw per day group. Mean plasma thyroid T3 hormone was statistically significantly decreased in the 13.5 and 45 mg/kg bw per day groups, whereas T4 hormone was decreased statistically significantly only in the highest dose group animals (45 mg/kg bw per day). Upon microscopic examination, thyroid samples from the two highest dose groups showed vacuolisation and increased colloidal volume of the follicles and the two lower doses were not reported to show microscopic changes. The Panel noted that no measurements of plasma nitrate levels to confirm nitrate exposure at the doses tested were carried out and that other studies testing much higher concentrations of potassium nitrate (up to 3,636 mg/L) did not report any effects on the thyroid functions (Boink et al., 1995). The Panel also noted that the description of the iodine source in this study is not clear because 'iode nitrate' is not a compound with a CAS number.

Sodium nitrate was administered in the drinking water to four groups of female rats ($n = 10$ per group, strain not specified) for 30 weeks at doses of 0, 50, 100, 250 or 500 mg nitrate/L (equivalent to 0, 2.5, 5, 12.5 or 25 mg/kg bw per day) (Eskiocak et al., 2005). In all dose groups, thyroid weights increased. Other effects varied between doses; for example, iodine uptake was decreased in dose groups 2.5 and 5 mg/kg bw per day, and increased in dose groups 12.5 and 25 mg/kg bw per day. TSH was decreased in dose groups 2.5, 12.5 and 25 mg/kg bw per day and did not change in dose group 5 mg/kg bw per day.

Potassium nitrate was administered in the drinking water to four groups of female Wistar rats ($n = 12$ per group) at doses of 50, 150 and 500 mg/L (equivalent to 2.5, 7.5 or 25 mg/kg bw per day) for 5 months to assess potential effects on the morphology and physiology of the thyroid (Chaoui et al., 2004). Control animals consumed *ad libitum* tap water containing approximately 13 mg nitrate/L (equivalent to 0.7 mg/kg bw per day). At the end of the study, animals were killed and their T3 and T4 total levels were measured by radioimmunology. Thyroid glands were collected and processed to microscopic analysis. Thyroid absolute weight and T3 levels increased significantly in animals from the two highest dose groups not in the 50 mg/L group. T4 levels also increased significantly but only in animals from the 500 mg/L group. Microscopically, thyroid follicles from the two highest dose groups showed an increased size and a flat epithelium shape.

Studies in volunteers

Human studies with nitrate exposure and effects on the thyroid are summarised in Appendix E, Table E.3.

In a study in 10 volunteers, oral intake of 15 mg/kg nitrate for 28 days did not lead to changes in iodine uptake and thyroid hormone levels (T3, rT3, T4, TSH), indicating that a dose largely exceeding the ADI had no influence on thyroid function (Hunault et al., 2007).

In 69 subjects, van Maanen et al. (1994) observed a difference in the volume of the thyroid between low and medium vs high nitrate exposure groups, showing development of thyroid

hypertrophy at nitrate water levels in the highest exposure group (N = 7) with 109.5 ± 68.1 mg/L and a total daily nitrate intake of 245 ± 92 mg. There was a significant correlation between thyroid volume and nitrate concentration ($p = 0.008$) in drinking water and thyroid volume and thyroglobulin ($p = 0.004$) and an inverse correlation between thyroid volume and serum TSH ($p = 0.0001$). The increased in thyroid volume in the high nitrate exposure group is accompanied by a tendency towards a decrease in TSH levels but free T4 was not increased. (van Maanen et al., 1994).

A study in Slovak children (10–13 years old) showed a slightly higher thyroid volume in children (n = 324) from a region with high nitrate drinking water supply (51–274 mg/L) compared to children from two regions with low nitrate drinking water supply (n = 168 and 596). In 13 of 324 (4.0%) of the children with high exposure, TSH level in blood was elevated (Tajtáková et al., 2006). In a group of 10-year-old children, the frequency of hypoechogenicity in the high nitrate area was higher (13.7%) than in the low nitrate area (4.7%) ($p < 0.01$). Thyroid volume assessed by ultrasound was significantly higher in children with a high nitrate intake in comparison with children with a low nitrate intake.

In a cross-sectional study from Bulgaria (Gatseva and Argirova, 2008), clinical examination of the thyroid status of children chronically exposed to high levels of nitrate (78 mg/L in drinking water) (n = 156) revealed that 13.5% had goitre, whereas, in the group with low exposure (8 mg/L in drinking water) (n = 163), 4.9% of the children had goitre. The difference was statistically significant.

In a study from Slovakia (Rádiková et al., 2008), goitre grade 1 was more frequent in pregnant women (n = 26) living in a village with high-nitrate levels in drinking water (93 mg/L) compared to pregnant women (n = 22) in a village with a low-nitrate level in drinking water (8 mg/L) (odds ratio (OR) = 5.294, 95% confidence interval (CI) = 1.003–27.939, $p = 0.0454$). In children aged 3–6 years from the two villages, no difference in thyroid status was observed.

In a population-based cross-sectional epidemiological survey (n = 3,772 participants, 20–79 years), the mean nitrate excretion in urine was 53.1 ± 0.8 mg/L. The proportion of goitre in subjects with and without high urine nitrate concentrations and no iodine deficiency was not different (35.5% and 34.7%, $p = 0.69$) (Below et al., 2008).

Within the National Health and Nutrition Examination Surveys (NHANES) that assess the health and nutrition status of adults and children across the United States, a representative sample of the participants with laboratory results was extracted across all age group to study the association between nitrate and free T4. Multivariate regression models by iodine status were conducted. After controlling for sample weights and stratum age, race/ethnicity, BMI, serum cotinine, estimated total caloric intake, post-menopausal status, premenarche status, oestrogen use, serum C-reactive protein, and hours of fasting before sample collection, urine nitrate was a significant predictor of free T4 for both non-pregnant (n = 307) with urinary iodine of less than 100 μg/L (coefficient for log nitrate = −0.0368, $p = 0.03$) and non-pregnant women (n = 564) with urinary iodine of 100 μg/L or more (coefficient for log nitrate of −0.0259, $p = 0.02$). No association between urinary nitrate and serum free T4 was found for pregnant women (n = 48) with urinary iodine of less than 100 μg/L (Suh et al., 2014).

A large cohort study (n = 21.977 women) showed that increasing intake of nitrate from dietary sources (estimated from a food frequency questionnaire (FFQ)) was associated with an increasing occurrence (ever) of self-reported hypothyroidism. Subjects in the highest quartile of dietary nitrate intake had a 24% increased risk of hypothyroidism (odds ratio (OR) = 1.24, 95% CI: 1.10–1.40, $p_{trend} = 0.001$) in comparison with subjects in lowest quartile of nitrate intake (41.1 mg nitrate-N per day, corresponding to 2.6 mg/kg bw per day vs Q1 ≤ 17.4 mg nitrate-N per day), corresponding to 1.1 mg/kg bw per day, after controlling for age, total calories, body mass index (BMI), residence, education and smoking status. The ORs in quartiles 2 and 3 were 1.13 (95% CI 1.01–1.27) and 1.19 (95% CI 1.06–1.33). The association was possibly stronger among those with intakes of vitamin C below the median compared to those with a higher intake. No association was found for drinking water and hypothyroidism (Ward et al., 2010). Intake of iodine was not assessed, but is considered to be adequate in the US population.

Overall, there was some evidence to relate exposure towards nitrate in drinking water with the development of an enlarged thyroid, and goitre. One study found a slight, but statistically significant association between self-reported hypothyroidism and estimated dietary nitrate intake.

3.6.7.4. Cardiovascular system and haematology

Webb et al. (2008) report the results of a study, separated in three phases with distinct recruitment for each phase, assessing the effects of dietary nitrate on lower arterial blood pressure, supplement

endothelial function during ischaemia and inhibit platelet aggregation. In the blood pressure (BP) study, 14 healthy volunteers were randomly assigned to drink 500 mL of either beetroot juice or water, within a 30-min period. The mean concentration of nitrate in the beetroot juice was 45.0 ± 2.6 mM/L (2.79 g/L; initial study) and 34.0 ± 0.1 mM/L (2.11 g/L; spitting study). Nitrite concentrations in the juice were below the LOD (< 50 nM/L). The BP was measured every 15 min for 1 h pre- and 3 h post-ingestion of the beetroot juice, then hourly for up to 6 h, with a final measurement at 24 h (a mean of three readings was calculated for each time interval). Blood samples were collected every 30 min for 2 h, then hourly up to 6 h, with a final sample collected at 24 h, to determine plasma nitrate and nitrite concentrations. In the interruption of enterosalivary circulation study, the effect of spitting out all saliva during, and for 3 h following juice ingestion on blood pressure and changes in plasma nitrate/nitrite concentrations, was also investigated in a separated crossover study in six healthy volunteers. For the assessment of platelet aggregation, blood was collected at baseline and at 2.5 h after ingestion of the beetroot juice. In the second phase of this study, endothelial function was assessed in 10 healthy subjects by measuring brachial artery diameter in the non-dominant arm of the study in response to the endothelium-dependent reactive hyperaemia response before and after an ischaemic insult. In this open-label crossover study, healthy subjects were randomised to 500 mL of beetroot juice 2 h before the ischaemia–reperfusion (I/R) sequence. Plasma nitrate concentration rapidly (~30 min) increased (approximately 16-fold) after 1.5 h and remained at the same level up to 6 h. Blood pressure was affected only after 1 h of treatment, and especially the systolic blood pressure remained unchanged after 24 h. Overall, the mean heart rate was not altered by the treatment. Interrupting the enterosalivary circulation blocked the rise in plasma nitrite concentration, and blocked the reduction of systolic blood pressure but had no effect on platelet aggregation. Platelet aggregation was inhibited 2.5 h after beetroot ingestion. Beetroot juice did not alter pre-ischaemic branchial dilation but completely prevented ischaemia-induced endothelial dysfunction ($p < 0.05$).

3.6.7.5. Allergy and immunotoxicity

A case-study of a 56-year-old man with no family history of atopic diseases presenting severe pruritus on the trunk, upper limbs and head, with no concomitant rash, was reported by Asero (2005). After elimination of the likelihood of known skin diseases associated with itching by appropriate investigations, an elimination diet excluding food additives was initiated. By the fifth day of the diet, a significant improvement in pruritus was noted. After 3 weeks of no symptoms, a more detailed study to identify the causative agent was undertaken 4 h after ingestion of 10 mg sodium nitrate, the onset of pruritus was recorded again, thus identifying sodium nitrate as the causative agent of the condition.

The effects of nitrate on immunological parameters were investigated in peripheral mononuclear blood cells (PBMCs) from healthy volunteers aged 18–55 years old (Ustyugova et al., 2002). PBMCs were cultured for 72 h in 10 ppm sodium nitrate and an enzyme-linked immunosorbent assay was performed to assess the cytokines interleukin (IL)-2, interferon-γ, tumour necrosis factor-β and IL-10. No clear trend in cytokine production was evident in response to sodium nitrate.

3.6.8. Epidemiological studies on cancer

Human carcinogenicity of nitrate and nitrite has been extensively reviewed by the IARC (2010), and the studies discussed in the IARC report have therefore not been included in this review. The interested reader is invited to consult the IARC report for details of all studies evaluated. The overall evaluation of the IARC (2010) was that there is inadequate evidence in humans for the carcinogenicity of nitrate in foods and there was inadequate evidence for the carcinogenicity in experimental animals. The overall conclusions in this opinion are based on the evaluation of publications by the Panel that appeared subsequent to this previous review by IARC on nitrate, nitrites and cancer (IARC, 2010), which considered publications until 2006, combined with the publications evaluated by IARC (2010). Only original studies that have data on nitrate or dietary NOCs intake (reviews were not included) and studies that have not been included in the IARC (2010) report were reviewed, and retrieved according to the protocol agreed by the Panel presented in Appendix G. It was found that a paper, already described in the IARC (2010) comprising a review of Knekt et al. (1999) (on head and neck cancer, as well as colorectal cancer (CRC)), was no described in terms of NDMA results, and thus it appears in the current review as well.

The epidemiological studies per cancer site were ranked according to study design as: cohort, case–control and ecological studies. Study descriptions were extended with information on processed meat items or specific animal foods, if possible. The evaluation of cancer in the different organs

follows the ordering of International Classification of Diseases (ICD-10, WHO): head and neck, oesophagus, stomach, colorectal, liver, pancreas, lung, breast, ovarian, prostate, renal, bladder, urinary tract, thyroid, non-Hodgkin's lymphoma, leukaemia, brain/glioma.

The evidence for human cancer from these studies was categorised as: (i) *there was no evidence,* if studies indicate no association with a specific cancer; (ii) *there was insufficient evidence,* to (unequivocally) link to a cancer (e.g. few studies, contradictory results, etc.); (iii) *there was some evidence,* for an association with a specific cancer (e.g. inconsistent results between cohort studies and case–control studies); and (iv) *there was evidence,* for an association with a specific cancer (e.g. consistent results from cohort studies and case–control studies).

In the protocol, all necessary items to for the risk assessment (causal inference) were listed to be included in the description of studies. In brief, while evaluating the studies it was taken into consideration the type of study (ecological, case–control, cohort), giving more weight to cohort studies; the quality of the exposure assessment, the power of the study, the presence of dose–response (e.g. by evaluating p for trend) and the good control for confounding factors. Study-specific limitations were also taken into account in the evaluation. For cohort studies, the number of cases identified during the follow-up and the time of follow-up and number of participants lost to follow-up were also considered.

From the literature search, 95 studies on nitrate, nitrite and/or NOC intake by oral route and head and neck, oesophageal, stomach, colorectal, liver, pancreatic, lung, breast, ovarian, prostate, renal, bladder, urinary tract, thyroid, non-Hodgkin's lymphoma, leukaemia, brain/glioma and total cancer were selected and screened for detailed evaluation. In total, 49 epidemiological studies were critically reviewed. The reasons of exclusion are summarised in Appendix G.

3.6.8.1. Head & neck cancer

No new data were found subsequent to the IARC evaluation (2010). For head and neck cancer, no conclusions can be drawn on nitrate because of lack of data.

3.6.8.2. Oesophageal cancer

Cohort studies

Cross et al. (2011) studied the relationship between intake of meat, meat components and meat cooking by-products and risk of oesophageal cancer subtypes. The National Institutes of Health-American Association of Retired Persons Diet and Health Study (NIH-AARP) Diet and Health Study recruited men and women, aged 50–71 years, from six states in the United States. At baseline (1995–1996), participants completed self-administered demographic and lifestyle questionnaires, including a 124-item FFQ. Approximately 6 months later, cancer-free participants were mailed a risk factor questionnaire, which elicited detailed information on meat intake and cooking preferences. A total of 566,402 participants returned the baseline questionnaire and 337,074 of these also returned the risk factor questionnaire. They estimated nitrate and nitrite intake from processed meats using a database of measured values from 10 types of processed meats (bacon, red meat sausage, poultry sausage, red and white luncheon meats, red and white cold cuts, ham, hot dogs), which represent 90% of processed meats consumed in the United States; these meats were also measured for NOCs, although they were all below the detectable limit. After excluding prevalent cancer cases and those with implausible nutrient values, the baseline analytical cohort consisted of 494,979 persons (295,305 men and 199,674 women), and the risk factor questionnaire cohort consisted of 303,156 persons (176,842 men and 126,314 women). During 10 years of follow-up, they accrued 215 ESCC and 630 EAC. In the subcohort of participants who returned the risk factor questionnaire (126,314 females and 176,842 males), there were 128 incident cases of ESCC and 377 EAC. Cox PH modelling was used as analysis technique. After controlling for age, race, total energy intake, smoking, family history of cancer, family history of diabetes, BMI, physical activity, alcohol, saturated fat, fruits and vegetables intake, red meat intake was positively associated with ESCC (RR Q5 vs Q1: 1.79; 95% CI: 1.07–3.01, p_{trend} = 0.019) but not with adenocarcinoma of the oesophagus. The malignancies investigated in this study were not found to be associated with white or processed meat. Heterocyclic amine intake was seen to be positively associated with malignancies. A borderline statistically significant increased risk for EAC was identified for those in the high quintile intake of heterocyclic amines (HR = 1.35, 95% .97–1.89, p_{trend} = 0.022; HR = 1.45, 95% CI: 0.99–2.12, p_{trend} = 0.463, respectively). A positive association was also present for haem iron intake and oesophageal adenocarcinoma (HR for top vs bottom quintile = 1.47, 95% CI: 0.99–2.2, p_{trend} = 0.063). No association was found between nitrate or nitrite

intake from meat and oesophageal cancer subtypes. Comparing highest vs lowest quintile of intake, the results for nitrate from meat and ESCC risk were: RR Q5 (median 0.298 mg/1,000 kcal) vs Q1 (0.024) = 1.30, 95% CI: 0.72–2.35, p_{trend} = 0.153; and for nitrate and EAC were: RR = 1.10, 95% CI: 0.75–1.60, p_{trend} = 0.350. Comparing highest vs lowest quintile of intake, the results for nitrite from meat and ESCC risk were: RR Q5 (med 0.199 mg/1,000 kcal) vs Q1 (0.012) = 1.21, 95% CI: 0.67–2.20, p_{trend} = 0.651; and for nitrite and EAC were: RR = 1.19, 95% CI: 0.84–1.68, p_{trend} = 0.029. None of the quintile HR estimates were significantly different from one, nor were continuous analyses. Strengths of the study include the large size enabling the investigation of tumour subtypes. The study lacked information on nitrate and nitrite intake from other foods, and nitrate intake from drinking water.

Keszei et al. (2013) studied the relationship between risks of oesophageal cancer subtypes and intake of NDMA, ENOC, haem iron, nitrite and nitrate in the Netherlands Cohort Study. This prospective cohort study started in September 1986; at baseline, 58,279 men and 62,573 women aged 55–69 years were recruited from 204 municipal population registries throughout the Netherlands. At baseline (1986), a self-administered questionnaire was completed by study participants on dietary habits and other risk factors of cancer. The dietary part consisted of a validated 150-item food frequency questionnaire. The cohort was followed for 16.3 years and 110 ESCC and 151 EAC were analysed along with 4,032 subcohort members in a case–cohort analysis. To calculate intakes, food composition values for nitrate and nitrite were obtained from analyses conducted by Dutch institutes in the period 1984–1989. Information about nitrate content in drinking water from all pumping stations in the Netherlands in 1986 was used to determine the nitrate concentration in drinking water for each home address by postal code, and to calculate nitrate intake from water (in fact water, coffee, tea and soup combined). Nitrite intake was assessed solely on the intake of processed meat as the nitrite content of vegetables and cheese was considered negligible in comparison with processed meat. Considered processed meat items were: all types of sausages, bacon, ham, cold cuts, croquettes and frankfurters. NDMA values in food items were initially extracted from published measurements in Dutch foods in the 1970s and 1980s. For food items for which NDMA values were not available from Dutch sources, although non-zero content was indicated in a comprehensive food composition database of nitrosamines (Jakszyn et al., 2004a,b), measurements made in food sources from Western or Northern Europe in the 1980s were used. An index of ENOC was calculated, as previously determined by Jakszyn et al. (2006) based on the haem iron intake. The correlation between ENOC and haem iron was 0.97. In the sex-specific statistical analyses, Cox PH modelling was used, controlling for age, smoking, BMI, educational level, energy intake, vegetable and fruit intake, total alcohol intake; in NDMA analyses, adjustment was made for alcoholic beverages excluding beer because beer was an important source of NDMA. The estimated mean (SD) values of intake in the subcohort (a random sample of the Netherlands Cohort Study) were for men and women, respectively: nitrate: 108 (SD 45) and 106 (44) mg/day, nitrite: 0.12 (0.16) mg/day and 0.08 (0.12) mg/day, NDMA (median): 0.084 and 0.044 μg/day, ENOC: 102 (25) and 93 (23) μg/day. When comparing men in the highest vs lowest tertile of intake, the results for nitrite and ESCC risk were: HR T3 (median 0.28 mg/day) vs T1 (0.03) = 1.92, 95% CI: 0.94–3.89, p_{trend} = 0.06; and for nitrite and EAC were: HR = 0.74, 95% CI: 0.43–1.28, p_{trend} = 0.30. Although none of the tertile HR estimates were significantly different from one, nor were any tests for trend, in continuous analyses, there was a significant association between nitrite and ESCC: HR = 1.19, 95% CI: 1.05–1.36 per 0.1 mg/day increment. In nitrite analyses in women, none of the tertile HR estimates were significantly different from one, nor were any tests for trend, or continuous analyses. For nitrate, in both men and women, none of the tertile HR estimates were significantly different from one, nor were any tests for trend, or continuous analyses. For NDMA intake, when comparing men in the highest vs lowest tertile of intake, the results for ESCC risk were: HR T3 (med 0.25 μg/day) vs T1 (0.04) = 2.43, 95% CI: 1.13–5.23, p_{trend} = 0.01; and for NDMA and EAC were: HR = 0.87, 95% CI: 0.52–1.45, p_{trend} = 0.63. For ESCC, HR estimates were significant in continuous analyses: HR = 1.15, 95% CI: 1.05–1.25 per 0.1 μg/day increment. In women, when comparing the highest vs lowest tertile of NDMA intake, the results for ESCC risk were: HR T3 (0.07 μg/day) vs T1 (0.03) = 1.21, 95% CI: 0.56–2.62, p_{trend} = 0.57; and for NDMA and EAC were: HR = 0.92, 95% CI: 0.40–2.14, p_{trend} = 0.90. For ESCC, there was a significant association with NDMA in continuous analyses (HR = 1.34, 95% CI: 1.04–1.71 per 0.1 μg/day increment). A combined analysis of men and women showed a significant positive association with ESCC (HR T3 vs T1 = 1.76, 95% CI: 1.07–2.90, p_{trend} = 0.01). Haem iron intake, and thus ENOC because of the high correlation, was also positively associated with ESCC men (HR T3 vs T1 = 2.23, 95% CI: 1.05–4.75, p_{trend} = 0.03) but not in women (HR T3 vs T1 = 0.71, 95% CI: 0.34–1.51, p_{trend} = 0.40). For other cancer subsites,

no significant associations were seen in men or women in multivariate analyses. These results suggest that NOCs may influence the risk of ESCC, especially in men, although there are no clear associations for EAC. Strengths of the study include the large size and long follow-up enabling the investigation of tumour subtypes. A potential weakness is the single measurement of diet, only at baseline. Nitrite intake was assessed solely on the intake of processed meat but nitrite content of vegetables and cheese was considered negligible in comparison with processed meat.

Case–control studies

Ward et al. (2008) conducted a population-based case–control study of adenocarcinoma of the oesophagus in Nebraska, United States. Cases were white men and women aged 21 years or older, newly diagnosed between 1988 and 1993, identified from the Nebraska Cancer Registry and confirmed by histological review. Controls were selected from a previous population-based case–control study of lymphatic and haematopoietic cancers in Nebraska, and were re-interviewed at the time of this study (1992–1994). Response rates were between 79% and 88%. Telephone interviews were conducted with subjects or their proxies for those who were deceased or too ill to participate. Proxy interviews were conducted for 76% and 61% of oesophagus cancer cases and controls, respectively. Nitrate concentrations in public drinking water supplies were linked to residential water source histories. Among those using private wells at the time of the interview, they measured nitrate levels in water samples from wells. Dietary nitrate and nitrite were estimated from a FFQ. They estimated OR and 95% CI using unconditional logistic regression, adjusting for gender, year of birth, and risk factors for oesophagus cancer (smoking, alcohol, BMI). Among those who primarily used public water supplies (84 oesophagus cancer cases, 321 controls), average nitrate levels were not associated with risk (highest vs lowest quartile: oesophagus OR = 1.3, 95% CI: 0.6–3.1, p_{trend} = 0.519). Increasing intake of nitrate plus nitrite from animal sources (details NA) was associated significantly with oesophagus cancer (OR Q4 (> 8.3 mg) vs Q1 (< 3.8) = 2.2, 95% CI 0.9–5.7, p_{trend} = 0.015) but not with nitrite from plant sources (median intake 0.52 mg/day). Increasing intake of nitrate from plant sources (median intake NO_3: 116.1 mg/day) was not associated with oesophagus cancer (OR Q4 (> 171.9 mg) vs Q1 (< 74.9) = 0.8, 95% CI: 0.3–1.8, p-trend = 0.121). The number of cases is small in this study. Although there was a relatively high response rate, percentage of proxy interviews is high, which could have led to information bias.

Liao et al. (2013) explored whether Mg levels in drinking water modified the effects of nitrate on oesophageal cancer mortality. A matched cancer case–control study was used to investigate the relationship between the risk of death from oesophageal cancer and exposure to nitrate in drinking water in Taiwan. All oesophageal cancer deaths of Taiwan residents from 2006 through 2010 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health. Controls were deaths from other causes and were pair-matched to cancer cases by gender, year of birth and year of death. In total, there were 3,024 cases and 3,024 controls. Information on the levels of nitrate and Mg in drinking water was collected from Taiwan Water Supply Corporation. The municipality of residence for cancer cases and controls was presumed to be the source of the subject's NO_3 and Mg exposure via drinking water. In the analysis, the subjects were categorised into tertiles of NO_3 exposure. Conditional logistic regression was used to estimate associations, adjusting for age, gender, marital status and urbanisation level of residence. Relative to individuals whose NO_3 exposure level was ≤ 1.68 mg/L, the adjusted OR (95% CI) for oesophageal cancer death was 1.05 (95% CI :0.91–1.19, p_{trend} = 0.79) for individuals who resided in municipalities served by drinking water with a NO_3 exposure > 2.92 mg/L. Evidence of an interaction was noted between drinking water NO_3 and Mg intake: the OR for those with highest tertile of nitrate and lowest half of Mg intake was 1.27 (95% CI : 1.03–1.57) compared to those in the lowest tertile of nitrate and highest half of Mg intake. Although including a large number of cases and controls, the study lacked information on nitrate intake from food, and the consumption volume of water was also not known in this study.

Ecological studies

Zhang et al. (2012) investigated, in China, the association between nitrogen compounds in drinking water with the incidence of ESCC in an ecological study by geographical spatial analysis. The incidence of ESCC is high in Shexian county, China. The study focuses on three nitrogen compounds in drinking water, namely nitrate, nitrite and ammonia, all of which are derived mainly from domestic garbage and agricultural fertiliser. The study surveyed 48 villages in the Shexian area with a total population of 54,716 subjects (661 ESCC cases). Logistic regression analysis was used to detect risk factors for ESCC incidence. Most areas with high concentrations of nitrate nitrogen in drinking water had a high

incidence of ESCC. Correlation analysis revealed a significant relationship between nitrate concentration and ESCC (r = 0.38, p = 0.01), but not with nitrite.

Summary

In a previous review, which considered publications until 2006 (IARC, 2010), one case–control study was described (Rogers et al., 1995) that reported a significantly inverse association between oesophageal cancer and ingested nitrate. Subsequently, two new cohort studies, two case–control studies, and one ecological study were published, often on oesophageal cancer subtypes. The cohort and case–control studies generally used multivariable analyses to adjust for confounders. Two cohort studies looked into histological subtypes. For ESCC and EAC, no significant associations were found with total nitrate (food, drinking water) (Keszei et al., 2013), nor with nitrate intake from meat (Cross et al., 2011). The two case–control studies did not find a significant association with nitrate from food or drinking water. The ecological study in China (Zhang et al., 2012) reported a statistically significant positive association between ESCC incidence and nitrate levels in drinking water.

Overall, the Panel noted that the information is still sparse for oesophageal cancer but, based on the stronger study designs, there was no evidence for an association with ingested nitrate and oesophageal cancer or its subtypes, oesophageal squamous cell carcinomas and oesophageal adenocarcinoma (ESCC and EAC).

3.6.8.3. Gastric cancer

Cohort studies

Cross et al. (2011) studied the relationship between intake of meat, meat components, and meat cooking by-products and risk of gastric cancer subtypes. The NIH-AARP Diet and Health Study recruited men and women, aged 50–71 years, from six states in the United States. At baseline (1995–1996), participants completed self-administered demographic and lifestyle questionnaires, including a 124-item FFQ. Approximately 6 months later, cancer-free participants were mailed a risk factor questionnaire, which elicited detailed information on meat intake and cooking preferences. A total of 566,402 participants returned the baseline questionnaire and 337,074 of these also returned the risk factor questionnaire. They estimated nitrate and nitrite intake from processed meats using a database of measured values from 10 types of processed meats (bacon, red meat sausage, poultry sausage, red and white luncheon meats, red and white cold cuts, ham, hot dogs), which represent 90% of processed meats consumed in the United States; these meats were also measured for NOCs, although they were all below the detectable limit. After excluding prevalent cancer cases, and those with implausible nutrient values, the baseline analytical cohort consisted of 494,979 persons (295,305 men and 199,674 women), and the risk factor questionnaire cohort consisted of 303,156 persons (176,842 men and 126,314 women). During 10 years of follow-up, they accrued 454 gastric cardia adenocarcinomas, and 501 gastric non-cardia adenocarcinomas. In the subcohort of participants who returned the risk factor questionnaire (126,314 females and 176,842 males), there were 255 gastric cardia cancers and 277 gastric non-cardia cancers. Cox PH modelling was used as analysis technique. After controlling for age, race, total energy intake, smoking, family history of cancer, family history of diabetes, BMI, physical activity, alcohol, saturated fat, fruits and vegetables intake, red meat intake was not associated with gastric (cardia or non-cardia) cancer. The malignancies investigated in this study were not found to be associated with white or processed meat. Heterocyclic amine intake was seen to be positively associated with malignancies; when individuals in the high quintile were compared with the low quintile, an increased risk for cardia cancer (HR = 1.44, 95% CI: 1.01–2.07) was found. No association was found between nitrate or nitrite intake from meat and gastric cancer subtypes. Comparing highest vs lowest quintile of intake, the results for nitrate from meat and gastric cardia adenocarcinoma (GCA) were: RR Q5 (median 0.298 mg/1,000 kcal) vs Q1 (0.024) = 0.81, 95% CI: 0.52–1.25, p_{trend} = 0.259; and for nitrate and gastric non-cardia adenocarcinoma (GNCA) were: RR = 1.04, 95% CI: 0.69–1.55, p_{trend} = 0.578. Comparing highest vs lowest quintile of intake, the results for nitrite from meat and GCA were: RR: 0.71, 95% CI: 0.47–1.08, p_{trend} = 0.250; and for nitrite and GNCA were: RR Q5 (med 0.199 mg/1,000 kcal) vs Q1 (0.012) = 0.93, 95% CI: 0.63–1.37, p_{trend} = 0.615. None of the quintile HR estimates were significantly different from one, nor were any tests for trend, or continuous analyses. Strengths of the study include the large size enabling the investigation of tumour subtypes. The study lacked information on nitrate and nitrite intake from other foods, and nitrate intake from drinking water.

Keszei et al. (2013) studied the relationship between risks of gastric cancer subtypes and intake of NDMA, ENOC, haem iron, nitrite and nitrate in the Netherlands Cohort Study. This prospective cohort study started in September 1986; at baseline, 58,279 men and 62,573 women aged 55–69 years were recruited from 204 municipal population registries throughout the Netherlands. At baseline (1986), a self-administered questionnaire was completed by study participants on dietary habits and other risk factors of cancer. The dietary part consisted of a validated 150-item food frequency questionnaire. The cohort was followed for 16.3 years, and 166 GCA and 497 GNCA cases were analysed along with 4,032 subcohort members in a case–cohort analysis. To calculate intakes, food composition values for nitrate and nitrite were obtained from analyses conducted by Dutch institutes in the period 1984–1989. Information about nitrate content in drinking water from all pumping stations in the Netherlands in 1986 was used to determine the nitrate concentration in drinking water for each home address by postal code, and calculate nitrate intake from water (i.e. water, coffee, tea and soup combined). Nitrite intake was assessed solely on the intake of processed meat as the nitrite content of vegetables and cheese was considered negligible in comparison with processed meat. Considered processed meat items were: all types of sausages, bacon, ham, cold cuts, croquettes, and frankfurters. NDMA values in food items were initially extracted from published measurements in Dutch foods in the 1970s and 1980s. For food items for which NDMA values were not available from Dutch sources, but nonzero content was indicated in a comprehensive food composition database of nitrosamines (Jakszyn et al., 2004a,b), measurements made in food sources from Western or Northern Europe in the 1980s were used. An index of ENOC was calculated, as previously determined by Jakszyn et al. (2006), based on the haem iron intake. The correlation between ENOC and haem iron was 0.97. In the sex-specific statistical analyses, Cox PH modelling was used, controlling for age, smoking, BMI, educational level, energy intake, vegetable and fruit intake, total alcohol intake; in NDMA-analyses, adjustment was made for alcoholic beverages excluding beer, because beer was an important source of NDMA. The estimated mean (SD) values of intake in the subcohort (a random sample of the Netherlands Cohort Study) were for men and women, respectively: nitrate: 108 (SD 45) and 106 (44) mg/day, nitrite: 0.12 (0.16) and 0.08 (0.12) mg/day, NDMA (median): 0.084 and 0.044 μg/day, ENOC: 102 (25) and 93 (23) μg/day. When comparing men in the highest vs lowest tertile of intake, the results for nitrite and GCA were: HR T3 (median 0.28 mg/day) vs T1 (0.03) = 1.18, 95% CI: 0.75–1.86, p_{trend} = 0.34; and for nitrite and GNCA were: HR = 1.23, 95% CI: 0.89–1.70, p_{trend} = 0.20. None of the tertile HR estimates were significantly different from one, nor were any tests for trend, or continuous analyses. In nitrite analyses in women, none of the tertile HR estimates were significantly different from one, nor were any tests for trend, or continuous analyses. For nitrate, in both men and women, none of the tertile HR estimates were significantly different from one, nor were any tests for trend, or continuous analyses. For NDMA intake, when comparing men in the highest vs lowest tertile of intake, the results for NDMA and GCA were: HR T3 (med 0.25 μg/day) vs T1 (0.04) = 0.94, 95% CI: 0.59–1.49, p_{trend} = 0.75; and for NDMA and GNCA were: HR = 1.31, 95% CI: 0.95–1.81, p_{trend} = 0.09. For GNCA, HR estimates were significant in continuous analyses: HR = 1.06, 95% CI: 1.01–1.10 per 0.1 μg/day NDMA increment. In women, when comparing the highest vs lowest tertile of NDMA intake, the results for GCA were: HR T3 (0.07 μg/day) vs T1 (0.03)= 1.02, 95% CI: 0.33–3.14, p_{trend} = 0.96; and for NDMA and GNCA, these were: HR = 0.90, 95% CI: 0.58–1.42, p_{trend} = 0.49. For gastric cancer subsites, no significant associations were seen with haem iron or ENOC in men or women in multivariate analyses. These results suggest that NOCs may influence the risk of GNCA in men, although there are no clear associations for GCA. The strengths of the study include the large size and long follow-up enabling the investigation of tumour subtypes. A potential weakness is the single measurement of diet, only at baseline. Nitrite intake was assessed solely on the intake of processed meat, but nitrite content of vegetables and cheese was considered negligible in comparison with processed meat.

Case–control studies

Ward et al. (2008) conducted a population-based case–control study of adenocarcinoma of the distal stomach in Nebraska, US. Cases were white men and women aged 21 years or older, newly diagnosed between 1988 and 1993, identified from the Nebraska Cancer Registry and confirmed by histological review. Controls were selected from a previous population-based case–control study of lymphatic and haematopoietic cancers in Nebraska, and were re-interviewed at the time of this study (1992–1994). Response rates were between 79% and 88%. Telephone interviews were conducted with subjects or their proxies for those who were deceased or too ill to participate. Proxy interviews were conducted for 80%, and 61% of stomach cancer cases and controls, respectively. Nitrate

concentrations in public drinking water supplies were linked to residential water source histories. Among those using private wells at the time of the interview, they measured nitrate levels in water samples from wells. Dietary nitrate and nitrite were estimated from a food-frequency questionnaire. They estimated ORs and 95% CI using unconditional logistic regression, adjusting for gender, year of birth, and risk factors for oesophagus cancer (smoking, alcohol, BMI). Among those who primarily used public water supplies (79 distal stomach cancer cases, 321 controls), average nitrate levels were not associated with risk (highest vs lowest quartile: stomach OR = 1.2, 95% CI 0.5–2.7, p_{trend} = 0.946). They observed the highest ORs for distal stomach cancer among those with higher water nitrate ingestion and higher intake of processed meat compared with low intakes of both; however, the test for positive interaction was not significant (p = 0.213). Increasing intake of nitrate plus nitrite from animal sources (details NA) was associated with elevated ORs for stomach cancer, but not significantly (OR Q4 (> 8.3 mg) vs Q1 (< 3.8): 1.6, 95% CI: 0.7–3.7, p_{trend} = 0.352) and not with nitrite from plant sources (median intake 0.52 mg/day). Increasing intake of nitrate from plant sources (median intake NO_3 = 116.1 mg/day) was not associated with stomach cancer (OR Q4 (> 171.9 mg) vs Q1 (< 74.9) 1.6, 95% CI: 0.7–3.6, p_{trend} = 0.266). The number of cases is small in this study. Although there was a relatively high response rate, the percentage of proxy interviews is high, which could have led to information bias.

Kim et al. (2007) studied whether the intake of nitrate relative to antioxidant vitamin rather than absolute intake of nitrate affects the risk of gastric cancer. In a case–control study in Korea using a 84-item FFQ, trained dieticians interviewed 136 gastric cancer cases (1997–98) and an equal number of hospital controls. Controls were selected from patients who had visited one of the clinics of orthopaedic surgery, ophthalmology, dermatology, plastic surgery or family medicine by matching sex and age (± 2 years) in the same hospital. To avoid a biased control selection, they also used gastrofiberscopy to confirm that the controls had no severe stomach problems. They estimated the individual daily nitrate intake from foods using the nitrate database reported recently for Korea. As an index of nitrate intake relative to antioxidant vitamins intake, they calculated the nitrate:antioxidant vitamin consumption ratio. Unconditional logistic regression was used to calculate ORs and the corresponding 95% CIs, adjusted for age, sex, socioeconomic status, family history of gastric cancer, duration of refrigerator use, and *H. pylori* infection, several foods, such as charcoal grilled beef, spinach, garlic, mushrooms, and a number of types of kimchi, which had exhibited a significant association with gastric cancer risk in their previous studies. The median daily nitrate intake from foods was very high: 458 mg/day in controls. Higher absolute intake of nitrate was not associated with gastric cancer risk (OR T3 (811 mg) vs T1 (240 mg) = 1.13, 95% CI: 0.42–3.06, p_{trend} = 0.842). However, the gastric cancer risk increased as the nitrate: antioxidant vitamin consumption ratio increased, particularly with a higher nitrate:vitamin E ratio and nitrate:folate ratios. It was concluded that gastric cancer risk was influenced by the intake of nitrate relative to antioxidant vitamins. The number of cases is small in this study. Response rates among cases and controls were not given, and so selection bias is not unlikely. Nitrate intake seems to be extremely high, but based on only an 84-item FFQ.

Hernández-Ramírez et al. (2009) conducted a case–control study in Mexico on gastric cancer in relation to the individual and combined consumption of polyphenols and NOC precursors (nitrate and nitrite). A population-based case–control study was carried out in Mexico City from 2004 to 2005 including 257 histologically confirmed gastric cancer cases and 478 controls, who were at least 20 years old. Cases were recruited in nine of the main tertiary care hospitals in Mexico City, where 60% of the GC cases are diagnosed; response rate was 97.7%. For each case, up to two healthy controls without a history of cancer, who resided in the same geographical area as the cases, were selected and matched to the cases by age (± 5 years) and gender; response rate was 94.3%. Intake of polyphenols, nitrate and nitrite were estimated using a 127-item FFQ. The nitrate and nitrite content for foods in this study was obtained from several non-Mexican sources (Europe, Korea). ORs and 95% CIs were estimated using unconditional logistic regression analysis, adjusting for age, gender, energy, schooling, *H. pylori* CagA status, chilli consumption, salt consumption, alcohol intake and, in additional models, vitamin C, vitamin E, fruits and vegetables, and polyphenols. Total nitrate was significantly inversely associated with gastric cancer risk (OR T3 (> 141.7 mg/day) vs T1 (< 90.4): 0.61, 95% CI: 0.39–0.96, p_{trend} = 0.035). Total nitrite was borderline significantly associated with gastric cancer risk (OR T3 (> 1.2 mg/day) vs T1 (< 1.0): 1.52, 95% CI 0.99–2.34, p_{trend} = 0.052). However, both nitrate from animal (not specified) sources (OR T3 (> 3.9 mg/day) vs T1 (< 1.7): 1.92, 95% CI 1.23–3.20, p_{trend} = 0.004) and nitrite from animal (details NA) sources (OR T3 (> 0.4 mg/day) vs T1 (< 0.2): 1.56, 95% CI: 1.02–2.4, p_{trend} = 0.030) were positively associated with gastric cancer risk. ORs around

two-old were observed among individuals with both low intake of cinnamic acids, secoisolariciresinol or coumestrol and high intake of animal-derived nitrate or nitrite, compared to high intake of the polyphenols and low animal nitrate or nitrite intake, respectively. Results were similar for both the intestinal and diffuse types of gastric cancer. The response rates among cases and controls are high, but the study lacked information on nitrate and nitrite in Mexican foods, and lacked information on nitrate from water, and did not present information on which animal foods the nitrate and nitrite estimates are based on.

Chiu et al. (2012) explored whether Ca and Mg levels in drinking water modified the effects of nitrate on gastric cancer mortality. A matched cancer case–control study was used to investigate the relationship between the risk of death from gastric cancer and exposure to nitrate in drinking water in Taiwan. All gastric cancer deaths of Taiwan residents from 2006 through 2010 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health. Controls were deaths from other causes and were pair-matched to cancer cases by gender, year of birth and year of death. In total, there were 2,832 cases and 2,832 controls. Information on the levels of nitrate (NO_3) and Ca and Mg (water hardness) in drinking water were collected from the Taiwan Water Supply Corporation. The municipality of residence for cancer cases and controls was presumed to be the source of the subject's NO_3 and Mg exposure via drinking water. In the analysis, the subjects were categorised into tertiles of NO_3 exposure. Conditional logistic regression was used to estimate associations, adjusting for age, gender, marital status and urbanisation level of residence. Relative to individuals whose NO_3 exposure level was < 1.68 mg/L (median), the adjusted OR (95% CI) for gastric cancer death was 1.16 (95% CI: 1.05–1.29) for individuals who resided in municipalities served by drinking water with a NO_3 exposure > 1.68 mg/L. Evidence of an interaction was noted between drinking water NO_3 and Ca and Mg intake: the OR for those with highest half of nitrate and lowest half of Ca intake was 1.70 (95% CI: 1.43–2.03) compared to those in the lowest half of nitrate and highest half of Ca intake ($P_{interaction} < 0.05$). The OR for those with highest half of nitrate and lowest half of Mg intake was 1.49 (95% CI: 1.24–1.80) compared to those in the lowest half of nitrate and highest half of Mg intake ($P_{interaction} < 0.05$). Thus, effect of nitrate seems higher in softer drinking water. Although a large number of cases and controls, the study lacked information on nitrate intake from food, and the consumption volume of water was also not known in this study.

Ecological studies

No studies available.

Summary

In a previous review, which considered publications until 2006 (IARC, 2010), one cohort study, two case–control studies and 15 ecological studies were described on nitrate in drinking water and gastric cancer. There was no clear evidence for an association from these studies. Two cohort studies and seven case–control studies were described on nitrate from food and stomach cancer risk. None of these studies found a positive association; one study reported an inverse association.

Subsequently, two new cohort studies, and four case–control studies were published, often on gastric cancer subtypes. The cohort and case–control studies generally used multivariable analyses to adjust for confounders. Two cohort studies looked into histological subtypes. For GCA and GNCA, no significant associations were found with total nitrate (food, drinking water) intake (Keszei et al., 2013; total 663 cases), nor with nitrate intake from meat (Cross et al., 2011; total 955 cases).

Although one case–control study in the United States found no association between distal stomach cancer and nitrate in drinking water or food, or nitrite (Ward et al., 2008), a Mexican case–control study reported significant positive associations with nitrate from animal foods (Hernández-Ramírez, 2009); these foods were not specified and therefore it is difficult to interpret this. In a Korean case–control study with very high nitrate intakes, no association was found with gastric cancer, although the risk increased with increasing ratios of nitrate to antioxidant vitamins (Kim et al., 2007). For gastric cancer death, a positive association with drinking water nitrate levels was reported in Taiwan (Chiu et al., 2012), but there was little control of confounders, the study had no information on total nitrate intake, and water consumption volumes are also unknown.

Overall, the Panel concluded based on the stronger study designs that there was no evidence from cohort studies for a positive association between ingested nitrate and gastric cancer or it subtypes gastric cardia and non-cardia adenocarcinoma (GCA and GNCA).

3.6.8.4. Colorectal cancer (CRC)

Cohort studies

Cross et al. (2010) conducted a large prospective study on colorectal cancer (CRC) risk and meat consumption, investigating several underlying mechanisms. The NIH-AARP Diet and Health Study recruited men and women, aged 50–71 years, from six states in the United States. At baseline (1995–1996), participants completed self-administered demographic and lifestyle questionnaires, including a 124-item FFQ. Approximately 6 months later, cancer-free participants were mailed a risk factor questionnaire, which elicited detailed information on meat intake and cooking preferences. A total of 566,402 participants returned the baseline questionnaire and 337,074 of these also returned to risk factor questionnaire. After excluding prevalent cancer cases, and those with implausible nutrient values, the baseline analytical cohort consisted of 494,979 persons (295,305 men and 199,674 women) and the risk factor questionnaire cohort consisted of 303,156 persons (175,369 men and 125,579 women). The meat and CRC study was limited to the latter group. With the FFQ, meat type (white/red/processed), meat cooking methods and doneness levels were monitored and recorded. Nitrate and nitrite intake from processed meats was estimated based on a database of previously measured content, representing 90% of available processed meats in the United States (bacon, red meat sausage, poultry sausage, red and white luncheon meats, red and white cold cuts, ham, hot dogs). A follow-up period of 7.2 years identified 2,719 incident cases of CRC. In Cox PH models, adjustment was made for gender, education, BMI and smoking, as well as intake of total energy, fibre and dietary Ca. Risk of CRC was positively related to intake of red meat (HR Q5 vs Q1 = 1.24, 95% CI: 1.09–1.42, $p_{trend} < 0.001$) and processed meat intake (HR Q5 vs Q1 = 1.16, 95% CI: 1.01–1.32, $p_{trend} = 0.017$) but inversely with white meat (HR Q5 vs Q1 = 0.85, 95% CI: 0.76–0.97, $p_{trend} = 0.017$). Furthermore, nitrate intake from processed meats had a significant positive association with CRC (HR Q5 (median 289.2 μg/1,000 kcal) vs Q1 (23.9) = 1.16, 95% CI: 1.02–1.32, $p_{trend} = 0.001$), whereas nitrite from processed meats did not (HR Q5 (median 194.1 μg/1,000 kcal) vs Q1 (11.9) = 1.11, 95% CI: 0.97–1.25, $p_{trend} = 0.055$). Assessment of combined intake of nitrate and nitrite from processed meat was associated with a higher risk for CRC (1.14, 95% CI: 1–1.3, p = 0.019), although the data were not shown. Total dietary nitrate intake revealed an inverse association in the highest quintile (data not shown) of dietary nitrate (HR = 0.82, 95% CI: 0.71–0.95, $p_{trend} = 0.111$) but no association for total nitrite (HR = 1.05, 95% CI: 0.92–1.21, $p_{trend} = 0.316$) and CRC.

Ferrucci et al. (2012) investigated the relationship between meat consumption and the risk of incident distal colon and rectal adenoma in the United States. Among male and female participants in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial who underwent baseline and follow-up sigmoidoscopy (n = 17,072), they identified 1,008 individuals with incident distal colorectal adenoma. All participants completed a self-administered baseline questionnaire on demographics, personal and family cancer history, medical history, and lifestyle habits. Participants in the screening arm also completed a 137-item FFQ on usual intake of foods and beverages during the past year. Nitrate and nitrite intake from processed meats was calculated using a database of laboratory measured values of these compounds in 10 types of processed meats representing 90% of processed meats assessed by a typical FFQ in the United States (bacon, red meat sausage, poultry sausage, red and white luncheon meats, red and white cold cuts, ham, hot dogs). They calculated ORs and 95% CIs for associations between meat and meat-related components and incident distal colorectal adenoma using multivariate logistic regression, controlling for age at baseline, study centre, gender, ethnicity, education, family history of CRC, BMI, use of non-steroidal anti-inflammatory drugs, physical activity and smoking status, as well as intakes of alcohol, dietary Ca, supplemental Ca, dietary fibre, and total energy. They observed suggestive positive associations for red meat, processed meat, haem iron, and nitrate/nitrite with distal colorectal adenoma. Comparing the highest intake of nitrate plus nitrite from processed meat compared with the lowest, they observed a nonsignificant positive association (OR Q4 (median 0.84 mg/1,000 kcal) vs Q1 (0.06) = 1.22, 95% CI: 0.94–1.53, $p_{trend} = 0.14$). This is one of the few studies on colorectal adenoma, although total dietary intake of nitrate and nitrite was not evaluated, and the study lacked information on nitrate from water.

Dellavalle et al., (2014) investigated the association between dietary nitrate and nitrite intake and risk of CRC in the Shanghai Women's Health Study, a cohort of 73,118 women ages 40–70 years, residing in Shanghai. Nitrate, nitrite and other dietary intakes were estimated from a 77-item FFQ administered at baseline. Over a mean of 11 years of follow-up, they identified 619 CRC cases (383

colon and 236 rectum). HR and 95% CI were estimated using Cox proportional hazard regression, adjusting for age, total energy intake, education, physical activity, dietary vitamin C intake, carotene and folate. The median daily intakes of dietary nitrate and nitrite were 300.7 mg (interquartile range = 214.5–412.5 mg) and 1.4 mg (interquartile range = 1.1–1.8 mg), respectively. The majority of dietary nitrate intake (median 298.6 mg/day) and nitrite intake (median 1.2 mg/day) was from plant sources. Total nitrate intake was not associated with CRC risk (HR Q5 (median 313.2 mg/day) vs Q1 (98.7) = 1.08, 95% CI: 0.73–1.59, p_{trend} = 0.39). Also, total nitrite intake was not associated with CRC risk (HR Q5 (median 1.23 mg/day) vs Q1 (0.56) = 1.05, 95% CI: 0.77–1.42, p_{trend} = 0.78). Among women with vitamin C intake below the median (83.9 mg/day), and hence a higher potential exposure to NOCs, the risk of CRC increased with increasing quintiles of nitrate intake (HR Q5 vs Q1 = 2.45, 95% CI: 1.15–5.18, p_{trend} = 0.02). There was no association with nitrate among women with a higher vitamin C intake (HR Q5 vs Q1 = 0.93, 95% CI: 0.44–1.96, p_{trend} = 0.69). They found no association between nitrite intake and risk of CRC overall or by intake level of vitamin C. The findings suggest that a high dietary nitrate intake among subgroups expected to have higher exposure to endogenously formed NOCs increases risk of CRC. This Chinese population seems to be exposed to much higher nitrate intake levels than in Europe or North America. Nitrate intake from drinking water sources was not considered in their assessment of exposure because they determined that exposures from tap water in Shanghai were low.

Case–control studies

Yang et al. (2007) investigated the relationship between nitrate in the public water supply and colon cancer mortality in a case–control study. Data on all deaths of Taiwan residents between 1999 and 2003 was provided by the provincial health department statistics bureau. The case group consisted of all eligible colon cancer deaths occurring in individuals between 50 and 69 years of age. In total, 2,234 cases were included; of the 2,234 cases, 1,310 were males and 924 were females. The control group consisted of all other deaths, excluding those deaths that were associated with gastrointestinal disease, head and neck cancer, lung, bladder and NHL. Controls were pair-matched to the cases by gender, year of birth and year of death. Detailed demographic information and residential district were recorded. Information on the levels of nitrate (NO_3) in drinking water was collected from the Taiwan Water Supply Corporation. The municipality of residence for colon cancer cases and controls was presumed to be the source of the subject's NO_3 exposure via drinking water. Controls had a mean NO_3 exposure of 1.95 mg/L (SD 1.95). In the analysis, the subjects were categorised into tertiles of NO_3 exposure. Conditional logistic regression was used to estimate associations, adjusting for age, gender, Ca levels in drinking water and urbanisation level of residence. Relative to individuals whose NO_3 exposure level was ≤ 0.97 mg/L (median 0), the adjusted OR (95% CI) for colon cancer death was 0.98 (0.83–1.16, p_{trend} = 0.22) for individuals who resided in municipalities served by drinking water with a NO_3 exposure > 2.13 mg/L (median 3.19, highest tertile). However, the study lacked information on nitrate intake from food, and the consumption volume of water was also not known.

Ward et al. (2007a) studied the associations of processed meat intake and associated compounds and risk of colorectal adenoma. They conducted a case–control study of 146 cases of colorectal adenoma, diagnosed at sigmoidoscopy or colonoscopy, and 228 polyp-free controls in Maryland. Response rates were 84% for cases and 74% for controls. Using unconditional logistic regression (adjusting for age, gender, pack-years of smoking and total caloric intake), they calculated ORs and found a positive association with processed meat (bacon, breakfast sausage, hot dogs/other sausage, ham steaks/pork chops, ham, bologna, salami and other luncheon meats and liverwurst) intake (OR Q4 vs Q1 = 2.0, 95% CI: 1.0–4.0, no p_{trend}). They estimated nitrate and nitrite intake from meat using published data from the literature as well as from actual measurements of meats analysed recently. No significant associations were found for nitrite from meats (OR Q4 (> 0.16 mg/day) vs Q1 (< 0.02) = 1.7, 95% CI: 0.9–3.2, no p_{trend}) but, for nitrite plus nitrate from meats, it reached significance (OR Q4 (> 0.48 mg/day) vs Q1 (< 0.09) = 2.0, 95% CI: 1.0–3.9, no p_{trend}). Additional adjustment for the heterocyclic amine (HCA), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) attenuated the association (OR = 1.6, 95% CI: 0.8–3.2) but other HCA and polycyclic aromatic hydrocarbons (PAHs) had minimal effect. Higher CYP2A6 activity was not associated with risk and there was no evidence of an interaction of CYP2A6 activity with nitrate and nitrite intake. The results suggest that nitrite and nitrate intake from processed meat intake increases the risk of colorectal adenoma after accounting for HCA and PAH. Response rates for cases and controls were relatively high, reducing the likelihood of selection bias. However, retrospective information was used for dietary

intake. The study lacked information on total nitrate and nitrite intake from food, and the consumption volume of water was also not known.

Kuo et al. (2007) investigated the relationship between nitrate in the public water supply and risk of death due to rectal cancer in a case–control study. Data on all deaths of Taiwan residents between 1999 and 2003 was provided by the provincial health department statistics bureau. The case group consisted of all eligible rectal cancer deaths occurring in individuals between 50 and 69 years of age. In total, 1,118 cases were included (456 females and 662 males). The control group consisted of all other deaths, excluding those deaths that were associated with gastrointestinal disease, head and neck cancer, lung, bladder and NHL. Controls were pair-matched to the cases by gender, year of birth, and year of death. Detailed demographic information and residential district were recorded. Information on the levels of nitrate (NO_3) in drinking water was collected from the Taiwan Water Supply Corporation. The municipality of residence for CBT cases and controls was presumed to be the source of the subject's NO_3 exposure via drinking water. In the analysis, the subjects were categorised into tertiles of NO_3 exposure. Conditional logistic regression was used to estimate associations, adjusting for age, gender, Ca levels in drinking water and urbanisation level of residence. Relative to individuals whose NO_3 exposure level was $\leq$ 0.80 mg/L (median 0), the adjusted OR (95% CI) for rectal cancer death was 1.36 (1.08–1.70, p_{trend} = 0.02) for individuals who resided in municipalities served by drinking water with a NO_3 exposure > 2.13 mg/L (median 3.19, highest tertile). However, the study lacked information on nitrate intake from food, and the consumption volume of water was also not known.

McElroy et al. (2008) investigated the association between nitrate exposure from drinking water and CRC risk in a population-based case–control study of 475 women aged 22–74 years with CRC, and 1,447 community controls (source: list of motor vehicle drivers and Medicare beneficiaries) living in rural Wisconsin, United States. Response rates among cases were 80% and 85% among controls. Drinking water nitrate exposure levels were interpolated to subjects' residences based on measurements that had taken place as part of a separate water quality survey in 1994. Information on selected risk factors for CRC was collected in a structured telephone interview. Logistic regression models were used to estimate the risk of CRC in the study, after adjusting for age, family history of CRC, CRC screening, smoking status and interview period. In the statistical analysis, there was no significant positive association between overall CRC and nitrate concentration in water: OR highest (> 44.3 mg/L) vs lowest (< 2.21 mg/L) category was 1.57 (95% CI: 0.97–2.52, no p_{trend}). However, for proximal colon cancer, this association was significant (OR = 2.76, 95% CI: 1.42–5.38, no p_{trend}). No significant association was observed for distal colon (OR = 1.23) or rectal cancer risk (OR = 1.26). A weakness of the study is the limited control for confounders; dietary nitrate or nitrite was not considered, and the consumption volume of water was also not known.

Chiu et al. (2010) explored whether Mg levels in drinking water modify the effects of nitrate on colon cancer mortality. A matched case–control study was used to investigate the relationship between the risk of death from colon cancer and exposure to nitrate in drinking water in Taiwan. All colon cancer deaths (n = 3,707) of Taiwan residents from 2003 to 2007 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health. Controls (n = 3,707) were deaths from non-gastrointestinal diseases, and were pair-matched to the cases by gender, year of birth and year of death. Information on the levels of nitrate (NO_3) and Mg in drinking water was collected from the Taiwan Water Supply Corporation. The municipality of residence for cases and controls was assumed to be the source of the subject's NO_3 and Mg exposure via drinking water. In the analysis, the subjects were categorised into three categories of NO_3 exposure (bottom two quartiles combined (low); 3rd quartile (medium); 4th quartile (high). Conditional logistic regression was used to estimate associations, adjusting for age, gender, marital status and urbanisation level of residence. Relative to individuals whose NO_3 exposure level was < 1.67 mg/L, the adjusted OR (95% CI) for colon cancer death was 1.16 (95% CI: 1.04–1.30) for individuals who resided in municipalities served by drinking water with a NO_3 exposure > 2.67 mg/L. There was a suggestion of interaction between drinking water nitrate and Mg intake, in that individuals with the highest NO_3 exposure and low Mg intake from drinking water had a 1.47-fold increased risk (95% CI: 1.21–1.47) of colon cancer, whereas those with the highest NO_3 exposure whose drinking water Mg intake was above the median had no statistically significant increased risk (OR = 1.04). Although the study has a large number of cases and controls, the study lacked information on nitrate intake from food, and the consumption volume of water was also not known.

Miller et al. (2013) studied associations between meat-related compounds and CRC risk by subsite in a population-based case–control study in Pennsylvania. All newly diagnosed cases, identified within

15 months of diagnosis, with histologically confirmed colon or rectal cancers were identified between June 2007 and May 2011 from the Pennsylvania State Cancer Registry. Controls residing in the same 19 county region were identified by random digit dialling. Of those contacted, 57% of eligible cases and 51% of eligible controls participated in the study. Participants (989 cases and 1,033 healthy controls) completed a 137-item FFQ with a meat-specific module. Within this module, the processed red meat category included bacon, sausage, cold cuts (ham, bologna, salami, pepperoni, beef luncheon meat, dried or chipped beef), beef jerky, corned beef, hot dogs, ham, and processed meat added to mixed dishes such as pizza. Multivariable logistic regression was used to examine associations between meat variables and CRC, adjusting for age, sex, total energy intake, BMI, fruit and vegetable intake, and past regular non-steroidal anti-inflammatory drug use. Among other compounds (meat mutagens), significant positive associations were observed for the intake of nitrites plus nitrate and proximal colon cancer (OR Q5 (> 496.6 μg/kcal) vs Q1 (< 114.6) = 1.57, 95% CI: 1.06–2.34, p_{trend} = 0.023). For CRC, this was not significant (OR Q5 vs Q1 = 1.19, 95% CI: 0.87–1.61, p_{trend} = 0.189), nor for total colon, rectal and distal rectal cancer. The response rates among case and controls were low, total dietary intake of nitrate and nitrite was not evaluated, the study lacked information on nitrate from water, and no separate data on nitrate and nitrite are presented. Retrospective information was used for dietary intake, increasing the likelihood of recall bias.

Zhu et al. (2014) examined the association between nitrate, nitrite and NOC intake, and CRC risk and possible effect modification by vitamins C and E and protein in a case–control study carried out in Newfoundland and Labrador and Ontario, Canada. Cases (identified from familial CRC registries in Newfoundland and Ontario) were diagnosed from 1999 to 2003 and were aged 20–74 years. Controls consisted of a random sample of each provincial population aged between 20 and 74 years who were selected using random digit dialling. Controls were frequency-matched with cases on sex and 5-year age strata. The overall response rates for the study were 65.0% for cases and 53.5% for controls. A total of 1,760 cases with pathologically confirmed adenocarcinoma and 2,481 population controls were asked to complete a 170-item self-administered FFQ to evaluate their dietary intakes 1 year before diagnosis (for cases) or interview (for controls). Median daily intake (among controls) was 124.8 mg for nitrate, 1.12 mg for nitrite and 0.20 μg for NDMA. Adjusted OR and 95% CI were calculated across the quintiles of nitrate, nitrite and NDMA intake and relevant food items using unconditional logistic regression. Multivariate adjustment was made for age, sex, total energy intake, BMI, cigarette smoking, alcohol consumption, physical activity, education attainment, household income, reported colon screening procedure, non-steroidal anti-inflammatory drug use, multivitamin supplement use, folate supplement use and province of residence. NDMA intake was found to be associated with a higher risk of CRC (OR Q5 (median 2.29 μg/day) vs Q1 (0.03) = 1.42, 95% CI: 1.03–1.96, p_{trend} = 0.005), specifically for rectal carcinoma (OR Q5 vs Q1 = 1.61, 95% CI 1.11–2.35, p_{trend} = 0.01). Nitrate intake was not associated with CRC (OR Q5 (median 264.14 mg/day) vs Q1 (56.94) = 0.89, 95% CI 0.68–1.16, p_{trend} = 0.43), nor was nitrite (OR Q5 (median 1.92 mg/day) vs Q1 (0.65) = 1.09, 95% CI 0.77–1.54, p_{trend} = 0.66), or with subsites. There was evidence of effect modification between dietary vitamin E and NDMA. Individuals with high NDMA and low vitamin E intakes had a significantly higher risk compared to those with both low NDMA and low vitamin E intakes (OR = 3.01, 95% CI 1.43–6.51, $P_{interaction}$ = 0.017). There was no interaction with vitamin C. The present results support the hypothesis that NOC intake may be positively associated with CRC risk in humans. Vitamin E, which inhibits nitrosation, could modify the effect of NDMA on CRC risk. The response rates among controls were low, the study lacked information on nitrate from water. Retrospective information was used for dietary intake, increasing the likelihood of recall bias.

Summary

In a previous review, which considered publications until 2006 (IARC, 2010), two cohort studies (Knekt et al., 1999; Weyer et al., 2001) and one case–control study (de Roos et al., 2003) on nitrate from diet and drinking-water were described. No association was found with dietary or drinking water nitrate in the Finnish and US cohort studies, or with dietary nitrate in the US case–control study, whereas, in the case–control study, a positive association with average nitrate levels in drinking water was only found among those with relatively low vitamin C intake.

Subsequently, three new cohort studies and seven case–control studies were published on colorectal carcinoma or adenoma. The cohort and case–control studies generally used multivariable analyses to adjust for confounders. One US cohort study (Cross et al., 2010) found that total dietary nitrate intake was significantly inversely associated with CRC risk. Nitrate from processed meats was significantly positively associated with CRC risk. In a cohort study among women from China, total

nitrate intake was not associated with CRC risk (Dellavalle et al., 2014). Among those with vitamin C intake below the median, there was a significant positive association between nitrate intake and CRC. However, it must be noted that the nitrate intake in the Chinese cohort was much higher than seen in Western cohorts: a median of around 300 mg/day in China vs values of around 100 and lower in European and American cohorts. In a US cohort study on risk of colorectal adenoma, no association was found with nitrate plus nitrite from processed meat (Ferrucci et al., 2012).

For total dietary nitrate, no association with CRC risk was seen in a case–control study (Zhu et al., 2014). One case–control study reported a statistically significant positive association between proximal colon cancer and nitrate plus nitrite from meats but not for other subsites; however, the response rates were low (Miller et al., 2013). One case–control study reported a significant positive association between colorectal adenoma and nitrate plus nitrite from meats but not for nitrite from meats (Ward et al., 2007a). Regarding drinking water nitrate levels, three case–control studies on CRC deaths were reported from Taiwan; for colon cancer death, one found no association (Yang et al., 2007), whereas another reported a statistically significant positive association (Chiu et al., 2010); for rectal cancer death, a significant positive association with drinking water nitrate was found (Kuo et al., 2007). However, these studies have no information on total nitrate intake, and water consumption volumes are also unknown. In a US case–control study on drinking water nitrate levels with limited control for confounders, a significant positive association was found with proximal colon cancer, but not with rectum cancer or CRC overall (McElroy et al., 2008). Dietary nitrate was not measured.

Overall, the Panel concluded based on the cohort studies that: there was no evidence for a positive association between dietary nitrate and colorectal cancer (CRC) or its subtypes; there was insufficient evidence that ingested nitrate from processed meat is associated with increased risk of CRC or its subtypes based only on one large cohort study; there was insufficient evidence for an association between drinking water nitrate and CRC.

3.6.8.5. Liver cancer

Cohort studies

Freedman et al. (2010) investigated the relationship between meat and associated exposures with hepatocellular carcinoma (HCC) incidence (n = 338) in 495,006 men and women of the NIH-AARP Diet and Health Study (295,332 men and 199,674 women). At baseline (1995 to 1996), participants completed self-administered demographic and lifestyle questionnaires, including a 124-item FFQ. Approximately 6 months later, cancer-free participants were mailed a risk factor questionnaire that elicited detailed information on meat intake and cooking preferences. A total of 566,402 participants returned the baseline questionnaire and 337,074 of these also returned the risk factor questionnaire. Follow-up ended on 31 December 2003). They estimated nitrate and nitrite intake from processed meats using a database of measured values from 10 types of processed meats, which represent 90% of processed meats consumed in the United States. HRs and 95% CIs for the fifth (Q5) vs the first (Q1) quintile were estimated from multivariable adjusted Cox proportional hazards regression models, adjusting for age, sex, total energy, BMI, education, ethnicity, alcohol, cigarette smoking, diabetes, physical activity, and fruit and vegetable intake. They reported inverse associations between white meat and risk of HCC (HR Q5 vs Q1 = 0.52, 95% CI: 0.36–0.77, $p_{trend} < 0.001$). Red meat was associated with a higher risk of HCC (HR Q5 vs Q1 = 1.74, 95% CI: 1.16–2.61, $p_{trend} = 0.024$). Nitrate intake from meat was not associated with risk of HCC (HR Q5 (> 0.22 mg/1,000 kcal) vs Q1 (< 0.05 mg/1,000 kcal) = 1.11, 95% CI: 0.67–1.84, $p_{trend} = 0.81$), nor was nitrite from meat (HR Q5 (> 0.14 mg/1,000 kcal) vs Q1 (< 0.02) = 0.93, 95% CI: 0.55–1.71, $p_{trend} = 0.15$). Strengths of the study include the large size enabling the investigation of liver cancer as one of the very few cohorts. The study lacked information on nitrate and nitrite intake from other foods, and nitrate intake from drinking water.

Summary

One cohort study has investigated the association between intake of nitrate and nitrite from processed meats and risk of hepatocellular carcinoma (Freedman et al., 2010). No significant associations were seen for both nitrate and nitrite in this large study with 338 HCC cases. The study lacked information on nitrate and nitrite intake from other foods, and nitrate intake from drinking water; thus, no definitive conclusions can be drawn because of lack of data. Overall, the Panel concluded that there was no evidence for an association between ingested nitrate and liver cancer, but there are insufficient data to draw conclusions.

3.6.8.6. Pancreatic cancer

Cohort studies

Aschebrook-Kilfoy et al. (2011), investigated dietary exposure to nitrate and nitrite in relation to the risk of pancreatic cancer in a prospective cohort study (NIH-AARP Diet and Health Study). Participants with no previous diagnosis of cancer (303,156), aged 50–71 years, completed a 124-item food frequency. In addition to the main questionnaire, after 6 months, a risk factor questionnaire, which elicited information on intake of meat, meat products and food rich in vitamin C, was mailed to participants. After a follow-up of 10 years, 1,728 incidence cases were identified via linkage to state cancer registries. The mean dietary nitrate intake in the cohort was 88 mg/day (SD 65), and the mean nitrite intake was 1.2 mg/day (SD 0.6). After controlling for age, race, total energy intake, smoking, family history of cancer, family history of diabetes, BMI, saturated fat, folate and vitamin C, no association was found for total intake of nitrate (Q5 median value = 94.8 mg/1,000 kcal vs Q1 median value = 19.3 mg/1,000 kcal), HR = 1.01, 95% CI: 0.85–1.20, p_{trend} = 0.58) and nitrite (Q5 median value = 0.90 mg/1,000 kcal vs Q1 median value = 0.45 mg/1,000 kcal, HR = 0.92, 95% CI: 0.78–1.08, p_{trend} = 0.31) and pancreatic cancer in both man and women. However, among men, an increased risk was observed for nitrate plus nitrite intake from processed meat sources (Q5 median value = 1.43 mg/1,000 kcal vs Q1 median value = 0.11 mg/1,000 kcal; HR = 1.18, 95% CI: 0.95–1.47, p_{trend} = 0.11), although it did not reach statistical significance. Because vitamins C and E and red meat intake affect endogenous formation of NOCs, a stratified analysis by vitamin C intake was conducted in men in the highest quintile of nitrate and nitrite from processed meat who also had a low vitamin C intake and had a higher risk of pancreatic cancer compared to those with a higher vitamin C intake, although these associations were not statistically significant (HR = 1.29, 95% CI: 0.92–1.80) and (HR = 1.12, 95% CI: 0.83–1.51). When the analysis was conducted separately for dietary sources, no increased risk was observed for plant (Q5 median value = 0.68 vs Q1 median value 0.25 mg/1,000 kcal, HR = 0.91, 95% CI: 0.76–1.09, p_{trend} = 0.32) and animal sources (Q5 median value = 0.36 vs Q1 median value 0.10 mg/1,000 kcal, HR = 0.96, 95% CI: 0.82–1.13, p_{trend} = 0.41). In a sensitivity analyses, participants who resided in areas with high nitrate levels in drinking water (≥ 10 mg/L) were excluded from the analysis (2.4% of the study population) and the results did not changed. Regarding nitrate plus nitrite intake during adolescence in man, an increased risk was found for the quintile 2 (median value = 0.65 mg/1,000 kcal) (HR = 1.39, 95% CI: 1.10–1.76), quintile 3 (HR = 1.25, 95% CI: 0.97–1.60), quintile 4 (HR = 1.46, 95% CI: 1.13–1.87) quintile 5 (Q5 = 3.33 mg/1,000 kcal, HR = 1.32, 95% CI: 0.99–1.76, p_{trend} = 0.11) vs Q1 (0.21 mg/1,000 kcal). The strength of the study is the good control for confounding factors and the subdivision of nitrate and nitrites by sources (animal and plants) and the limitations of the study are the high levels of missing information for meat exposure during adolescence leading to information bias (61%). Out of the 1,728 pancreatic cases identified, only 658 men out of 1,055 cases and 397 women out of 625 cases completed a risk factor questionnaire. This could be another limitation of the study.

Ecological

Yang et al. (2009) conducted an ecological study to investigate the relationship between nitrate levels in drinking water and risk of pancreatic cancer in Taiwan. The case group consisted of all eligible pancreatic cancer deaths occurring in individuals between 50 and 69 years of age (mean age 61.2 years) (n = 2,412). The control group consisted of all other deaths excluding those deaths attributed to by malignant neoplasms of stomach, bladder, colon and rectum, lung, oesophagus, head and neck and non-Hodgkin's lymphoma (NHL), and matched to cases by sex, year of birth and year of death (n = 2412). Information on the levels of NO_3–N in each municipality's treated drinking-water supply was obtained from the Taiwan Water Supply Corporation. The mean nitrate concentration in the drinking water of the pancreatic cancer cases was 0.44 and 0.43 mg/L among the controls. No association was found for levels of nitrate exposure (≥ 0.48 mg/L) and pancreatic cancer death (OR = 1.10, 95% CI: 0.96–1.27), after adjusting for age, sex and urbanisation. The limitation of the study is the study design (ecological), which does not allow the assessment of whether there was a true exposure–outcome relationship because there was no individual exposure data (e.g. family history of cancer, dietary nitrate intake). Death by diabetes was included in the control series that might flatten risk estimates towards the null. Moreover, death as an outcome has many limitations (e.g. access to medical care).

Summary

In a previous report (IARC, 2010), one cohort study (Weyer et al., 2001) and three case–control studies (Howe et al., 1990; Baghurst et al., 1991; Coss et al., 2004) were described regarding the association between nitrate intake and pancreatic cancer. No association was reported between dietary intake of nitrate and pancreatic cancer in all four studies. Subsequently, a cohort study conducted in the USA (Aschebrook-Kilfoy et al., 2011) and an ecological study conducted in Taiwan (Yang et al., 2009) were published. No association was found between pancreatic cancer and ingested nitrate from both diet and drinking water. Overall, the Panel concluded that there was no evidence for an association between ingested nitrate and pancreatic cancer.

3.6.8.7. Lung cancer

Case–control studies

No studies on nitrate and lung cancer available.

Summary

One cohort study has investigated the association between intake of nitrate from food and drinking water and the risk of lung cancer (Weyer et al., 2001) and found no association, as was described in the IARC 2010 review. Subsequently, no new studies have been reported.

Overall, the Panel considered that, for lung cancer, information is still too sparse to draw meaningful conclusions, although there was no evidence that nitrate intake is associated with increased risk of lung cancer.

3.6.8.8. Breast cancer

Cohort studies

Inoue-Choi et al. (2012) evaluated in a prospective cohort study (22 years) the interaction of dietary and water nitrate intake with total folate intake on breast cancer risk in the Iowa Women's Health Study. A self-administered FFQ (127 items) and a health and lifestyle questionnaire were completed by 34,388 post-menopausal women (mean age 61.6 years, SD 4.2 years). Nitrate intake from public water was assessed using a historical database on Iowa municipal water supplies. In total, 2,875 incident breast cancers were identified by record linkage with the State Health Cancer Registry of Iowa. The average dietary intakes of nitrate and nitrite were 123.5 and 1.2 mg/day, respectively. No increased risk was found for nitrate (Q5 $\geq$ 165.6 mg/day vs $\leq$ 65.2 mg/day; HR = 0.86, 95% CI: 0.74–1.01, p_{trend} =0.31) or nitrite intake (Q5 $\geq$ 1.5 mg/day vs $\leq$ 0.8 mg/day; HR = 1.05, 95% CI: 0.86–1.29, p_{trend} = 0.28) and cancer risk. A protective effect was found for nitrate-folate ratio (Q5 $\geq$ 0.47 mg/day vs $\leq$ 0.17 mg/day, HR = 0.87, 95% CI: 0.74–1.03; p_{trend} = 0.04) and cancer risk, after controlling for age, total energy intake, education, BMI, WHR, smoking, physical activity, alcohol intake, family history of breast cancer, age of menopause, age at first live birth, oestrogen use, total folate intake (except for nitrate:folate ratio), vitamin C, E, flavonoids, intake of cruciferous and red meat. A statistical interaction was seen between water nitrate intake and total folate intake (p = 0.05). Among women with adequate or higher total folate intake ($\geq$ 400 μg/day), breast cancer risk was statistically significantly increased in women using public water with the highest quintile of nitrate (Q5 $\geq$ 33.5 mg/2 L vs Q1 $\leq$ 2.8 mg/day, HR = 1.40, 95% CI: 1.05–1.87, p_{trend} = 0.04) and in those using private wells (HR = 1.38, 95% CI: 1.05–1.82) compared to those using public water with the lowest quintile of water nitrate intake; whereas, such an association was not observed among women with low total folate intake (< 400 μg/day). A major strength of this study is the large sample size and the number of confounding factors taken into account in the analysis. Limitations are the lack of data regarding individual water consumption and the fact that total folate was given by the summed of diet plus vitamins supplements (70% of people used dietary supplementation).

Case–control

Yang et al. (2010) conducted a hospital case–control study to investigate the relationship between dietary intake of nitrate relative to antioxidant vitamins is associated with breast cancer in women from South Korea. Cases (n = 362) were histologically confirmed and matched to controls (n = 362) by age and menopausal status. Health questionnaires on the family history of cancer, menstrual and reproductive history, exercise, smoking and drinking habits were filled in by all participating patients aged 30–65 years, mean age 46.1 years (SD 8.5) among cases and 46.0 years (SD 8.6) among

controls. In addition, a 121-item quantitative FFQ was used by trained interviewers to assess nutrient intake over the past 12 months. Daily nitrate intake was thereafter estimated by using available nitrate databases of a total of 137 items commonly consumed in Korea, based on the National Survey Report. The mean intakes of nitrate for cases and controls were 421 and 424 mg/day, respectively. In the multivariable analysis, an increased risk was found for high dietary intake of nitrate (Q5 ≥ 578 vs Q1 ≤ 234.2 mg/day, OR = 1.54, 95% CI: 0.88–2.70, p_{trend} = 0.265), although with wide CIs. High ratio between nitrate and folate intakes (Q5 ≥ 1.79 vs Q1≤ 1.0, OR = 2.03, 95% CI: 1.16–3.54, p_{trend} = 0.052) was associated with twice the risk of breast cancer, after controlling for education, parity, oral contraceptive use, multivitamin supplement use, number of children, breast feeding, menopause, soy protein, mushroom and dietary fat. The limitation of the study is the lack of data on nitrate from different dietary sources (animal, plants) and water sources and the low response rate of controls (60%) in comparison with cases (85%). The strength of the study is the good control for confounding factors.

Brody et al. (2006) conducted a study on 824 Cape Cod (Massachusetts) women diagnosed with breast cancer in 1988–1995 and 745 controls who lived in homes served by public drinking water supplies and never lived in a home served by a Cape Cod private well. Women who were permanent residents of Cape Cod for at least 6 months at the time of an invasive breast cancer diagnosis in 1988–1995 and whose diagnosis was reported to the MCR were eligible cases. Controls were selected from women who were permanent residents of Cape Cod for at least 6 months in 1988–1995. They were frequency matched to cases on date of birth in decades and vital status. Residential nitrate exposure was assessed by using nitrate nitrogen (nitrate-N) levels measured in public wells and pumping volumes for the wells. After controlling for diagnosis/reference year, age at diagnosis/reference year, birth decade, study, vital status, previous breast cancer diagnosis, age at first birth, family history of breast cancer and education, an increased risk, although not statistically significant, was found between breast cancer and average annual excess nitrate-N in drinking water (≥ 1.2 vs 0.3 mg/L, OR = 1.2, 95% CI 0.5–3.1). No increased risk was observed for the number of years exposed to nitrate-N over 1 mg/L (OR = 0.90, 95% CI 0.5–1.5 for ≥ 8 vs 0 years). The main limitations of the study are as following: the exposure (individual volume of water consumption), the lack of data on dietary intake of nitrate and the low response rate of cases (74%) and controls (68%).

Summary

In a previous report (IARC, 2010), one cohort study (Weyer et al., 2001) conducted in Iowa was evaluated concerning breast cancer. No association between high levels of nitrate in both drinking water and nitrate from dietary intake was observed. After the IARC evaluation, one cohort study (Inoue-Choi et al., 2012) and two case–control studies (Brody et al., 2006; Yang et al., 2010) were published. In the cohort study conducted by Inoue-Choi et al. (2012), no association was found for breast cancer and total nitrate intake. However, among women with adequate or high folate intake, a positive association was found for high nitrate levels and breast cancer (Inoue-Choi et al., 2012). In both case–control studies, a non-statistically significant positive association was found between dietary nitrate (Yang et al., 2010) and nitrate from drinking water and breast cancer (Brody et al., 2006).

Overall, the Panel concluded that there was no evidence for an association between dietary nitrate and breast cancer or nitrate in drinking water and breast cancer.

3.6.8.9. Ovarian cancer

Cohort studies

Aschebrook-Kilfoy et al. (2012b) conducted a large US cohort study (NIH-AARP Diet and Health Study) among 617,119 women aged 50–71 years to investigate dietary nitrate and nitrite and epithelial ovarian cancer. After exclusions, 151,316 subjects were included in the study. A total of 709 epithelial ovarian cases cancer were identified through Cancer Registries during a 10-year follow-up. A baseline questionnaire and a FFQ that included 124 food items were mailed to all participants. At baseline, the mean dietary intake of nitrate and nitrite was 91.9 mg/day (SD 68.6) and 1.1 mg/day (SD 0.5), respectively. Women in the highest intake quintile of dietary nitrate had an increased risk (Q5 median = 126.5 vs Q1 median = 22.2 mg/1,000 kcal, HR = 1.31, 95% CI: 1.01–1.68, p_{trend} = 0.06) of epithelial ovarian cancer, after controlling for age, education, total energy intake, cigarette smoking status, race, family history of cancer, BMI, parity, menopausal status, age at menarche and vitamin C intake. Although total nitrite intake was not associated with risk (Q5 median = 0.93 vs 0.47 mg/1,000 kcal, HR = 0.93, 95% CI: 1.50–1.18, p_{trend} = 0.31), a positive association between high

nitrite from animal sources and epithelial ovarian cancer risk (Q5 median = 0.33 vs 0.09 mg/1,000 kcal, HR = 1.34, 95% CI: 1.05–1.69, p_{trend} = 0.02) was observed. In contrast, neither nitrite from plants (Q5 median = 0.73 vs 0.27 mg/1,000 kcal, HR = 1.03, 95% CI: 0.81–1.32, p_{trend} = 0.93), nor processed meat sources (Q5 median = 0.14 vs Q1 median = 0.01 mg/1,000 kcal, HR = 0.97, 95% CI: 0.76–1.23, p_{trend} = 0.63) was associated with ovarian cancer risk. Residential nitrate estimates for drinking water were also taken into account in the analysis but the results did not change. The strength of the study is its prospective design and control of many possible confounders. The limitation of the study is the lack of control for family history of ovarian cancer.

Inoue-Choi et al. (2015) conducted a study to evaluate the association between nitrate and nitrite intake and postmenopausal ovarian cancer risk in the Iowa Women's Health Study. Among 28,555 postmenopausal women and after 14 years of follow-up, 315 epithelial ovarian cancers were identified through the State Health Registry. However, only 190 cases were included in the water nitrate analysis (145 using public water supplies and 45 using private wells). Dietary intake at baseline was assessed using a food frequency questionnaire (126 food items). Nitrate-nitrogen (NO_3-N) and total trihalomethane levels for Iowa public water utilities were linked to residences and average levels were computed based on each woman's duration at the residence. Median NO_3-N levels for women drinking from public water supplies were 1.08 mg/L (range 0.01–25.34 mg/L). Mean dietary nitrate and nitrite intake was 123.3 mg/day (Sd 83.4) and 1.2 mg/day (SD 0.5), respectively. After adjusting for age, BMI, family history of ovarian cancer, number of live births, age at menarche, age at menopause, age at first live, oral contraceptive use, oestrogen use and history of unilateral oophorectomy and total trihalomethane levels, an increased risk was found for ovarian cancer among women with high exposure of NO_3-N ($\geq$ 2.98 vs $\leq$ 0.472 mg/L, HR = 2.03, 95% CI: 1.22–3.38, p_{trend} = 0.003) from public drinking water. The risk associated with high nitrate levels was lower among women with high vitamin C intake. Higher dietary nitrate intake was associated with lower ovarian cancer risk (Q5 $\geq$ 165.54 vs Q1 $\leq$ 65.43 mg/day, HR = 0.61, 95% CI: 0.40–0.95, p_{trend} = 0.02), whereas dietary nitrite intake was not associated with ovarian cancer risk (Q5 $\geq$ 1.537 vs Q1 $\leq$ 0.80 mg/day, HR = 1.03, 95% CI: 0.58–1.84, p_{trend} = 0.50). However, an increased risk, although not statistically significant, was found for high nitrite intake from processed meats (Q5 $\geq$ 0.2 vs Q1 = 0 mg/day, HR = 1.65, 95% CI: 0.93–2.94, p_{trend} = 0.04). The strength of the study is its prospective design and control of many possible confounders. The limitation of the study is the small number of cancer cases included in the analysis (after exclusions) that could have led to selection bias.

Summary

In a previous report (IARC, 2010), one cohort study on ovarian cancer and nitrate intake was described. No association was observed for levels of nitrate from dietary intake, but a non-statistically significant positive association was observed for nitrate in drinking water. After the IARC evaluation, two cohort studies in USA were conducted to investigate dietary nitrate and ovarian cancer (Aschebrook-Kilfoy et al., 2012b; Inoue-Choi et al., 2015). In one of the cohort studies, a statistically significant positive association was found between dietary nitrate intake and ovarian cancer (Aschebrook-Kilfoy et al., 2012a,b), whereas, in the other one, a negative association was found (Inoue-Choi et al., 2014).

Overall, information is still sparse and results contradictory. The Panel concluded that there was insufficient evidence for an association between nitrate and ovarian cancer.

3.6.8.10. Prostate cancer

Cohort studies

Sinha et al. (2009) investigated the relationship between meat consumption, PAHs, haem iron, nitrite, nitrate and the risk of prostate cancer in a cohort of 175,343 US men aged 50–71 years. Self-administrated risk factors questionnaires, including a FFQ (124-item) and information on cooking methods used for different meats, were filled by participants mailed to 196,851 subjects. After 9 years of follow-up, 10,313 incident cases and 419 fatal cases of prostate cancer were identified through cancer registries. Levels of HCAs (2-amino-3,4,8 trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), MeIQx and 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP)), levels of benzo(a)pyrene (BaP) and mutagenic activity (a measure of total mutagenic potential incorporating all meat-related mutagens) were measured from meats with known cooking details. After adjusting for age, total energy intake, ethnicity, education, marital status, family history of prostate cancer, undergoing prostate-specific antigen testing in the past 3 years, history of diabetes, BMI, smoking history, physical activity, alcohol,

Ca, tomatoes, α-linolenic acid, vitamin E, zinc and selenium, elevated risks were associated with red meat (Q5 median = 66.1 g/1,000 kcal, HR = 1.12, 95% CI: 1.04–1.21, p_{trend} = 0.002) and processed meat (Q5 median = 24.6 g/1,000 kcal, HR = 1.07, 95% CI: 1.00–1.14, p_{trend} = 0.040) and haem iron (Q5 median = 336.8 μg/1,000 kcal, HR = 1.09, 95% CI: 1.02–1.17, p_{trend} = 0.003). No increased risk were found for nitrite (Q5 median 0.215 vs Q1 median 0.017 mg/1,000 kcal, HR = 1.05, 95% CI: 0.99–1.12, p_{trend} = 0.14) and nitrate from meat (Q5 median 0.314 vs 0.032 mg/1,000 kcal, HR = 1.06, 95% CI: 0.99–1.13, p_{trend} = 0.11). However elevated risk were observed for advanced prostate cancer for both high nitrite (HR = 1.24, 95% CI: 1.02–1.51, p_{trend} = 0.03) and nitrate intake (HR = 1.31, 95% CI: 1.07–1.61, p_{trend} = 0.03), haem iron (HR = 1.28, 95% CI: 1.03–1.58, p_{trend} = 0.02) and red meat intake (HR = 1.31, 95% CI: 1.05–1.65, p_{trend} = 0.04). The strength of the study is the prospective design and the large size. The limitation of the study is the lack of adjustments for other dietary factors (nitrosation inhibitors) that may also influence the risk of prostate cancer.

Case–control

Wu et al. (2013) conducted a nested case–control study within the Health Professionals Follow-up Study (n = 51,529 men) to investigate whether plasma nitrate levels were associated with risk of prostate cancer. Baseline blood samples were collected among 18,018 participants who provided blood specimens and incident cases of prostate cancer were identified (n = 630). The eligibility criteria for a control was to be alive and free of cancer at the date that the matched case was diagnosed and to have had a prostate-specific antigen test after the date of the blood draw and before the matched case was identified. Each case was matched with one control by age, the time of the blood draw, the season of the blood draw and the year of the blood draw. Baseline plasma levels of nitrate were measured in the 630 cases and 630 matched controls. Questionnaires on anthropometric variables, medical conditions, lifestyle factors and a FFQ (131-item) were administered. The median values for cases and controls of nitrate and nitrate were 37.71 μmol/L (interquartile range = 29.39–51.47) and 39.01 μmol/L (interquartile range = 30.10–49.77) respectively. Baseline levels of plasma nitrate were not associated with risk of prostate cancer (Q5 RR = 0.97, 95% CI: 0.65–1.44, p_{trend} = 0.9) after adjustment for family history of prostate cancer, history of smoking, hypertension, BMI, history of diabetes, vigorous physical activity, total calorie intake, hours since last meal, intake of vegetables, red and processed meat and history of vasectomy. When analyses were restricted to men fasting more than 6 h, the trend was similar. Furthermore, plasma nitrate was inversely associated with advanced-stage prostate cancer (RR = 0.30, 95% CI: 0.09–0.99, p_{trend} = 0.05) for the fasting data set. No effect modification was observed by smoking. Some sensitivity analysis were conducted such as exclusion of cases and matched controls identified in the first 5 years after blood draw to exclude the possibility of reverse causation and also a stratified analysis according to smoking status. Among non-smokers, a similar association was found. The limitation of this study is that it did not into consideration the disease status of controls except for diabetes and hypertension and/or drug use that could have affected nitrate plasma levels and values for quintiles are not provided. The strength of the study is the study design and statistical analysis, including the sensitivity analysis.

Summary

In a previous report (IARC, 2010), two ecological studies were reviewed in relation to nitrate and prostate cancer. A study in Spain, examined mortality rates for prostatic cancer and nitrate levels and found a positive non-statistically significant association for high nitrate levels in drinking water (> 50 mg/L) (Morales et al., 1993), whereas the ecological study conducted in Germany showed no association between prostate cancer and nitrate from drinking water (Volkmer et al., 2005). After the IARC report, one cohort study (Sinha et al., 2009) and a case–control study (Wu et al., 2013) were conducted. Sinha (2009) investigated the relationship between meat consumption, PAHs, haem iron, nitrite, nitrate and the risk of prostate cancer. No association was found for nitrate from meat and prostate cancer. However, a positive association, with a statistically significant trend in risk across quintiles, was found for advanced prostate cancer. Wu et al. (2013) conducted a nested case–control study within the 'Health Professionals Follow-up and showed no association between levels of plasma nitrate and prostate cancer.

Overall, the Panel concluded that there was insufficient evidence for an association between nitrate intake and prostate cancer.

3.6.8.11. Renal cancer

Cohort studies

Daniel et al. (2012b) investigated the risk of renal cell cancer in relation to meat, nitrate, nitrite and meat mutagens intake such as HCA and PAHs, in a prospective study (NIH-AARP Diet and Health study). Participants completed a self-administered questionnaire about demographics, diet, and lifestyle (n = 492,186). A 124-item FFQ was used to assess dietary intake. Cancer cases were ascertained through linkage with the cancer registries. After 6 months, a second self-administrated questionnaire with queries for meat cooking methods and doneness levels was sent to all participants. Meat cooked by methods such as grilling or pan-frying results in the formation of HCA and PAHs (e.g. PhIP; BaP). Haem iron (pro-oxidant involved in carcinogenesis) in red and processed meats may further increase endogenous NOC formation. After a mean follow up of 9 years, a total of 1,814 cases of renal cell carcinoma (RCC) were identified. In the multivariable analysis, an increased risk was observed for participants in the highest compared with the lowest quintiles of total red meat intake (Q5 median = 62.2 g/1,000 kcal, HR = 1.19, 95% CI: 1.01–1.40, p_{trend} = 0.06). No association was identified between combined nitrate and nitrite intake and risk of renal cell cancer (Q5 median = 0.29 mg/1,000 kcal, HR = 0.93, 95% CI: 0.78–1.12) after adjusting for age, sex, total energy intake, other types of meat intake, education, marital status, family history of cancer, race, BMI, smoking status, history of diabetes, history of hypertension, and intakes of alcohol, fruit and vegetables. Meat intake was significantly correlated with intake of haem iron (r = 0.82), PhIP (r = 0.42), MeIQx (r = 0.52) and BaP (r = 0.36). Intake of BaP (Q5 median = 44 ng/1,000 kcal, HR = 1.23, 95% CI: 1.01–1.48, p_{trend} = 0.03) and PhIP (Q5 median = 123.6 ng/1,000 kcal, HR = 1.30, 95% CI: 1.07–1.58, p_{trend} = 0.04) was associated with elevated risk of RCC. The strength of the study was the large sample and the assessment of other known carcinogens present in meat. The limitation of the study is that the intake of nitrate and nitrite was combined and based on estimates from only 10 types of processed meats (e.g. bacon, cold cuts, ham, hot dogs and sausage) instead of the whole diet including fresh meat and milk and milk products. The latter could have led to a possible information bias.

Dellavalle et al. (2013) prospectively investigated the association between nitrate and nitrite intake from dietary sources and the risk of RCC within the NIH-AARP Diet and Health Study. Among 491,841 participants (293,248 men and 198,593 women, mean age 50–71 years), 1,816 RCC cases were identified after a mean follow up of 9 years. The study was an expansion of the study conducted by Daniel et al. (2012b) to include nitrate and nitrite from a variety of dietary sources (e.g. fresh meat and dairy products). RCC cases were identified via linkage with state cancer registries. Nitrate and nitrite intake was estimated from a semi-quantitative 124-item FFQ and intake was calculated for animal and plant sources separately. Daily mean dietary nitrate intake in the study population was 51.0 mg/1,000 kcal (SD 36.3). Mean daily dietary nitrite intake was 0.7 mg (SD 0.2). Nitrite from animal sources accounted for 33% of daily mean intake of nitrite (0.2 mg/1,000 kcal), with the remainder of nitrite intake derived from plant sources. Approximately 40% of nitrite from animal sources was derived from processed meats (0.1 mg/1,000 kcal). The daily average combined nitrate and nitrite intake from processed meat sources was 0.7 mg/1,000 kcal. No increased risk was found for total nitrate (Q5 $\geq$ 70.94 vs Q1 $\leq$ 24.9 mg/1,000 kcal, HR = 0.98, 95% CI: 0.84–1.14, p_{trend} = 0.98) and nitrite intake (Q5 $\geq$ 0.82 vs Q1 $\leq$ 0.52 mg/1,000 kcal, HR = 1.02, 95% CI: 0.87–1.19, p_{trend} = 0.47). No increased risk was found for nitrate from plant sources (Q5 $\geq$ 0.58 vs Q1 $\leq$ 30 mg/1,000 kcal, HR = 0.89, 95% CI: 0.76–1.04, p_{trend} = 0.44). An increased risk (Q5 $\geq$ 0.31 vs Q1 $\leq$ 0.13 mg/1,000 kcal, HR = 1.28, 95% CI: 1.10–1.49, p_{trend} < 0.01) of RCC among participants was found for the highest quintile of nitrite from animal sources intake compared with those in the lowest quintile after controlling for age, sex, caloric intake, race, smoking status, family history of cancer, BMI, alcohol intake, education, history of hypertension and history of diabetes. Similar associations were observed when nitrite (from processed meat (Q5 $\geq$ 0.16 vs Q1 $\leq$ 0.03 mg/1,000 kcal, HR = 1.16, 95% CI: 1.00–1.35, p_{trend} = 0.04) and nitrite, from other animal sources excluding processed meat (Q5 $\geq$ 0.19 vs Q1 $\leq$ 0.08 mg/1,000 kcal, HR = 1.23, 95% CI: 1.06–1.43, p_{trend} = 0.02). A sensitivity analyses that excluded participants who resided in areas with high nitrate levels was conducted but the risk did not change. No association was observed between nitrite intake from plant sources or total nitrite intake and risk of total RCC. No statistical interaction was observed for vitamin C and/or vitamin E. The strength of the study is the large sample and the good control for confounding factors, as well as the number of stratified analysis conducted.

Case–control

Ward et al. (2007b) conducted a population-based case–control study (201 cases and 1,244 controls) to evaluate drinking water and dietary sources of nitrate and nitrite as risk factors for RCC. Eligible cases were white residents of Iowa aged 40–85 years who were newly diagnosed with histologically confirmed RCC. Of 463 eligible RCC cases, only 201 cases participated and had complete information on nitrate from drinking water and diet. Controls were frequency matched by gender, race and 5-year age groups to the distribution of the six cancers combined (bladder, brain, colon, kidney, rectum and pancreas), resulting in a matching ratio for the RCC cases of approximately 6:1. The mailed questionnaire assessed major RCC risk factors, including demographics, height and weight, smoking history, and questions about physician diagnosed hypertension and bladder or kidney infections. Dietary intake was assessed by a 55-item FFQ. There was no association of RCC with high nitrate level in drinking water from public supplies (> 2.78 vs< 0.62 mg/L, OR = 0.89, 95% CI: 0.57–1.39) after controlling for age, gender, BMI and average population size. No increased risk was found for neither high intake of dietary nitrate (Q4 ≥ 122.01 vs Q1 < 59.32 mg/day, OR = 0.41, 95% CI: 0.28–0.60), nor high intake of nitrites (Q4 ≥ 1.26 vs Q1 < 0.70 mg/day, OR = 0.82, 95% CI: 0.50–1.33) after controlling for age, sex, sodium, total calories. High intake of nitrites from animal source was also not associated with an increased risk (Q4 ≥ 0.48 vs Q1 < 0.18 mg/day, OR = 1.00, 95% CI: 0.63–1.59) after controlling for age, sex, sodium and total fat. However, high nitrate exposure from water (> 5 mg/L) was associated with an increased risk among subgroups with above the median red meat intake (≥ 1.2 vs < 1.2 servings per day, OR = 1.91, 95% CI: 1.04–3.51) or below the median vitamin C intake (OR = 1.90, 95% CI: 1.01–3.56) after controlling for age, gender, average population size of residences, BMI and total calories. The strength of the study was the information regarding individual consumption of drinking water plus nitrate and nitrite from dietary sources. The limitation of the study is the high number of subjects excluded from the analysis such as subjects with 10 mg/L of nitrate and with greater than five missing food items. The imputation method they used for missing information on foods or levels of nitrate may be also a limitation.

Summary

In a previous report (IARC, 2010), one ecological study was reported on nitrate and renal cancer mortality and found no association between renal cancer and nitrate from drinking water (Volkmer, 2005). After the IARC report, a case–control study (Ward et al., 2007b) and two cohort studies (Daniel et al., 2012b; Dellavalle et al., 2013) were conducted. Ward et al. (2007b) conducted a population-based case–control study to evaluate drinking water and dietary sources of nitrate and nitrite as risk factors for RCC and found no association. Daniel et al. (2012b) found no association between combined nitrate and nitrite intake and the risk of renal cell cancer. Dellavalle et al. (2013) investigated the association between nitrate and nitrite intake from dietary sources and the RCC within the NIH-AARP Diet and Health Study and found no association.

Overall, the Panel concluded that there was no evidence for an association between dietary nitrate and nitrate in drinking water and renal cancer.

3.6.8.12. Bladder cancer

Cohort studies

Ferrucci et al. (2010) investigated the association between meat and meat components (nitrate, nitrites, heterocyclic amines and polycyclic aromatic hydrocarbons) and bladder cancer, within a large prospective NIH-AARP Diet and Health Study (n = 300,933). Each participant, at baseline, completed a self- administrated questionnaire on demographic, lifestyle, including a 124-item FFQ and medical data. Six months after the baseline questionnaire, participants completed a mailed risk-factor questionnaire with questions on meat cooking methods (grilled, pan-fried, microwaved and broiled) and doneness levels (well done/very well done, and medium/rare) for a total of 125,574 females and 175,359 males (50–71 years). Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) was used to estimate HCAs: DiMeIQx, MeIQx and PhIP. BaP, a marker of overall PAH exposure, was also estimated for each participant. After 8 years of follow-up, 854 bladder cancers were identified through record linkage with state cancer registries. An increased risk, although not statistically significant (Q5 median 61.6 vs Q1 median 9.5 g/1,000 kcal, HR = 1.22, 95% CI: 0.96–1.54, p_{trend} = 0.07), was observed for high meat consumption of red meat, after controlling for age, sex, smoking, intake of fruits, vegetables, beverages and total energy intake. High nitrate intake (Q5 = median 95.4 vs 19.7 mg/1,000 kcal, HR = 0.80, 95% CI: 0.58–1.10, p_{trend} = 0.28) was not

associated with an increased risk of bladder cancer. A 28% increased risk (Q5 median = 0.91 vs Q1 median 0.46 mg/1,000 kcal, HR = 1.28, 95% CI: 1.02–1.61, p_{trend} = 0.06) was observed for high nitrite intake. When nitrites was divided by dietary sources, no increased risk was found for nitrites from animal (Q5 median 0.36 vs Q1 median 0.10 mg/1,000 kcal, HR = 1.09, 95% CI: 0.87–1.36, p_{trend} = 0.21) plant sources (Q5 median 0.69 vs Q1 median 0.25 mg/1,000 kcal, HR = 1.16, 95% CI: 0.90–1.50, p_{trend} = 0.18) or (Q5 median 0.19 vs Q1 median 0.10 mg/1,000 kcal, HR = 1.07, 95% CI: 0.85–1.36, p_{trend} = 0.79). Nevertheless, an increased risk was also observed for combined nitrate and nitrite levels from processed meat (Q5 median 0.95 vs Q1 median 0.06 mg/1,000 kcal, HR = 1.29, 95% CI: 1.00–1.67, p_{trend} = 0.11) but not for nitrite alone (HR = 1.07, 95% CI: 0.85–1.36, p_{trend} = 0.79). A suggestive increased risk was seen for nitrate from processed meat (Q5 median 0.29 vs Q1 median 0.02 mg/1,000 kcal, HR = 1.20, 95% CI: 0.95–1.51, p_{trend} = 0.06), although it did not reached statistical significance. Adjustment for other possible confounders and excluding individuals, who may high nitrate intake from drinking, did not alter risk estimates. DiMeIQx, MeIQx and BaP were not associated with bladder cancer. However, an, increased risk, although not statistical significant, was observed for high levels of PhIP (HR = 1.19, 95% CI: 0.95–1.48, p_{trend} = 0.06). No association was seen for doneness and white meat. The strength of this study is its large size and the good control for confounders. No values for mean nitrate or nitrite in the population were provided.

Catsburg et al. (2014) examined the role of dietary sources of NOCs and NOC precursors as potential bladder cancer risk factors using data from the Los Angeles Bladder Cancer Study, a population-based case–control study (1,660 bladder cancer cases and 1,586 controls). Bladder cancer cases were identified through the Los Angeles County Cancer Surveillance Program, the population-based Surveillance, Epidemiology and End Results (SEER) cancer registry of Los Angeles County. Controls were frequency matched by age (within 5 years), gender and race/ethnicity (non-Hispanic white, Hispanic, African American). In-person structured interviews were conducted in participants' homes. The questionnaire included information on demographic characteristics, height, weight, lifetime use of tobacco and alcohol, usual adult dietary habits, lifetime occupational history, prior medical conditions and prior use of medications. Forty food groups were included in the dietary section of the structured questionnaire. Mean age of cases and controls was 54.4 years. In the multivariate analysis, weekly intake of salami/pastrami/corned beef (rich in amines and nitrosamines), was associated with a 30% increased risk (OR = 1.33, 95% CI: 1.02–1.74, p_{trend} = 0.008) of risk of bladder cancer after adjusting for smoking, race, BMI, education, food servings, history of diabetes, vegetable intake and intake of vitamin A, C and carotenoid. Among non-smokers the risk was even stronger (OR = 1.95, 95% CI: 1.10–3.46, p_{trend} = 0.006). No association was found for nitrate intake (Q5 ≥ 148.4 vs Q1 ≤ 64.3 mg/day, OR = 0.90, 95% CI: 0.60–1.35, p_{trend} = 0.598) or nitrite intake (Q5 ≥ 533 vs Q1 ≤ 234 μg/day, OR = 0.89, 95% CI: 0.66–1.20, p_{trend} = 0.921) or nitrosamine (Q5 ≥ 54.5 vs Q1 14.6 ng/day, OR = 1.03, 95% CI: 0.78–1.36, p_{trend} = 0.984). An increased risk, although not statistically significant, was found for nitrites (OR = 1.56, 95% CI: 0.85–2.87, p_{trend} = 0.063) and nitrosamine (OR = 1.52, 95% CI: 0.86–2.66, p_{trend} = 0.281) among non-smokers. No increased risk was found for nitrate (OR = 0.96, 95% CI: 0.60–1.54, p_{trend} = 0.759) nitrites (OR = 0.77, 95% CI: 0.54–1.08, p_{trend} = 0.341) and nitrosamines (OR = 0.96, 95% CI: 0.69–1.33, p_{trend} = 0.701) in ever smokers. High intake of haem iron (≥ 5.2 mg) was also associated with an increased risk of bladder cancer among non-smokers (OR = 1.97, 95% CI: 1.16–3.33, p_{trend} = 0.010). When considering NOC precursors, risk was consistently higher among subjects with concurrent high intake of nitrate (≥ 103 mg/day) and high intake of the different meats, known as sources of amines and nitrosamines, such as liver (OR = 1.48, 95% CI: 1.09–2.01, p_{trend} = 0.001), salami/pastrami/corned beef (OR = 1.37, 95% CI: 0.94–2.00, p_{trend} = 0.035) and hot-dogs/polish sausage (OR = 1.36, 95% CI: 0.91–2.04, p_{trend} = 0.06). The strength of this study is the study design: a population case–control study with the control of many confounders. No data regarding the response rate among controls was provided.

Case–control

Zeegers et al. (2006) conducted a case-cohort study within the Netherlands Cohort Study (58,279 man and 62,573 women 55–69 years of age), to evaluate the association between nitrate exposure and bladder cancer. After 9.3 years, 955 cases were identified by computerised record linkage with the cancer registries in the Netherlands and the Dutch national database of pathology reports (PALGA). Because of incomplete or inconsistent dietary data or missing data on nitrate exposure in drinking water, only 871 cases were included in the analysis. A subcohort sample of 4,359 members was randomly selected after identification of all cohort members. All participants completed a mailed questionnaire on risk factors for cancer, including a FFQ (150-item). Nitrate intake from water was

calculated from the amount of water consumed derived from the questionnaire combining information from nitrate content in drinking water from all 364 pumping stations in the Netherlands. Mean total nitrate among cases was 109.8 mg/day (SD 43.4) and 109.4 mg/day (SD 44.3) among controls. No increased risk (Q5 ≥ 140.8 vs≤ 72.7 mg, RR = 1.09, 95% CI: 0.84–1.42, p_{trend} = 0.77) was found for total nitrate intake after adjusting for sex, age and smoking. No increased risk was found for nitrate in drinking water (Q5 > 7.7 vs ≤ 0.9 mg, RR = 1.06, 95% CI: 0.82–1.38, p_{trend} = 0.24) and nitrate in food (Q5 > 135.3 vs Q1 ≤ 69.0 mg, RR = 1.04, 95% CI: 0.80–1.36, p_{trend} = 0.96), after controlling for sex, age, smoking and nitrate exposure from food (for drinking water analyses only) or nitrate exposure from drinking water (for food analyses only). Dietary intake of vitamins C and E and cigarette smoking status was not effect modifiers. The limitations of the study were that food nitrate content was not sub-divided by plant and animal foods and there was a lack of control for some food items such as animal food products in the analysis. The strength of the study is the study design that decreases the possibility of information bias. It is superior to a simple case–control study.

Ecological

Chiu et al. (2007) conducted an ecological study to investigate the association between bladder cancer death and nitrate exposure from drinking water in Taiwan. Records of all deaths from bladder cancer (n = 513 cases; 339 males, 174 females; 62.7 years) between 1999 and 2003 were obtained from the Taiwan provincial health department. Controls consisted of all deaths, excluding death from genitourinary diseases, gastric, oesophageal, head and neck cancer, and NHL. Cases were matched to controls by sex, year of birth and year of death. Data on nitrate levels in 252 municipalities were obtained from the Taiwan provincial drinking water supplier. The mean concentration of nitrate in the drinking water for individuals that died from bladder cancer was reported to be 0.52 mg/L (SD 0.47) and, for those that died from other causes (controls), the nitrate concentration in drinking water was reported to be 0.43 mg/L (SD 0.45). Each subject's source of nitrate exposure was presumed to arise exclusively from the water supplied by the municipality of residence. Under these conditions, after controlling for age, gender, urbanisation and level of residence, an increased risk of death from bladder cancer was observed for high nitrate levels in drinking water (Q3 ≥ 0.48 vs ≤ 0.18 mg/L, OR = 1.96, 95% CI: 1.41–2.72, p_{trend} < 0.001). The limitation of the study is the study design (ecological), which does not allow assessment of whether there was a true exposure-outcome relationship because there was no individual exposure data. There is no control for confounding factors such as smoking and diet.

Summary

In a previous report (IARC, 2010), five studies, conducted in different geographic areas (Denmark, Spain, Slovakia, USA, Canada) in relation to drinking water and/or diet in relation to bladder cancer were reported. Among those, three ecological studies showed no association between bladder cancer and nitrate in drinking water (Gulis et al., 2002; Jensen, 1982; van Leeuwen et al., 1999) and one reported a non-statistically significant positive association (Morales et al., 1992). A cohort study (IOWA Health Study) that evaluated nitrate in both the diet and drinking water in relation to the incidence of bladder cancer showed a twofold increased risk for nitrate in drinking water and a non-statistically significant positive association with dietary nitrate (Weyer et al., 2001). After the IARC report, one ecological study (Chiu et al., 2007), two cohort studies (Zeegers et al., 2006; Ferrucci et al., 2010) and one case–control study (Catsburg et al., 2014) were conducted. Chiu et al. (2007) conducted an ecological study to investigate the association between bladder cancer death and nitrate exposure from drinking water and found a positive association. Ferrucci et al. (2010) investigated the association between meat and meat components (nitrates, nitrite, heterocyclic amines and PAHs) and bladder cancer, within a large prospective NIH-AARP Diet and Health Study. In this study, high nitrate intake was not associated with bladder cancer. However, a non-statistically significant positive association was seen for nitrate from processed meat (Ferrucci et al., 2010). Zeegers et al. (2006) conducted a cohort study within the Netherlands Cohort Study to evaluate the association between nitrate exposure in both diet and drinking-water and bladder cancer, and found no association. A population-based case–control study showed no association between dietary nitrate intake and bladder cancer (Catsburg et al., 2014).

Overall, the Panel concluded that there was insufficient evidence of an association between dietary nitrate and nitrate in drinking water and bladder cancer.

3.6.8.13. Thyroid cancer

Cohort studies

Ward et al. (2010) investigated the association between nitrate intake and the risk of developing thyroid cancer in a cohort of women in Iowa (aged 55–69 years). Initially, a total of 41,836 women participated in the initial survey, constituting the study cohort. After excluding women who had used multiple sources of water or used their public or private well supply for 10 years or less and women living in communities for which no nitrate measurement data and women with missing dietary data, the cohort numbered 20,631. Data on demographics, anthropometry, reproductive history, hormone use, family history of cancer, residence location, physical activity, smoking, alcohol consumption, medical conditions and the primary source of their drinking water, as well as how long they drank that type of water. FFQs were employed to assess dietary intake (126 items). After 19 years of follow-up, 45 women were diagnosed with thyroid cancer. The cohort was traced annually for cancer incidence by linkage of personal identifiers to the State Health Registry of Iowa's cancer database. After adjusting for age, total calories, vitamin C intake and residence, women with longer consumption of water (≥ 5 years) exceeding 5 mg/L nitrate-N had 2.5-fold increased risk of thyroid cancer (HR = 2.59, 95% CI: 1.09–6.19, p_{trend} = 0.04) in comparison with women with less than 1 year of exposure of water exceeding 5 mg/L nitrate-N. High intake of nitrate from dietary sources (Q4 > 41.1 vs Q1 ≤ 17.4 mg/day, HR = 2.85, 95% CI: 1.00–8.11, p_{trend} = 0.046) was also associated with an increased risk. The strength of this study was the large sample size, the long follow-up time and the nitrate data in both drinking water and diet. The limitation of this study was the large number of subjects excluded from the analysis (around 50%) and the lack of control for occupation. No mean or median values for nitrate were given for the whole study population.

Aschebrook-Kilfoy et al. (2013b) evaluated nitrate and nitrite intake and the risk of thyroid cancer in the Shanghai Women's Health Study (SWHS) (n = 73,317 women, aged 40–70 years). In-person interviews were used to collect information on demographic characteristics, medical information, lifetime residential and occupational history, life-style and dietary habits. Dietary intake was assessed using a food frequency questionnaire (77 food items). After approximately 11 years of follow-up, 164 incident thyroid cancer cases were identified through the Shanghai Cancer Registry. The mean age was 52.0 years, the median daily nitrate was 309 mg/day and median nitrite was 1.4 mg/day, respectively. Nitrate intake was not associated with thyroid cancer risk (Q4 median = 251 vs Q1 median 109 mg/1,000 kcal, HR = 0.93, 95% CI: 0.42–2.07, p_{trend} = 0.40). A twofold increased risk was found for total dietary nitrite intake (Q4 median 1.1 vs Q1 median 0.6 mg/1,000 kcal, HR = 2.05, 95% CI: 1.20–3.51, p_{trend} = 0.36), after adjusting for age, education, history of thyroid diseases, vitamin C, carrot and folate intake. An increased risk was found for nitrite from animal sources (Q4 median 0.2 vs Q1 median 0.1 mg/1,000 kcal, HR = 1.59, 95% CI: 1.00–2.52, p_{trend} = 0.02). An increased risk, although not statistically significant, was seen for nitrite from plant sources (Q4 median 1.0 vs Q1 median 0.5 mg/1,000 kcal, HR = 1.30, 95% CI: 0.76–2.4, p_{trend} = 0.7). The risk was stronger for nitrite from processed meats (Q4 median 0.1 vs Q1 median 0.0 mg/1,000 kcal, HR = 1.96, 95% CI: 1.28–2.99, p_{trend} < 0.01). To evaluate the consistency of the results found regarding total nitrate and nitrite, they were stratified by age, BMI, education, red meat intake and vitamin C. However, no interaction was seen and the results did not change. The strength of the study is the nature of the study (cohort), the high level of complete baseline and the completeness of follow-up (92% of the initial sample). Although the study controlled for many confounders, smoking was collected but not considered while running the models and that could be a great limitation.

Kilfoy et al. (2011) conducted a cohort (NIH-AARP) Diet and Health Study and evaluated dietary nitrate and nitrite intake and thyroid cancer risk overall and for subtypes. In total, 490,194 men and women aged 50–71 years were included in the study. During an average of 7 years of follow-up, 370 incident cases were identified through cancer registries and the US National Death Index Plus. A baseline questionnaire ascertained information on possible risk factors, including dietary intake (food frequency questionnaire of 124 items and the use of individual and multivitamin supplements). Mean dietary intake of nitrate was 88 mg/day (SD 65) and nitrite was 1.2 mg/day (SD 0.6). An interaction between nitrate intake and sex was observed. Among men, a twofold increased risk was observed in the highest quintile of nitrate intake (Q5 median 94.8 vs Q1 median 19.4 mg/day, RR = 2.28, 95% CI: 1.29–4.04, p_{trend} < 0.01). An increased risk, although not statistically significant was found for nitrite intake among men (Q5 median 0.9 vs Q1 median 0.5 mg/day, RR = 1.36, 95% CI: 0.78–2.37, p_{trend} = 0.26), after controlling for age, smoking status, race, calories, alcohol, family history cancer, physical activity, education, BMI, vitamin C, beta-carotene and folate consumption. Among women,

nitrate intake was not associated with thyroid risk (Q5 median 94.8 vs Q1 median 19.4 mg/day, RR = 0.69, 95% CI: 0.42–1.15, p_{trend} = 0.61), but an increased risk, although not statistical significant, was found for nitrite (Q5 median 0.9 vs Q1 median 0.5 mg/day, RR = 1.19, 95% CI: 0.71–1.98, p_{trend} = 0.40). The strength of the study is the prospective design, the large sample size and the high number of confounding factors taken into consideration. The limitation is the lack of information of nitrites by different dietary sources and the lack of control for other potential environmental and dietary risk factors for thyroid cancer.

Summary

In a previous report (IARC, 2010), no study was described on nitrate and thyroid cancer. After The IARC report, three cohort studies were conducted. Ward et al. (2010) investigated the association between nitrate intake and the risk of developing thyroid cancer in a cohort of women in Iowa and showed a positive association between dietary nitrate and nitrate in drinking water and thyroid cancer with a trend in risk across quartiles. Kilfoy et al. (2011) conducted a cohort study (NIH-AARP) to evaluate dietary nitrate and nitrite intake and thyroid cancer risk. Among men, but not among women, a positive association was observed for high nitrate intake with a trend in risk across quintiles. Aschebrook-Kilfoy et al. (2013b) evaluated nitrate and nitrite intake and the risk of thyroid cancer in the Shanghai Women's Health Study (SWHS) and found no association for nitrate intake.

Overall, the Panel concluded that there was insufficient evidence to link dietary nitrate and thyroid cancer.

3.6.8.14. Non-Hodgkin's lymphoma (NHL)

Cohort studies

Daniel et al. (2012a) investigated meat intake in relation to NHL risk in a large US cohort (NIH-AARP Diet and Health Study, n = 567,169). Participants completed a food frequency (124-items) and a lifestyle questionnaire (n = 492,186) and a subcohort (n = 302,162) also completed a questionnaire on meat-cooking methods and doneness levels. Over a mean of 9 years of follow-up, 3,611 incident cases of NHL were identified through original state cancer registries. Intake of nitrate and nitrite was estimated by using a database of measured values from 10 types of processed meat, constituting 90% of the processed meat types consumed in the United States. Nitrate and nitrites intake from processed meat sources were not associated with total NHL risk, after controlling for age, sex, education, family history of any cancer, race, BMI, smoking status and physical activity, as well as intake of alcohol, fruit, vegetables, and total energy (Q5 median value = 0.47 vs Q1 median value = 0.04 mg/1,000 kcal, HR = 1.02, 95% CI: 0.88–1.18, p_{trend} 0.68). Haem iron, heterocyclic amines and polycyclic aromatic compounds were not associated with an increased risk of NHL. The strength of the study is the large sample and the assessment of other known carcinogens present in meat. The limitation of the study is the estimates of nitrites and nitrate that were based only on processed meat instead of the whole diet including fresh meat and milk and milk products.

Aschebrook-Kilfoy et al. (2012a) conducted a prospective study among 832 female cases of NHL identified between 1996 and 2000 through the Connecticut Tumour Registry to test the hypothesis that nitrate and nitrite intake affects NHL survival. After exclusions for missing data, 568 patients (mean age 61.6 years) with NHL were included in the study and followed-up until 2008 (mean follow-up 4.06 years). The median daily intake of nitrate (95.9 mg/day) and nitrite (1.1 mg/day) were used as the cut-off points for high and low intake. During the follow-up time, 250 patients died. A FFQ (120 foods) was used to assess dietary intake. No association was found for high nitrate intake (Q4 $\geq$ 141.0 vs Q1 < 62.8 mg/day; HR = 1.0, 95% CI: 0.7–1.5 p_{trend} = 0.88) and NHL mortality and nitrites (Q4 $\geq$ 1.4 vs Q1 < 0.8 mg/day; HR = 1.0, 95% CI: 0.6–1.6, p_{trend} = 0.69) and NHL mortality, after controlling for age, caloric intake, family history of cancer and vitamin C. The limitation of the study is the study design that does not allow assessment of whether there was a true exposure–outcome relationship. There is a lack of control for important confounders in the multivariate analysis such as staging, comorbidities and therapy.

Case–control

Kilfoy et al. (2010) investigated the association between nitrate and nitrite intake and the risk of NHL in a population case–control study of women from Connecticut. Out of 832 eligible patients, a total of 594 patients with histological confirmed NHL cases, aged 21–84 years (mean age 62 years) were included in the study. Population-based controls (710 individuals) from the same area were

recruited, and matched by age (± 5 years). In-person interviews were conducted to assess health behaviour and history, and all participants were sent a 120-item FFQ to assess dietary intake. Intake of nitrate and nitrite was divided into high and low intake according to the median consumption. The median intake in their study was similar to the NCI-SEER (Ward et al., 1996) (median nitrate intake 114 mg/day, median nitrite intake 0.91 mg/day). The mean nitrate intake among cases and controls were 116.5 mg/day (SE 83.0) and 112.1 mg/day (SE 75.1) and the mean dietary nitrite was 1.2 mg/day (SE 0.6) and 1.1 mg/day (SE 0.5), respectively. An increased risk of NHL was found for higher dietary nitrite intake (OR = 1.37, 95% CI: 1.04–1.79) after adjusting for age, family history of NHL, total daily energy intake, vitamin C, vitamin E and protein intake. No association was found between dietary nitrate and NHL risk, after controlling for potential confounders (OR = 1.09, 95% CI: 0.86–1.39). After stratifying for NHL histological type, a particular increased risk was observed for T-cell lymphoma (OR = 2.38, 95% CI: 1.12–5.06) and nitrites. After stratification by sources of nitrites, the increased risk remained only for nitrites from animal sources (OR = 1.35, 95% CI: 1.05–1.75) but not from nitrites from plants (OR = 1.11, 95% CI: 0.86–1.44). No effect modification was observed for vitamin C, vitamin E and protein intake and risk of overall NHL. The strength of the study was the sample size. The limitation of the study is the low response rate among cases (71%) and controls (69% < 65 years and 47% for subjects ≥ 65 years) and the lack of control for other potential confounding factors. Low and high nitrate and nitrite levels of intake were based on median consumption however the median values were not given in the manuscript.

Aschebrook-Kilfoy et al. (2013a) conducted a case–control study (348 cases and 470 controls) in Nebraska to investigate the association between dietary nitrate and nitrite intake and risk of NHL. Information on demographic, medical, environmental and lifestyle factors was collected by telephone interviews. A self-administrated FFQ was sent by post (117 food items). The mean nitrate intake among cases was 100.3 mg/day (SD 70.6 mg/day) and the mean nitrite intake was 1.4 mg/day (SD 0.7). The mean nitrate intake among controls was 103.0 mg/day (SD 68.0) and the mean nitrite intake was 1.3 mg/day (SD). No increased risk of NHL was found for high intake of total nitrate (Q4 median 88.3 vs Q1 22.2 mg/1,000 kcal, OR = 0.8, 95% CI: 0.5–1.3, p_{trend} = 0.6). A non-significant increased risk of NHL was found for high intake of total nitrite (Q4 median 0.86 vs Q1 22.2 mg/1,000 kcal, OR = 1.3, 95% CI: 0.8–1.9, p_{trend} = 0.4) and the increased risk seems to be related to nitrites from animal sources (Q4 median 0.41 vs Q1 0.16 mg/1,000 kcal, OR = 1.3, 95% CI: 0.8–1.9, p_{trend} = 0.3) but not from plant sources (Q4 median 0.53 vs Q1 0.26 mg/1,000 kcal, OR = 0.9, 95% CI: 0.6–1.4, p_{trend} = 0.9). Among women but not among men, a statistical significant increased risk was observed for high intake of nitrites from animal sources (Q4 median 0.41 vs 0.16 mg/1,000 kcal, OR = 1.9, 95% CI: 1.0–3.4), after controlling for age, BMI, education, family history of cancer, vitamin C and total daily energy intake. No increased risk was found for either nitrite (Q4 median 0.21 vs Q1 0.02 mg/1,000 kcal, OR = 1.0, 95% CI: 0.6–1.5, p_{trend} = 0.9) or nitrite plus nitrate (Q4 median 1.51 vs Q1 median 0.14 mg/1,000 kcal, OR = 1.0, 95% CI: 0.7–1.6, p_{trend} = 0.9) from processed meats. Other potential confounding factors (farming status, physical activity, alcohol consumption and the use of hair dyes) were also taken into consideration in the analysis but they did not change risk estimates. The response rate among cases and controls were 73.2% and 76.8%, respectively. No significant associations were observed for nitrate or nitrite by NHL subtype. The limitation of the study is the low response rate among cases and controls and the strength of the study is the good control for confounding factors and the risk estimates shown by different dietary sources.

Ecological

Chang et al. (2009) investigated the relationship between nitrate exposure from drinking water in Taiwan and mortality attributed to NHL. Data on all death of Taiwan from 2000 to 2006 were obtained from the Taiwan Provincial Department of Health. Death from NHL (cases) were compared with death from all other causes of death (controls). In total, 1,716 NHL were matched by sex, age and year of death with controls. Data on the nitrate–nitrogen concentration in water was obtained from Taiwan Water Supply Corporation. Nitrate intake was assumed to occur via the water supply of the municipality of residence of each participant. The levels of nitrate of each municipality were used as an indicator of exposure to nitrate for an individual residing in that municipality. The mean nitrate concentration in the drinking water of the NHL cases and controls was 0.45 mg/L (SD 0.47) and 0.41 (SD 0.41), respectively. No association was found between nitrate exposure and increased risk (≥ 0.48 vs≤ 0.18 mg/L; OR = 1.05, 95% CI: 0.89–1.24, p_{trend} = 0.75) of death from NHL, after controlling for sex, age and urbanisation level of residence. The limitation of the study is the study design (ecological) which does not allow us to assess whether there was a true exposure–outcome

relationship because there was no individual exposure data. There is no control for confounding factors.

Summary

In a previous report (IARC, 2010), five ecological studies (Weisenburger et al., 1987; Law et al., 1999; van Leeuwen et al., 1999; Gulis et al., 2002; Cocco et al., 2003), three case–control studies (Ward et al., 1996, 2006; Freedman et al., 2000) and one cohort study were described in relation to nitrate and NHL (Weyer et al., 2001). Out of the five ecological studies two studies, one in Europe (Gulis et al., 2002) and one in USA (Weisenburger et al., 1987) showed a positive association between nitrate levels in drinking water and NHL. Out of the three case–control studies reported in the IARC review (Ward et al., 1996, 2006; Freedman et al., 2000) one study conducted in Nebraska observed a positive association between NHL and nitrate in drinking water. In the cohort study, no association was observed for dietary nitrate (Weyer et al., 2001). After the IARC report, two cohort studies, two case–control studies and one ecological study were conducted to investigate the relationship between nitrate and NHL. Daniel et al. (2012a) showed no association between nitrate and nitrite from processed meat sources and NHL risk. In another cohort study in which the outcome was mortality for NHL, no association was observed for dietary nitrate (Aschebrook-Kilfoy et al., 2012a). Two case–control studies conducted in the United States reported no association between NHL and dietary nitrate (Kilfoy et al., 2010; Aschebrook-Kilfoy et al., 2013a). An ecological study conducted in Taiwan, found no association between NHL death and nitrate in drinking water (Chang et al., 2009).

Overall, the Panel concluded that there was no evidence of an association between dietary nitrate and Non-Hodgkin's lymphoma (NHL) and insufficient evidence to associate nitrate from drinking water and NHL.

3.6.8.15. Leukaemia

No new studies were found since IARC, 2010.

Summary

In a previous report (IARC, 2010), two ecological studies (Wu et al., 1993; Foster et al., 1997) and one cohort study (Weyer et al., 2001) were reviewed in relation to nitrate and the risk of leukaemia (Wu et al., 1993; Foster et al., 1997; Weyer et al., 2001). The ecological study conducted in the UK showed no association between levels of nitrate in public water supplies and incidence of leukaemia (Foster et al., 1997), whereas the study conducted in China showed a positive association between urinary levels of nitrate and mortality for leukaemia (Wu et al., 1993). In the cohort study, a non-statistically significant positive association was observed for dietary nitrate (Weyer et al., 2001). No other studies were conducted to investigate the relationship between nitrate and risk of leukaemia. Overall, the Panel, based on the IARC 2010 evaluation, considered that there was insufficient evidence that dietary nitrate and nitrate in drinking water are associated with leukaemia.

3.6.8.16. Brain cancer

Adult glioma

Cohort studies

Michaud et al. (2009), conducted a prospective study of meat intake and dietary nitrates, nitrites and nitrosamines and risk of adult glioma in the United States. They examined the relationship between intakes of meats, nitrate, nitrite and two nitrosamines (NDMA and NPYR) and glioma risk in a prospective analysis. Data from three US prospective cohort studies (Nurses' Health Study (NHS) I, NHS II, and male Health Professionals Follow-Up Study (HPFS)) were combined for this analysis. In total, 335 glioma cases were diagnosed during 24 years of follow-up. Habitual dietary intake was assessed with FFQs; these were initially collected in 1986 for 49,935 men (HPFS), in 1980 for 92,468 women (NHS I), and in 1991 for 95,391 women (NHS II), and diet was generally updated every 4 years. For the NHS I, they used a 61-item semiquantitative FFQ (including dietary items and vitamin use) at baseline in 1980, which was expanded to 130 items (including food, beverages and vitamin use) in 1984, 1986 and every 4 years thereafter. For the HPFS and NHS II cohorts, baseline dietary intake was assessed by using a 131-item FFQ. The questions on meat intake (other than fish) were very similar on the 61-item FFQs and the 131-item FFQs; both had the same number of questions with similar meat items included in each. Considered processed meat items were: bacon, hot dogs,

sausage, salami and bologna. Nitrate, nitrite and nitrosamine values were calculated based on the published values of these nutrients in various foods over different periods in time, preferably from US data, and from Europe otherwise. The median daily intake of nitrate was around 150 mg in HPFS, 96 mg in NHS I and 137 mg in NHS II. The median daily intake of nitrite was around 1.6 mg in HPFS, 1.4 mg in NHS I and 2.0 mg in NHS II. The median daily intake of NDMA was around 0.07 μg in HPFS, 0.06 μg in NHS I and 0.06 μg in NHS II. Cox proportional hazards models were used to estimate incidence RRs and 95% CIs. Estimates from each cohort were pooled by using a random effects model, and only combined effects were presented. After controlling for age, calendar period and caloric intake (and additionally vitamin C and E, coffee and tea), the risk of glioma was not elevated among individuals in the highest intake category of red meat (RR = 1.09, 95% CI: 0.62–1.93, p_{trend} = 0.57), total processed meats (RR = 0.92, 95% CI: 0.48–1.77, p_{trend} = 0.99), nitrate (RR Q5 vs Q1 (cut-offs are not presented because these vary for the three cohorts) = 1.02, 95% CI: 0.66–1.58, p_{trend} = 0.81), nitrite (RR Q5 vs Q1 = 1.26, 95% CI: 0.89–1.79, p_{trend} = 0.23), NDMA (RR Q5 vs Q1 = 0.88, 95% CI: 0.57–1.36, p_{trend} = 0.73) or NPYR (RR T3 vs T1 = 0.81, 95% CI: 0.62–1.05, p_{trend} = 0.93) compared with the lowest category. No effect modification was observed by intake of vitamins C or E or other antioxidant measures. It was concluded there was no suggestion that the intake of meat, nitrate, nitrite or nitrosamines is related to the risk of glioma. Other factors typically considered as potential confounders in cancer analyses (e.g. smoking, BMI, and fruit and vegetables) were not included in the models because they were not considered as risk factors for glioma in these cohorts. The strengths of this study are the inclusion of three large prospective cohort studies among men and women, with multiple measurements of dietary intake per person, coupled with a long follow-up. The study lacked information on nitrate intake from drinking water.

Dubrow et al. (2010) studied adult glioma risk relative to endogenous N-nitroso compound (NOC) formation in the prospective NIH-AARP Diet and Health Study. The NIH-AARP Diet and Health Study recruited men and women, aged 50–71 years, from six states in the United States. At baseline (1995–1996), participants completed self-administered demographic and lifestyle questionnaires, including a 124-item FFQ. A total of 566,402 participants returned the baseline questionnaire. After excluding prevalent brain cancer cases, those who had questionnaires completed by proxy respondents and those with implausible nutrient values, the final analytical cohort consisted of 545,770 participants (322,347 men and 223,423 women). The FFQ was used in association with published information (from United States and Canada preferably) on nitrate content of various foods to estimate daily nitrate intake. The daily median intake of nitrate was estimated to be 40.95 mg/1,000 kcal, for nitrite this was 0.65 mg/1,000 kcal. Daily intake of vitamins C and E was also estimated. After a mean follow-up of 7.2 years, they identified 585 incident glioma cases. Cox proportional hazards models were used. After controlling for age, race, total energy intake, education, height and family history of cancer (smoking was not associated with glioma), they found significant positive trends for nitrite intake from plant sources (HR for quintile 5 (med 0.68 mg/1,000 kcal) vs Q1 (0.25) = 1.59, 95% CI: 1.20–2.10, p_{trend} = 0.028) and total nitrite intake (HR Q5 (med 0.90 mg/1,000 kcal) vs Q1 (0.45) = 1.32, 95% CI: 1.01–1.71, p_{trend} = 0.089) and, unexpectedly, for fruit and vegetable intake (HR = 1.42, 95% CI: 1.08–1.86, p_{trend} = 0.0081). Processed meat consisted of red and white meat sources of bacon, sausage, luncheon meats, cold cuts, ham and hot dogs. No significant trend in glioma risk for consumption of processed red meat (HR = 1.05, 95% CI: 0.80–1.37, p_{trend} = 0.44), nitrate (HR Q5 (94.85 mg/1,000 kcal) vs Q1 (19.35) = 1.28, 95% CI: 0.97–1.70, p_{trend} = 0.14) or vitamin C or E was detected. Examination of interactions between dietary intakes (e.g. nitrite and vitamin C) and a limited analysis of diet at ages 12–13 years provided no support for the NOC hypothesis. Accordingly, the study suggests that the consumption of processed or red meat, nitrite or nitrate does not meaningfully increase adult glioma risk and that the consumption of fruit and vegetables, fruit and vegetable subgroups, vitamin C or vitamin E does not meaningfully protect against adult glioma risk. The strength of this study is the inclusion of a large cohort of men and women. The study lacked information on nitrate intake from drinking water.

Childhood brain tumours

Case–control studies

Weng et al. (2011) investigated the relationship between nitrate in the public water supply and the risk of childhood brain tumours in a case–control study. Data on all deaths of Taiwan residents between 1999 and 2008 was provided by the provincial health department statistics bureau. All eligible individuals with malignant tumours of the brain or cranial nerves between 0 and 19 years old were included in the cancer group. In total, 457 deaths (cases) in accordance with the criteria were included

(190 females and 267 males). Controls were deaths from other causes and were pair-matched to the cases by gender, year of birth and year of death. Detailed demographic information and residential district were recorded. Information on the levels of nitrate, Ca and Mg in drinking water was collected from the Taiwan Water Supply Corporation. The municipality of residence for childhood brain tumours cases and controls was presumed to be the source of the subject's NO_3, Ca and Mg exposure via drinking water. In the analysis, the subjects were categorised into one of the two NO_3 exposure categories: low (less than or equal to median among controls; $\leq$ 1.37 mg/L) and high (greater than median among controls; $>$ 1.37 mg/L). Conditional logistic regression was used to estimate associations, adjusting for age, gender and urbanisation level of residence. Relative to individuals whose NO_3 exposure level was $\leq$ 1.37 mg/L, the adjusted OR (95% CI) for childhood brain tumour occurrence was 1.4 (1.07–1.84) for individuals who resided in municipalities served by drinking water with a NO_3 exposure $>$ 1.37 mg/L. No significant effect modification was observed by Ca and Mg intake via drinking water. These data suggest that exposure to NO_3 in drinking water is associated with a higher risk of childhood brain tumour development in Taiwan. However, the study lacked information on nitrate intake from food, and the consumption volume of water was also not known.

Summary

Adults

In a previous review, which considered publications until 2006 (IARC, 2010), six case–control studies on adult brain cancer (often glioma) and nitrate in food or drinking water were reviewed. No associations were found with nitrate. Subsequently, two cohort studies were published. Michaud (2009) found no significant associations with adult glioma (335 incident cases) in three cohorts of men and women with dietary nitrate, with no effect modification by vitamin C or E or other antioxidants. In the other cohort study (with 585 incident cases), no association was found with glioma risk for dietary nitrate, nor was there any interaction with vitamin C or E (Dubrow, 2010). According to Dubrow et al. (2010), their study suggests that the consumption of nitrate does not meaningfully increase adult glioma risk.

Overall, the Panel concluded that there was no evidence that nitrate intake is associated with increased glioma in adults.

Childhood brain tumours

In a previous review, which considered publications until 2006 (IARC, 2010), four case–control studies on childhood brain tumours and nitrate in drinking water were reviewed. No associations were found with nitrate. Subsequently, only one case–control study has been reported; this was based on 457 deceased childhood brain tumour cases in Taiwan in relation to nitrate levels in drinking water (Weng, 2011). This study reported a statistically significant positive association with nitrate, although the consumption volume of water was not known, and the study lacked information on nitrate intake from food.

Overall, the Panel concluded that there was no evidence that nitrate intake is associated with increased risk of childhood brain tumours.

3.7. Discussion

Nitrates are used together with nitrites in the curing mixtures for meat processing to produce and fix the colour, to inhibit microbial growth and to develop characteristic flavours. Potassium or sodium nitrate is also used in the manufacture of certain types of cheeses for the prevention of blowing defect by microorganisms (Korenekova et al., 2000). Regarding stability in food, most information is related to vegetables, where nitrate naturally occur, in which the decrease observed during storage is mainly due to its conversion to nitrite. Washing and thermal treatment of vegetables in water can also cause a reduction of nitrate concentration (Tamme et al., 2009). When used as an additive, nitrate is reduced by microorganisms during processing and storage to nitrite that forms nitrous anhydride, which participates in the nitrosamine formation. Nitrate also contributes to the characteristic pink colour of cured meat products by its conversion to nitrite and the reaction of the formed nitric acid and NO with myoglobin.

In humans, dietary nitrate is rapidly and extensively absorbed through the gastrointestinal tract (Iijima et al., 2002). Absorbed nitrate is recirculated through the salivary glands where bacterial metabolism converts the secreted nitrate into nitrite. Some gastric nitrite is absorbed into the general circulation where it can oxidise haemoglobin to form methaemoglobin while being reduced to nitric oxide. Nitrate biotransformation comprises nitrate reduction, nitrite formation, nitrite reoxidation to nitrate, and formation of methaemoglobin or NO, in a dynamic equilibrium (Gladwin et al., 2005;

Lundberg et al., 2008). Due to the very low gastric pH, very little further reduction of nitrate to nitrite occurs in man (Mirvish, 1975). It has been shown that nitrate is nearly all excreted by the kidneys.

The acute toxicity of nitrate observed in the available studies was low. The main acute toxicological effect of nitrate intoxication is secondary to nitrite-dependent formation of methaemoglobin. Individuals with a deficiency in glucose-6-phosphate dehydrogenase, a common trait among some Asian populations, might be at higher risk of developing methaemoglobinaemia. Examples of acutely induced methaemoglobinaemia have been described in Section 3.6.2. Another population subgroup that is more susceptible to the effects of methaemoglobin formation is infants under 3 months of age.

Short-term and subchronic toxicity studies in rats showed, overall, that nitrate intake of up to 5% in the diet (equivalent to 4,500 mg sodium nitrate/kg bw per day) did not result in adverse effects in rats. At higher dose levels, animals showed signs of methaemoglobinaemia leading to the death of the animals.

In vitro studies on sodium and potassium nitrate in bacteria and mammalian cells did not provide evidence of a genotoxic potential. In mammals, no reliable indication of genotoxicity was obtained in mice and rats exposed to nitrate by the oral route, both in somatic and in germ cells. Although the database was limited, the Panel concluded that the available experimental data indicated that nitrate salts do not raise concern for genotoxicity.

Chronic toxicity and carcinogenicity studies with sodium and potassium nitrate were available. In studies with mice, sodium nitrate did not show any difference in tumour incidences compared to controls. Four non-standard studies in rats and pigs assessed haematological parameters or effects on thyroid and thyroid-related hormones (Boink et al., 1995; JECFA, 1996; Zaki et al., 2004; Mukhopadhyay et al., 2005; Azeez et al., 2011). Overall, the Panel considered that nitrate did not affect adrenal and thyroid glands function in animals and it was not carcinogenic in animal studies.

The more recent toxicity study, addressing carcinogenicity aspects, was a non-standard study by Del Negro et al. (2008). The study investigated the carcinogenic potential of hydrochloric acid, pepsin and sodium nitrate on oropharyngeal mucosa in rats. The study has been included for completeness and supported the lack of carcinogenicity of nitrate itself; however, due to the combined treatment and subchronic duration of the study, it could not be considered for ADI determination.

No effects were observed in a reproductive/developmental toxicity screening study (OECD TG 422) in rats administered potassium nitrate by gavage at doses up to 1,500 mg/kg bw per day. No developmental toxicity was observed in mice, rats, hamsters or rabbits receiving doses up to 400, 1,980, 280 or 206 mg potassium nitrate/kg bw per day by gavage, respectively. In a reproductive toxicity study in mice given potassium nitrate in drinking water, effects were observed on sperm count and testicular enzymes at the highest dose tested, at which also sperm abnormalities were observed; the NOAEL in this study was 122 mg potassium nitrate/kg bw per day. Histopathological changes in testis, epididymis and other sex organs were reported in this study. A conclusion could not be reached since the duration of the dosing in males in this screening study was limited and the number of animals tested was low.

Overall, the Panel noted that although some effects were observed in sperm analysis and reproductive organs in this limited study in mice, no indications of reproductive toxicity were observed at higher doses in a rat study conducted according to OECD guideline TG 422.

Human non-cancer effects were observed in the thyroid in several studies, suggesting that nitrate exposure altered human thyroid gland function by competitively inhibiting thyroidal iodide uptake. A large cohort study (n = 21,977 women) showed that increasing intake of nitrate from dietary sources was associated with an increasing occurrence of hypothyroidism (Ward et al., 2010). In addition, in several studies, an enlarged thyroid or even goitre was observed when the intake of nitrate via drinking water was high. Other studies, however, showed no effects. Overall, there was some evidence to relate exposure to nitrate with the development of enlarged thyroid, goitre and hypothyroidism. The exposure levels were in the same range as that within which methaemoglobinaemia was observed.

Another non-cancer effect in humans was the lowering of blood pressure, which was due to the conversion of nitrate to vasoactive nitric oxide (NO). IARC (2010) reported epidemiological studies on type I diabetes mellitus and exposure to nitrates and/or nitrites. In most of the studies reported by IARC, exposure to nitrates in children or the mothers was associated with a decreased risk of type I diabetes or no significant effects were identified in children. IARC (2010) also reported epidemiological studies suggesting association between exposure to nitrates in drinking-water and spontaneous abortions, intrauterine growth restrictions and birth effects. However, IARC noticed that those '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'.

The methaemoglobin formation reported in animal studies can also be observed in humans. This effect occured both, upon acute exposure as well as after chronic exposure, to nitrate. The effect was

a consequence of the endogenous production of nitrite from ingested nitrate as described in the ADME section (Section 3.6.1).

Potential carcinogenicity of nitrate and nitrite in humans has been extensively reviewed by IARC (2010), and the epidemiological studies discussed in the IARC report have therefore not been described in this opinion. The interested reader is invited to consult the IARC report for details of all these studies. The overall conclusions of the Panel were based on IARC evaluation of the epidemiological studies on nitrate, nitrite and cancer published until 2006 (IARC, 2010) and on the evaluation of epidemiological studies published subsequently.

The summary evidence for an association between nitrate exposure and each type of human cancer was categorised by the Panel as: (i) *there was no evidence* for an association, if studies indicate no association with a specific cancer; (ii) *there was insufficient evidence,* to (unequivocally) link to a cancer (e.g. few studies, contradictory results, etc.); (iii) *there was some evidence* for an association with a specific cancer (e.g. inconsistent results between cohort studies and case–control studies); (iv) *there was evidence,* for an association with a specific cancer (e.g. consistent results from cohort studies and case–control studies).

The Panel concluded that *there was no evidence* for a positive association between: ingested nitrate and oesophageal cancer and its subtypes oesophageal squamous cell carcinomas and oesophageal adenocarcinoma (ESCC and EAC); ingested nitrate and gastric cancer or its subtypes gastric cardia adenocarcinoma (GCA) and gastric non-cardia adenocarcinoma (GNCA); dietary nitrate and colorectal cancer (CRC) or colon or rectum cancer; ingested nitrate and pancreatic cancer; ingested nitrate and lung cancer; dietary nitrate and non-Hodgkin lymphoma (NHL); ingested nitrate and breast cancer; ingested nitrate and renal cell cancer; and ingested nitrate and adult glioma or childhood brain tumours.

There was insufficient evidence for a positive association between: nitrate from processed meat and colorectal cancer (CRC) or its subtypes; drinking water nitrate and CRC or its subtypes; drinking water nitrate and non-Hodgkin lymphoma (NHL); ingested nitrate and leukaemia; ingested nitrate and ovarian cancer; ingested nitrate and bladder cancer; ingested nitrate and prostate cancer; and ingested nitrate and thyroid cancer.

There were insufficient data to draw conclusions on: ingested nitrate and head and neck cancer and ingested nitrate and liver cancer.

In its 1990 evaluation, the SCF established an ADI of 5 mg/kg bw per day for sodium nitrate (SCF, 1992) based on the NOAEL of 2,500 mg sodium nitrate/kg bw per day the highest dose tested in a long-term rat study by Maekawa et al. (1982) and using a safety factor of 500 (SCF, 1992, 1997). The SCF justification of the safety factor was based on the fact that the rat was not considered a good model for man due to the absence of salivary secretion of nitrate and its oral conversion to nitrite. In 1995, the SCF set an ADI of 0–5 mg/kg bw per day for sodium nitrate (equivalent to 0–3.7 mg/kg bw per day as nitrate ion) based on the same study (SCF, 1997). However, the Panel noted the high incidence of tumours in all groups within the study, including the controls, and considered that this could have affected the sensitivity of the study to detect adverse effects attributable to nitrate.

In its evaluation, JECFA commented that, in the two separate long-term feeding studies in rats, doses of 370 and 1,820 mg/kg bw per day, expressed as nitrate ion, failed to produce any adverse effects (JECFA, 1996). JECFA considered, however, that the study in which a dose of 1,820 mg/kg bw per day was reported was solely a carcinogenicity study and could not be considered for deriving a NOEL. Overall, JECFA considered that 'on the basis of the NOEL of 370 mg of nitrate ion/kg bw per day in the long-term study in rats and a safety factor of 100, an ADI of 0–5 mg/kg bw per day, expressed as sodium nitrate, or 0–3.7 mg/kg bw per day expressed as nitrate ion, could be allocated'. JECFA further did a back calculation from nitrite to nitrate and concluded that 'if the proportion of nitrate converted to nitrite in humans is taken as 5% (mol/mol) for normally responding individuals and 20% for those showing a high level of conversion and the NOEL for nitrite (6 mg/kg bw/day expressed as nitrite ion) is used, the "transposed" NOELs for nitrate, expressed as nitrate ion, would be 160 and 40 mg/kg bw/day, respectively. As these figures are derived in part from human pharmacokinetic data, the use of a safety factor of less than 100 is justified. If the data on individuals showing a high level of conversion are used, a safety factor of 10 would be justified because intraindividual differences have already been taken into account. On the basis of the "transposed" NOEL for nitrate of 160 mg/kg bw per day for normally responding persons (5% rate of conversion) and a safety factor of 50, an ADI of 0–3.2 mg/kg bw, expressed as nitrate ion, could be allocated. These two methods of deriving the ADI for nitrate thus resulted in similar figures, and the Committee at its forty-fourth meeting therefore retained the previously established ADI of 0–3.7 mg/kg bw, expressed as nitrate ion' (JECFA, 1996).

The Panel noted that the study on which the original JECFA ADI of 3.7 mg nitrate ion/kg bw per day was established using the NOEL of 370 mg nitrate ion/kg bw per day and an uncertainty factor of 100 was probably the study of Lehman from 1958, the full report of which was not available to the Panel. Summarised information obtained by the Panel (Documentation provided to EFSA n.6) on the Lehman's study lead to the conclusion that sodium nitrate at doses up to 10% in the feed (equivalent to 5,000 mg/kg bw per day) did not induce tumour increases related to the treatment. However, the Panel was not able to independently evaluate the full study and therefore considered that there was uncertainty about the basis for this ADI. The Panel noted that in 1996, JECFA itself appeared to have reservations concerning the evidence underlying the ADI and also used an indirect derivation from nitrite with a 5% conversion of the nitrate dose to nitrite and an uncertainty factor of 50 to derive an ADI of 3.2 mg nitrate ion/kg bw per day. JECFA (1996) concluded that the existing ADI of 3.7 mg nitrate ion/kg bw per day should be retained since the values obtained in its two evaluations were similar. The Panel noted that the rationale for using an uncertainty factor of 50 was not explicitly stated but the Panel presumed that the uncertainty factor incorporated kinetic variability, meaning this component of the uncertainty factor was not required. However, the Panel could not exclude that JECFA has modified the uncertainty factor because of smaller dynamic variability in the response. The Panel noted that using the current WHO/IPCS CSAF approach this uncertainty factor would be modified by a factor 2.5 and 4, depending on the basis of these corrections which would give a total uncertainty factor between 25 and 40 rather than 50 (resulting in potential ADI values of 4 to 6 mg/kg bw per day). The Panel agreed with JECFA view that the values obtained by either approach were essentially identical since they were derived from empirical NOELs which were determined by the doses independently chosen for each study (nitrate and nitrite) and which were not designed to be identical. Despite the reservations expressed above over, the reliability of study used to derive an ADI by the SCF, the Panel noted that the numerical values of the SCF and JECFA ADIs were broadly comparable.

The human studies on goitre and the single study on hypothyroidism were considered not sufficient for deriving reference points for a health-based guidance value.

Hence, in the absence of other adverse effects and the unavailability of the original 1958 study used by JECFA and the database currently available, the Panel decided that the most relevant approach for assessing the toxicity of nitrate would be methaemoglobinaemia induced by nitrite formed from nitrate excreted in the saliva, once absorbed. The Panel review the reported information on secretion estimates of nitrate into the mouth in humans and observed that most estimates available varied between 20% and 25% of the dose (Spiegelhalder et al., 1976, Bartholomew and Hill, 1984). Additionally, the Panel noted that these studies were quite old and had limitations (it might be possible to obtain more accurate estimates nowadays). The Panel therefore considered that adequate, well conducted modern studies might decrease the uncertainty associated with this estimate.

The Panel noted that available estimates of the ratio of concentrations of nitrite to nitrate varied within and between individual subjects. The nitrate-to-nitrite conversion was estimated to range from 5% to 36% (e.g. Spiegelhalder et al., 1976; Wagner et al., 1983; Bartholomew and Hill, 1984; Bos et al., 1988; Granli et al., 1989; Shapiro et al., 1991; Jin et al., 2013; Bondonno et al., 2015; Hohensin et al., 2016; Montenegro et al., 2016; Woessner et al., 2016). The Panel noted that the available studies were carried out in diverse and differing populations. The Panel considered that to reflect the uncertainties in the underlying data and interindividual variability in conversion, it was appropriate to use a range of values for the conversion percentage of nitrate to nitrite in the saliva.

The Panel considered that any single estimate of the overall conversion of ingested nitrate to nitrite would not be reliable. The Panel did not consider it was possible to derive a single value as ADI from the data available.

Based on the secretion rates of nitrate (20–25%) into the saliva and the range of conversion rates of nitrate to nitrite (5–36%) in the mouth, a range for the overall conversion percentage between 1% and 9% would be estimated. Using this range and considering the ADI of nitrite (0.07 mg nitrite ion/kg bw per day), the ADI values estimated for nitrate would be between 1.05 and 9.4 mg nitrate ion/kg bw per day. The current ADI established by the SCF (1997) (3.7 mg nitrate ion/kg bw per day) falls within those estimates. The Panel considered that, as a point estimate, the current ADI was likely to be as accurate as this range.

The Panel also took into consideration the lack of overt toxicity data reported in the available studies in animals and that there was no cancer concerns overall from epidemiological studies in humans. Despite the uncertainty associated with the ADI set by the SCF (1997) due to the inability to examine its basis thoroughly, the Panel considered that currently there was insufficient evidence to withdraw this ADI.

The Panel recognised that further data from human studies were warranted. These studies could target the secretion of nitrate in the saliva and the conversion of nitrate to nitrite in the mouth, as well as the subsequent effect of increased nitrite blood concentrations, namely increase in methaemoglobinaemia, or assess the effects of nitrate on thyroid function in a well conducted study. Since these data might also affect evaluations in other areas of EFSA's remit, the Panel recognised that an integrated risk assessment would be required to best define further data requirements.

The Panel noted that nitrates (E 251–E 252) were authorised for use in a wide range of foods and it was therefore not expected that brand-loyalty would result in higher exposure in the general population. The Panel therefore selected the non-brand loyal scenario refined scenario as the most relevant exposure scenario for the safety evaluation of these food additives.

From its *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008, in the *non-brand-loyal scenario*, mean exposure to nitrates (expressed as nitrate ion) from their use as food additives (E 251–E 252) ranged from 0.01 mg/kg bw per day in infants to 0.09 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates (expressed as nitrate ion) ranged from 0.05 mg/kg bw per day in the elderly to 0.22 mg/kg bw per day in toddlers.

From these exposure scenarios considered for exposure assessment of nitrates (E 251–E 252) from their use as food additives, the most important contributors to the total mean exposure for all population groups were meat products (preserved meat and sausages) and cheese, whereas fish and fishery products contributed less.

From the exposure scenario considering the exposure to nitrates (expressed as nitrate ion) from all sources (food additives, natural presence and contamination), applying reduction factors for vegetables, mean exposure ranged from 0.97 mg/kg bw per day in the elderly to 4.15 mg/kg bw per day in toddlers. The 95th percentile of exposure to nitrates ranged from 1.59 mg/kg bw per day in the elderly to 8.73 mg/kg bw per day in children.

The main contributing food categories from the exposure scenario considering all sources (food additives, natural presence and contamination across surveys and population groups, range min–max), were vegetables and vegetable-based foods for all population groups (0–29%). In particular, the main contributing food categories were starchy roots and tubers in infants, toddlers and children (4–35%), whereas the leafy vegetables (0.4–47%) and prepared salads (0–44%) were the main contributors in children, adolescents, adults and the elderly. In infants, also foods for infants and toddlers (4–33%) made an important contribution to the total mean exposure to nitrates from all sources.

The Panel estimated that, when comparing all sources (food additives, natural presence and contamination), including reduction factors for vegetables, using the same refined exposure methodology (non-brand-loyal consumer scenario for general population), the contribution of nitrates (E 251–E 252) from their use as food additives is less than 5% of contribution to the overall exposure to nitrates from all sources in any scenario and for any population group (approximately 2% in average, range 0.4–4.4%).

The Panel noted that exposure to nitrates from their use as food additives was below the ADI of 3.7 mg/kg bw per day by using the most appropriate refined exposure scenario (non-brand loyal). The estimates for the high level consumers in all population groups were approximately 15% or less of the current ADI. As such, there would not be a safety concern from the current uses and use levels of nitrate as a food additive.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to nitrates (E 251–E 252) as a food additive in European countries for the regulatory maximum level exposure scenario and for the refined scenario considering that it was not possible to include a number of restrictions.

Based on a *refined estimated exposure scenario*, the exposure to nitrate resulting from its use as a food additive does not lead to an exceedance of the ADI of nitrates.

The Panel noted that total dietary exposure to nitrate from all sources exceeded the current ADI in all populations considered. Assessing whether or not the total dietary intake of nitrate was a safety concern was outside the scope of this re-evaluation and beyond the remit of the ANS Panel.

The formation of NOCs is a key step when considering the carcinogenic risk of nitrites (EFSA ANS Panel, 2017). In order to be able to estimate the amount of nitrosamines formed from nitrate, its conversion to nitrite needs to be quantifiable. In view of the large variability observed among the populations on the proportion of nitrite that can be formed from nitrate in the mouth, the Panel was unable to derive a point estimate for nitrates with sufficient reliability as a starting point for the calculation of the amount of endogenously formed nitrosamines from this source.

4. Conclusions

The available data did not indicate genotoxic potential for sodium and potassium nitrate. Furthermore, carcinogenicity studies in mice and rats were negative. Therefore, the Panel concluded that an ADI for nitrate *per se* could be derived from general toxicity data.

The Panel concluded that, although there was some evidence that nitrate intake from drinking water was associated with goitre, the single study on the association between self-reported hypothyroidism and estimated dietary nitrate intake (Ward et al., 2010) was considered insufficient for deriving a reference point for an ADI.

The Panel considered the derivation of an ADI for nitrate based on the formation of methaemoglobin, following the conversion of nitrate, excreted in the saliva, to nitrite. However, there were large variations in the data available on the conversion of nitrate to nitrite in the saliva in humans, making any estimate of the overall conversion of ingested nitrate to nitrite unreliable. Therefore, the Panel did not consider it was possible to derive a single value from the data available.

The range of ADI values that could be derived from the range of values for conversion of ingested nitrate to nitrite would also cover the existing ADI set by the SCF (1997) (0–3.7 mg nitrate ion/kg bw per day). The Panel considered that even using the highest nitrate-to-nitrite conversion factor of 9% a dose corresponding to the ADI of 3.7 mg/kg bw per day will be converted into 0.25 mg nitrite ion/kg bw per day. With this dose, the methaemoglobin levels will increase above background levels (1–3%) which is measurable, but not clinically significant. Furthermore, ingestion of 0.25 mg/kg bw per day nitrite ion would lead to endogenous N-nitroso compounds (ENOCs) production of 8.22×10^{-7} mg/kg bw per day calculated using the formula given in the Guideline for Canadian Drinking Water (Health Canada, 2013). Then, the MoE between the produced amount of ENOCs and the $BMDL_{10}$ of *N*-nitroso-dimethylamine (NDMA) (0.027 mg/kg bw per day) would be 3.2×10^4 which is still above the MoE of 10,000 considered by the Scientific Committee as of low concern from a public health point of view (EFSA, 2005; EFSA Scientific Committee, 2012b).

In concluding this, the Panel also took into consideration the lack of overt toxicity reported in the available studies in animals and that there was no cancer concerns overall from epidemiological studies in humans. Despite the uncertainty associated with the ADI set by the SCF (1997), the Panel concluded that currently there was insufficient evidence to withdraw this ADI.

The Panel concluded that exposure to nitrate resulting solely from its use as a food additive, estimated to be less than 5% of the overall exposure to nitrate in food based on the *refined estimated exposure scenario*, did not lead to an exceedance of the ADI. However, if all sources of dietary nitrate exposure are considered (food additive, natural presence and contamination), the ADI will be exceeded for all age groups at the mean and highest exposure.

5. Recommendations

The Panel recommended further human studies with a better control of confounding factors (e.g. radiation exposure dietary iodine intake and other anions that compete with iodide uptake in the thyroid) are needed to confirm the findings in thyroid gland.

The Panel recommended that additional experimental studies in humans measuring the excretion of nitrate into the saliva and its conversion to nitrites and the consequent methaemoglobin formation should be conducted in order to reduce uncertainties.

The Panel recommended that further studies on the levels of nitroso compounds formed in different meat products with known ingoing amounts of nitrates/nitrites added, with appropriate controls and with specified levels of detection (LOD) and levels of quantification (LOQ) for potentially formed nitroso- compounds would be necessary.

The Panel recommended that the European Commission considers lowering the current limits for toxic elements (lead, mercury and arsenic) in the EU specifications for nitrates (E 251–E 252) in order to ensure that nitrates (E 251–E 252) as a food additive will not be a significant source of exposure to those toxic elements in food.

Documentation provided to EFSA

1) MARS Chocolate UK. Data submitted to EFSA on 23 April 2010.
2) BASF. Characterisation and particle size distribution of Natriumnitrat E 250 mit SiO_2. Data submitted to EFSA on 21 June 2010.

3) FDA, 2010. Potassium Nitrate PAFA.
4) FDA, 2010. Sodium Nitrate PAFA.
5) FDA, 2010. FAP3T2840.
6) FDA, 2017. Pathological study of rats fed up to 10% sodium nitrate in the diet for 2 years, January 2017.
7) FDE (Food Drink Europe), 2013. Data on usage levels of nitrates (E 251- E 252) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2013). Submitted to EFSA on 29 November 2013.

References

Alavantić D, Šunjevarić I, Pečevski J, Bozin D and Cerović G, 1988a. In vivo genotoxicity of nitrates and nitrites in germ cells of male mice. I. Evidence for gonadal exposure and lack of heritable effects. Mutation Research/ Genetic Toxicology, 204, 689–695.

Alavantić D, Sunjevarić I, Cerović G, Bozin D and Pecevski J, 1988b. In vivo genotoxicity of nitrates and nitrites in germ cells of male mice. II. Unscheduled DNA synthesis and sperm abnormality after treatment of spermatids. Mutation Research, 204, 697–701.

Andrade R, Viana CO, Guadagnin SG, Reyes FGR and Rath S, 2003. A flow-injection spectrophotometric method for nitrate and nitrite determination through nitric oxide generation. Food Chemistry, 80, 597–602.

AOAC, 2005. *Official Methods of Analysis of AOAC International, 17th*. AOAC International, Gaithersburg, MD.

Armenteros M, Aristoy MC and Toldrá F, 2012. Evolution of nitrate and nitrite during the processing of dry-cured ham with partial replacement of NaCl by other chloride salts. Meat Science, 91, 378–381.

Aschebrook-Kilfoy B, Cross AJ, Rachael Z, Stolzenberg-Solomon RZ, Schatzkin A, Hollenbeck AR, Sinha R and Ward MH, 2011. Pancreatic cancer and exposure to dietary nitrate and nitrite in the NIH-AARP Diet and Health Study. American Journal of Epidemiology, 174, 305–315.

Aschebrook-Kilfoy B, Ward MH, Zheng T, Holford TR, Boyle P, Leadere B and Zhang Y, 2012a. Dietary nitrate and nitrite intake and Non-Hodgkin lymphoma survival. Nutrition and Cancer, 64, 488–492.

Aschebrook-Kilfoy B, Ward MH, Gierach GL, Schatzkin A, Hollenbeck AR, Sinha R and Cross AR, 2012b. Epithelial ovarian cancer and exposure to dietary nitrate and nitrite in the NIH-AARP Diet and Health Study. European Journal of Cancer Prevention, 21, 65–72.

Aschebrook-Kilfoy B, Ward MH, Dave BJ, Smith SM, Weisenburger DD and Chiu BC, 2013a. Dietary nitrate and nitrite intake and risk of non- Hodgkin lymphoma. Leukemia and Lymphoma, 54, 945–950.

Aschebrook-Kilfoy B, Shu XO, Gao YT, Ji BT, Yang G, Li HL, Rothman N, Chow WH, Zheng W and Ward MH, 2013b. Thyroid cancer risk and dietary nitrate and nitrite intake in the Shanghai women's health study. International Journal of Cancer, 132, 897–904.

Asero R, 2005. Food additive-induced chronic pruritus: further evidence. Clinical and Experimental Dermatology, 30, 719–720.

Azeez OH, Mahmood MB and Hassan JS, 2011. Effect of nitrate poisoning on some biochemical parameters in rats. Iraqi Journal of Veterinary Sciences, 25, 47–50.

Baghurst PA, McMichael AJ, Slavotinek AH, Baghurst KI, Boyle P and Walker AM, 1991. A case-control study of diet and cancer of the pancreas. American Journal of Epidemiology, 134, 167–179.

Bako IG, Mabrouk MA and Abubakar A, 2009. Antioxidant effect of ethanolic seed extract of Hibiscus sabdariffa Linn (Malvaceae) alleviate the toxicity induced by chronic administration of sodium nitrate on some haematological parameters in Wistar rats. Advance Journal of Food Science and Technology, 1, 39–42.

Bartholomew B and Hill MJ, 1984. The pharmacology of dietary nitrate and the origin of urinary nitrate. Food and Chemical Toxicology, 22, 789–795.

Bartos HR and Desforges JF, 1966. Red blood cells DPNH-dependent diaphorase levels in infants. Pediatrics, 37, 991–993.

Below H, Zöllner H, Völzke H and Kramer A, 2008. Evaluation of nitrate influence on thyroid volume of adults in a previously iodine-deficient area. International Journal of Hygiene and Environmental Health, 211, 186–191.

Bernini R, Picconi F, Iacovacci V and Abballe F, 2001. Analisi di salumi mediante cromatografia ionica – II: nitrito e Nitrato. Industrie Alimentari, XL, 741–746.

Boink BTJ, Dormans JAMA and Speijers GJA, 1995, The role of nitrite and/or nitrate in the etiology of the hypertrophy of the adrenal zona glomerulosa of rats. Health aspects of nitrates and its metabolites (particularly nitrite). Proceedings International Workshop. Bilthoven, Netherlands, 8–10 November 1994. Council of Europe Press, Bilthoven, The Netherlands, pp. 213–227.

Boink ABTJ, Beekhof PK, Dormans JAMA and Speijers GJA, 1996. On the etiology of nitrite-induced hypertrophy of the zona glomerulosa of rats: I. The possible role of nitrate. Report No. 235802003 of the National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands, pp. 1–19, as referred to in JECFA, 2005.

Boink ABTJ, Dormans JAMA, Speijers GJA and Vleeming W, 1999. Effects of nitrates and nitrites in experimental animals. Special Publication-Royal Society of Chemistry, 237, 317–326.

Bondonno CP, Liu AH, Croft KD, Ward NC, Puddey IB, Woodman RJ and Hodgson JM, 2015. Short-term effects of a high nitrate diet on nitrate metabolism in healthy individuals. Nutrients, 7, 1906–1915.
Bos PMJ, Van Den Brandt PA, Wedel M and Ockhuizen T, 1988. The reproducibility of the conversion of nitrate to nitrite in human saliva after a nitrate load. Food and Chemical Toxicology, 26, 93–97.
Bouchikhi B, Mavelle T and Debry G, 1999. Effect of the addition of nitrate to milk on the formation of volatile N-nitrosamines and apparent total N-nitroso compounds. European Food Research and Technology, 209, 88–92.
Brody JG, Aschengrau A, McKelvey W, Swartz CH, Kennedy T and Rudel RA, 2006. Breast cancer risk and drinking water contaminated by wastewater: a case control study. Environmental Health, 6, 28.
Butt SB, Riaz M and Iqbal MZ, 2001. Simultaneous determination of nitrite and nitrate by normal phase ion-pair liquid chromatography. Talanta, 55, 789–797.
Casanova JA, Gross LK, Mcmullen SE and Schenck FJ, 2006. Use of Griess reagent containing vanadium(III) for post-column derivatization and simultaneous determination of nitrite and nitrate in baby food. Journal of AOAC International, 89, 447–451.
Catsburg CE, Gago-Dominguez M, Yuan JM, Castelao JE, Cortessis VK, Pike MC and Stern M, 2014. Dietary sources of N-nitroso compounds and bladder cancer risk: findings from the Los Angeles bladder cancer study. International Journal of Cancer, 134, 125–135.
CEN (European Committee for Standardization), 1997. Foodstuffs – Determination of nitrate and/or nitrite content. Part 1: General considerations. EN 12014-1:1997.
CEN (European Committee for Standardization), 2005a. Foodstuffs – Determination of nitrate and/or nitrite content. Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite. EN 12014-3:2005.
CEN (European Committee for Standardization), 2005b. Foodstuffs – Determination of nitrate and/or nitrite contentPart 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products. EN 12014-4:2005.
Chang CC, Tsai SS, Wu TN and Yang CY, 2009. Nitrates in municipal drinking water and non-Hodgkin lymphoma: an ecological cancer case-control study in Taiwan. Journal of Toxicology and Environmental Health, Part A, 73, 330–338.
Chaoui A, Zaki A, Talibi A, Chait A, Derouiche A, Aboussaouira T, Benabdjlil F and Himmi T, 2004. Effets des nitrates inorganiques sur l'aspect morphofonctionnel de la glande thyroide chez le rat femelle. Therapie, 59, 471–475.
Chasco J, Lizaso G and Beriain MJ, 1996. Cured colour development during sausage processing. Meat Science, 44, 203–211.
Chiu HF, Tsai SS and Yang CY, 2007. Nitrate in drinking water and risk of death from bladder cancer: an ecological case-control study in Taiwan. Journal of Toxicology and Environmental Health A, 70, 1000–1004.
Chiu HF, Tsai SS, Wu TN and Yang CY, 2010. Colon cancer and content of nitrates and magnesium in drinking water. Magnesium Research, 23, 81–89.
Chiu HF, Kuo CH, Tsai SS, Chen CC, Wu DC, Wu TN and Yang CY, 2012. Effect modification by drinking water hardness of the association between nitrate levels and gastric cancer: evidence from an ecological study. Journal of Toxicology and Environmental Health A, 75, 684–693.
Chow CK, Chen CJ and Gairola C, 1980. Effect of nitrate and nitrite in drinking water on rats. Toxicology Letters, 6, 199–206, as referred to in JECFA, 1996.
Chung JC, Chou SS and Hwang DF, 2004. Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures. Food Additives & Contaminants, 21, 317–322.
Chung SWC, Tran JCH, Tong KSK, Chen MYY, Xiao Y, Ho YY and Chan CHY, 2011. Nitrate and nitrite levels in commonly consumed vegetables in Hong Kong. Food Additives & Contaminants, 4, 34–41.
Cocco P, Broccia G, Aru G, Casula P, Muntani S, Cantor KP and Ward MH, 2003. Nitrate in community water supplies and incidence of non Hodgkin's lymphoma in Sardinia, Italy. Journal of Epidemiology and Community Health, 57, 510–511.
Cortas NK and Wakid NW, 1991. Pharmacokinetic aspects of inorganic nitrate ingestion in man. Pharmacology and Toxicology, 68, 192–195.
Coss A, Cantor KP, Relf JS, Lynch JS and Ward MH, 2004. Pancreatic cancer and drinking water and dietary sources of nitrate and nitrite. American Journal of Epidemiology, 159, 693–701.
Croitoru MD, 2012. Nitrite and nitrate can be accurately measured in samples of vegetal and animal origin using an HPLC-UV/VIS technique. Journal of Chromatography B, 911, 154–161.
Cross AJ, Ferrucci LM, Risch A, Graubard BI, Ward MH, Park Y, Hollenbeck AR, Schatzkin A and Sinha R, 2010. A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. Cancer Research, 70, 2406–2414.
Cross AJ, Freedman ND, Ren J, Ward MH, Hollenbeck AR, Schatzkin A, Sinha R and Abnet CC, 2011. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. American Journal of Gastroenterology, 106, 432–442.
Czarniecka-Skubina E and Gołaszewska B, 2001. Wpływ procesu kulinarnego na jakos′c′ wybranych warzyw. Z˙ ywnos′c′, 2, 102–115.

Daniel CR, Sinha R, Park Y, Graubard BI, Hollenbeck AR, Morton LM and Cross AJ, 2012a. Meat intake is not associated with risk of non-Hodgkin lymphoma in a large prospective cohort of U.S. men and women. Journal of Nutrition, 142, 1074–1080.

Daniel CR, Cross AJ, Graubard BI, Park Y, Ward MH, Rothman N, Hollenbeck AR, Chow WH and Sinha R, 2012b. Large prospective investigation of meat intake related mutagens and risk of renal cell carcinoma. American Journal of Clinical Nutrition, 95, 155–162.

Del Negro A, Araújo MR, Tincani AJ, Meirelles L, Martins AS and Andreollo NA, 2008. Experimental carcinogenesis on the oropharyngeal mucosa of rats with hydrochloric acid, sodium nitrate and pepsin. Acta Cirurgica Brasileira, 23, 337–342.

Dellavalle CT, Daniel CR, Aschebrook-Kilfoy B, Hollenbeck AR, Cross AJ, Sinha R and Ward MH, 2013. Dietary intake of nitrate and nitrite and risk of renal cell carcinoma in the NIH-AARP Diet and Health Study. British Journal of Cancer, 108, 205–212.

DellaValle CT, Xiao Q, Yang G, Shu XO, Aschebrook-Kilfoy B, Zheng W, Li HL, Ji B, Rothman T, Chow T, Gao Y and Ward MH, 2014. Dietary nitrate and nitrite intake and risk of colorectal cancer in the Shanghai Women's Health Study. International Journal of Cancer, 134, 2917–2926.

De Mey E, Drabik-Markiewicz G, Peeters C, Derdelinckx G, Paelinck H and Impens S, 2009. Impact of cadaverine in volatile N-nitrosamine formation during the heating of a lean meat model. In Communications in agricultural and applied biological sciences (Vol. 4, No. 74, pp. 127-132). Universiteit Gent.

De Mey E, De Maere H, Goemaere O, Steen L, Peeters MC, Derdelinckx G, Paelinck H and Fraeye I, 2014. Evaluation of N-nitrosopiperidine formation from biogenic amines during the production of dry fermented sausages. Food and Bioprocess Technology, 7, 1269–1280.

Drabik-Markiewicz G, Van den Maagdenberg K, De Mey E, Deprez S, Kowalska T and Paelinck H, 2009. Role of proline and hydroxyproline in N-nitrosamine formation during heating in cured meat. Meat Science, 81, 479–486.

Drabik-Markiewicz G, Dejaegher B, De Mey E, Impens S, Kowalska T, Paelinck H and Vander Heyden Y, 2010. Evaluation of the influence of proline, hydroxyproline or pyrrolidine in the presence of sodium nitrite on N-nitrosamine formation when heating cured meat. Analytica chimica acta, 657, 123–130.

Drabik-Markiewicz G, Dejaegher B, De Mey E, Kowalska T, Paelinck H and Vander Heyden Y, 2011. Influence of putrescine, cadaverine, spermidine or spermine on the formation of N-nitrosamine in heated cured pork meat. Food chemistry, 126, 1539–1545.

Dubrow R, Darefsky AS, Park Y, Mayne ST, Moore SC and Kilfoy B, 2010. Dietary components related to N-nitroso compound formation: a prospective study of adult glioma. Cancer Epidemiology, Biomarkers & Prevention, 19, 1709–1722.

EFSA (European Food Safety Authority), 2003. The effects of nitrites/nitrates on the microbiological safety of meat products. BIOHAZ Panel. EFSA Journal 2003;2(3):14, 1–31 pp. https://doi.org/10.2903/j.efsa.2004.14

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Committee on a request from EFSA related to harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA Journal 2005;3(10):282, 31 pp. https://doi.org/10.2903/j.efsa.2005.282

EFSA (European Food Safety Authority), 2007. Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 1–22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2010a. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2010b. Statement on possible public health risks for infants and young children from the presence of nitrates in leafy vegetables. EFSA Journal 2010;8(12):1935, 42 pp. https://doi.org/10.2903/j.efsa.2010.1935

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal, 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. https://doi.org/10.2903/j.efsa.2012.2760. Available online: http://www.efsa.europa.eu/efsajournal

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambre C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent-Massin D, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, van den Brandt P, Fortes C, Merino L, Toldra F, Arcella D, Christodoulidou A, Barrucci F,

Garcia A, Pizzo F, Battacchi D and Younes M, 2017. Re-evaluation of potassium nitrite (E 249) and sodium nitrite (E 250) as food additives. EFSA Journal 2017;15(6):4786, 155 pp. https://doi.org/10.2903/j.efsa.2017.4786

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2008. Scientific opinion nitrates in vegetables. EFSA Journal 2008;6(6):689, 79 pp. https://doi.org/10.2903/j.efsa.2008.689

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009a. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009b. Scientific opinion on nitrite as undesirable substances in animal feed. EFSA Journal 2009;7(4):1017, 47 pp. https://doi.org/10.2903/j.efsa.2009.1017

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010a. Scientific Opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010b. Statement on possible public health risks for infants and young children from the presence of nitrates in leafy vegetables. EFSA Journal 2010;8(12):1935, 1–42 pp. https://doi.org/10.2903/j.efsa.2010.1935

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012a. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012b. Scientific Opinion on lead dietary exposure in the European population. EFSA Journal 2012;10(7):2831, 59 pp. https://doi.org/10.2903/j.efsa.2012.2831

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012c. Scientific Opinion on cadmium dietary exposure in the European population. EFSA Journal 2012;10(1):2551, 59 pp. https://doi.org/10.2903/j.efsa.2012.2551

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2014. Scientific Opinion on dietary exposure to inorganic arsenic in the European population. EFSA Journal 2014;12(3):3597, 68 pp. https://doi.org/10.2903/j.efsa.2014.3597

EFSA Scientific Committee, 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579. Available online: www.efsa.europa.eu

EFSA Scientific Committee, 2012b. Scientific Opinion on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Journal 2012;10(3):2578. 5 pp. https://doi.org/10.2903/j.efsa.2012.2578. Available online: www.efsa.europa.eu/efsajournal

Ensafi AA, Reazei B and Nouroozi S, 2004. Simultaneous spectrophotometric determination of nitrite and nitrate by flow injection analysis. Analytical Sciences, 20, 1749–1753.

Eskiocak S, Dundar C, Basoglu T and Altaner S, 2005. The effects of taking chronic nitrate by drinking water on thyroid functions and morphology. Clinical and Experimental Medicine, 5, 66–71.

Fang YJ, Gao ZX, Yan SL, Wang HY and Zhou HY, 2005. A dip-and-read test strip for the determination of nitrite in food samples for the field screening. Analytical Letters, 38, 1803–1811.

FDRL (Food and Drug Research Laboratories), 1972a. Teratologic evaluation of FDA 71-7 (sodium nitrate). Report nr. FDABF-GRAS-042. Food and Drug Research Laboratories, Inc., Maspeth, NY.

FDRL (Food and Drug Research Laboratories), 1972b. Teratologic evaluation of FDA 71-8 (potassium nitrate). Report nr. FDABF-GRAS-041. Food and Drug Research Laboratories, Inc., Maspeth, NY.

Ferrucci LM, Sinha R, Ward MH, Graubard BI, Hollenbeck AR, Kilfoy BA, Schatzkin A, Michaud DS and Cross AJ, 2010. Meat and components of meat and the risk of bladder cancer in the NIH-AARP Diet and Health Study. Cancer, 116, 4345–4353.

Ferrucci LM, Sinha R, Huang WY, Berndt SI, Katki HA, Schoen RE, Hayes RB and Cross AJ, 2012. Meat consumption and the risk of incident distal colon and rectal adenoma. British Journal of Cancer, 106, 608–616.

Fitzhugh OG and Nelson AA, no date. Unpublished report.

Foster AM, Prentice AG, Copplestone JA, Cartwright RA and Ricketts C, 1997. The distribution of leukaemia in association with domestic water quality in South West England. European Journal of Cancer Prevention, 6, 11–19.

Freedman DM, Cantor KP, Ward MH and Helzlsouer KJ, 2000. A case-control study of nitrate in drinking water and non-Hodgkin's lymphoma in Minnesota. Archives of Environmental Health, 55, 326–329.

Freedman ND, Cross AJ, McGlynn KA, Abnet CC, Park Y, Hollenbeck AR, Schatzkin A, Everhart JE and Sihna R, 2010. Association of meat and fat intake with liver disease and hepatocellular carcinoma in the NIH-AARP cohort. Journal of the National Cancer Institute, 102, 1354–1365.

FSANZ, 2011. Survey of nitrates and nitrites in food and beverages in Australia. Food Standards Australia New Zealand, available online: http://www.foodstandards.gov.au/consumer/additives/nitrate/documents/Survey%20of%20nitrates%20and%20nitrites%20Attach%201.pdf

Gangolli SD, Van Den Brandt PA, Feron VJ, Janzowsky C, Koeman JH, Speijers GJA, Spiegelhalder B, Walker R and Wishnok JS, 1994. Nitrate, nitrite and N-nitroso compounds. European Journal of Pharmacology, 292, 1–38.

Gao Q, 2002. Uric acid-hexacyanoferrate (III)-luminol chemiluminescence system for the determination of trace nitrite. Chinese Journal of Analytical Chemistry, 30, 812–814.

Gao F, Zhang L, Wang L, Shike She S and Zhu C, 2005. Ultrasensitive and selective determination of trace amounts of nitrite ion with a novel fluorescence probe mono[6-N(2-carboxy-phenyl)]-β-cyclodextrin. Analytica Chimica Acta, 533, 25–29.

Gatseva PD and Argirova MD, 2008. Iodine status and goitre prevalence in nitrate-exposed schoolchildren living in rural Bulgaria. Public Health, 122, 458–461.

Ghasemi J, Jabbari A, Amini A, Oskoei AG and Abdolahi B, 2004. Kinetic spectrophotometric determination of nitrite based on its catalytic effect on the oxidation of methyl red by bromate. Analytical Letters, 37, 2205–2214.

Girard JP, 1991. El ahumado. In: Girard JP (ed.). *Tecnologiu de la came y de 10s productos crirnicos*. Zaragoza, Acribia. pp. 183–229.

Gladwin MT, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, Cannon RO, Kelm M, Wink DA, Espey MG, Oldfield EH, Pluta RM, Freeman BA, Lancaster JR, Feelisch M and Lundberg JO, 2005. The emerging biology of the nitrite ion. Nature Chemical Biology, 1, 308–314.

Gloria MBA, Vale SR, Vargas OL, Barbour JF and Scanlan RA, 1997. Influence of nitrate levels added to cheesemilk on nitrate, nitrite, and volatile nitrosamine contents in Gruyere cheese. Journal of agricultural and food chemistry, 45, 3577–3579.

Gorsdorf S, Appel EK, Engeholm C and Obe G, 1990. Nitrogen dioxide induces DNA single-strand breaks in cultured Chinese hamster cells. Carcinogenesis, 11, 37–41.

Govoni A, Jansson EA, Weitzberg E and Lundberg JO, 2008. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide, 19, 333–337.

Granli T, Dahl R, Brodin P and Bockman OC, 1989. Nitrate and nitrite concentrations in human saliva: variations with salivary flow-rate. Food and Chemical Toxicology, 27, 675–680.

Green LC, Tannenbaum SR and Fox JG, 1982. Nitrate in human and canine milk. The New England Journal of Medicine, 306, 1367–1368.

Greenblatt M and Mirvish SS, 1973. Dose-response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. Journal of the National Cancer Institute, 50, 119–124, as referenced by JECFA, 1995 and IARC, 2010.

Gulis G, Czompolyova M and Cerhan JR, 2002. An ecologic study of nitrate in municipal drinking water and cancer incidence in Trnava District, Slovakia. Environmental Research, 88, 182–187.

Haynes WM, 2010. *CRC Handbook of Chemistry and Physics*. 91st Edition. CRC Press, Boca Raton, FL, pp. 4-83–4-90.

Haynes WM, ed., 2015. *Handbook of Chemistry and Physics*, 95th Edition (2014), Internet version. CRC Press, Taylor and Francis Group, Boca Raton. Available online: http://www.hbcpnetbase.com/

He D, Zhang ZW, Huang Y and Hu Y, 2007. Chemiluminescence microflow injection analysis system on a chip for the determination of nitrite in food. Food Chemistry, 101, 667–672.

Health Canada, 2011. Guidelines for Canadian Drinking Water Quality. Guideline Technical Document. N-Nitrosodimethylamine (NDMA). Available online: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/water-eau/ndma/ndma-eng.pdf

Health Canada, 2013. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Nitrate and Nitrite. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario (Catalogue No H144-13/2-2013E-PDF).

Hernández-Ramírez RU, Galván-Portillo MV, Ward MH, Agudo A, González CA, Oñate-Ocaña LF, Herrera-Goepfert R, Palma-Coca O and López-Carrillo L, 2009. Dietary intake of polyphenols, nitrate and nitrite and gastric cancer risk in Mexico City. International Journal of Cancer, 125, 1424–1430.

Herrmann SS, Granby K and Duedahl-Olesen L, 2015. Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages. Food Chemistry, 174, 516–526.

Hohensinn B, Haselgrübler R, Müller U, Stadlbauer V, Lanzerstorfer P, Lirk G, Höglinger O and Weghuber J, 2016. Sustaining elevated levels of nitrite in the oral cavity through consumption of nitrate-rich beetroot juice in young healthy adults reduces salivary pH. Nitric Oxide, 60, 10–15.

Honikel K-O, 2008. The use and control of nitrate and nitrite for the processing of meat products. Meat Science, 78, 68–76.

Hord NG, Ghannam JS, Garg HK, Berens PD and Bryan NS, 2011. Nitrate and nitrite content of human, formula, bovine, and soy milks: implications for dietary nitrite and nitrate recommendations. Breastfeeding Medicine, 6, 393–399.

Höring H, 1985. Sum Einfluss von subchronischer Nitratapplikation mit Trinkwasser auf die Schilddrüse der Ratte (Radio jod test). Gesundheit und Urnwelt, 4, 1–15, as referred to in JECFA, 1996.

Höring H, Dobberkay HJ and Seffner W, 1988. Antithyreoidale Umweltchemikalien [Environmental antithyroid chemicals]. Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete, 34, 170–173, as referred to in JECFA, 1996.

Howe GR, Jain M and Miller AB, 1990. Dietary factors and risk of pancreatic cancer: results of a Canadian population-based case-control study. International Journal of Cancer, 45, 604–608.

Hsu J, Jayashree A and Lee NA, 2009. Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. Food Chemistry, 115, 334–339.

Huang L, Borniquel S and Lundberg JO, 2010. Enhanced xanthine oxidoreductase expression and tissue nitrate reduction in germ free mice. Nitric Oxide, 15, 191–195.

Hunault CC, Lambers AC, Mensinga TT, van Isselt JW, Koppeschaar HP and Meulenbelt J, 2007. Effects of sub-chronic nitrate exposure on the thyroidal function in humans. Toxicology Letters, 175, 64–70.
Hunault CC, van Velzen AG, Sips AJ, Schothorst RC and Meulenbelt J, 2009. Bioavailability of sodium nitrite from an aqueous solution in healthy adults. Toxicology Letters, 190, 48–53.
Iammarino M and Di Taranto A, 2012. Nitrite and nitrate in fresh meats: a contribution to the estimation of admissible maximum limits to introduce in directive 95/2/EC. International Journal of Food Science & Technology, 47, 1852–1858.
Iammarino M, Di Taranto A and Cristino M, 2013. Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: results of five years of official controls and monitoring. Food Chemistry, 140, 763–771.
IARC (International Agency for Research on Cancer), 1998. *Some N-nitroso Compounds*. Vol. 17, IARC, Lyon.
IARC (International Agency for Research on Cancer), 2010. *Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 94. IARC, Lyon.
Iijima K, Henry E, Moriya A, Wirz A, Kelman AW and McColl KE, 2002. Dietary nitrate generates potentially mutagenic concentrations of nitric oxide at the gastroesophageal junction. Gastroenterology, 122, 1248–1257.
Inoue-Choi M, Ward MH, Cerhan JR, Weyer PJ, Anderson KE and Robien K, 2012. Interaction of nitrate and folate on the risk of breast cancer among postmenopausal women. Nutrition and Cancer, 64, 685–694.
Inoue-Choi M, Jones RR, Anderson KE, Cantor KP, Cerhan JR, Krasner S and Ward MH, 2015. Nitrate and nitrite ingestion and risk of ovarian cancer among postmenopausal women in Iowa. International Journal of Cancer, 137, 173–182.
Inui N, Nishi Y, Taketomi M and Mori M, 1979. Transplacental action of sodium nitrite on embryonic cells of Syrian golden hamster. Mutation Research, 66, 149–158.
Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology, 22, 623–636.
ISO (International Standard Organization), 2004a. ISO 14673-1:2004 (IDF 189-1: 2004) Milk and milk products – Determination of nitrate and nitrite contents – Part 1: Method using cadmium reduction and spectrometry.
ISO (International Standard Organization), 2004b. ISO 14673-2:2004 (IDF 189-2: 2004) Milk and milk products – Determination of nitrate and nitrite contents – Part 2: Method using segmented flow analysis (Routine method).
ISO (International Standard Organization), 2004c. ISO 14673-3:2004 (IDF 189-3: 2004) Milk and milk products – Determination of nitrate and nitrite contents – Part 3: Method using cadmium reduction and flow injection analysis with in-line dialysis (Routine method).
Jahreis G, Schone F, Ludke H and Hesse V, 1987. Growth impairment caused by dietary nitrate intake regulated via hypothyroidism and decreased somatomedin. Endocrinologia Experimentalis, 21, 171–180, as referred to in JECFA 1996.
Jakszyn P, Agudo A, Ibez R, Garc¡a-Closas R, Pera G, Amiano P and Gonzalez CA, 2004a. Development of a food database of nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. Journal of Nutrition, 134, 2011–2014.
Jakszyn P, Ibáñez R, Pera G, Agudo A, García-Closas R, Amiano P and González CA, 2004b. *Food Content of Potential Carcinogens*. Catalan Institute of Oncology, Barcelona. Available online: http://epic-spain.com/libro.html
Jakszyn P, Bingham S, Pera G, Agudo A, Luben R, Welch A, Boeing H, Del Giudice G, Palli D, Saieva C, Krogh V, Sacerdote C, Tumino R, Panico S, Berglund G, Simán H, Hallmans G, Sanchez MJ, Larra¤aga N, Barricarte A, Chirlaque MD, Quir¢s JR, Key TJ, Allen N, Lund E, Carneiro F, Linseisen J, Nagel G, Overvad K, Tjonneland A, Olsen A, Bueno-de-Mesquita HB, Ock MO, Peeters PH, Numans ME, Clavel-Chapelon F, Trichopoulou A, Fenger C, Stenling R, Ferrari P, Jenab M, Norat T, Riboli E and Gonzalez CA, 2006. Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. Carcinogenesis, 27, 1497–1501.
Jansson EA, Huang L, Malkey R, Govoni M, Nihlén C, Olsson A, Stensdotter M, Petersson K, Holm L, Weitzberg E and Lundberg JO, 2008. A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. Nature Chemical Biology, 4, 411–417.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1965. Specifications for the identity and purity of food additives and their toxicological evaluation: Food colours and some antimicrobials and antioxidants. Eighth Report of JECFA. WHO Technical Report Series No. 309.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974. Toxicological evaluation of certain food additives with a review of general principles and of specifications. Seventeenth report of JECFA. WHO Technical Report Series No. 539. FAO Nutrition meetings Report Series No. 53.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth report of JECFA. WHO Technical Report Series 859.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Nitrate. *Safety Evaluation of Certain Food Additives (Food additives Series 35)*. World Health Organization, Geneva. Available online: http://www.inchem.org/documents/jecfa/jecmono/v35je14.htm
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002. Evaluation of certain food additives and contaminants. Fifty-ninth report of JECFA. Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Reports Series 913. 20–32. Available online: http://whqlibdoc.who.int/trs/WHO_TRS_913.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003a. Nitrate (and potential endogenous formation of N-nitroso compounds). WHO Food Additives Series, 50. Available online: http://www.inchem.org/documents/jecfa/jecmono/v50je06.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003b. Nitrate and Nitrite (Intake assessment). WHO Food Additive series, 50. Available online: http://www.inchem.org/documents/jecfa/jecmono/v50je07.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003c. Nitrite (and potential endogenous formation of N-nitroso compounds). WHO Food Additives Series, 50. Available online: http://www.inchem.org/documents/jecfa/jecmono/v50je05.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Sodium nitrate. Joint FAO/WHO ExpertCommittee on Food Additives. Available online: http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-416.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006b. Potassium nitrate. Joint FAO/WHO ExpertCommittee on Food Additives Available online: http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-416.pdf

Jensen OM, 1982. Nitrate in drinking-water and cancer in Northern Jutland, Denmark, with special reference to stomach cancer. Ecotoxicology and Environmental Safety, 6, 258–267.

Jhiang SM, Cho JY, Ryu KY, DeYoung BR, Smanik PA, McGaughy VR, Fischer AH and Mazzaferri EL, 1998. An immunohistochemical study of Na+/I− symporter in human thyroid tissues and salivary gland tissues. Endocrinology, 139, 4416–4419.

Jin L, Qin L, Xia D, Liu X, Fan Z, Zhang C, Gu L, He J, Ambudkar I, Deng D and Wang S, 2013. Active secretion and protective effect of salivary nitrate against stress in human volunteers and rats. Free Radical Biology and Medicine, 57, 61–67.

Jones JA, Ninnis JR, Hopper AO, Ibrahim Y, Merritt TA, Wan KW, Power G and Blood AB, 2014. Nitrite and nitrate concentrations and metabolism in breast milk, infant formula, and parenteral nutrition. Journal of Parenteral and Enteral Nutrition, 38, 856–866.

Joshi CS, Shukla MR, Patel K, Joshi JS and Sahu O, 2015. Environmentally and economically feasibility manufacturing process of potassium nitrate for small scale industries: a review. International Letters of Chemistry, Physics and Astronomy, 41, 88–99.

Kazemzadeh A, 2005. Development of new optical nitrite detector. Asian Journal of Chemistry, 17, 767–773.

Keszei AP, Goldbohm RA, Schouten LJ, Jakszym P and van den Brandt PA, 2013. Dietary N-nitroso compounds, endogenous nitrosation, and the risk of oesophageal and gastric cancer subtypes in the Netherlands Cohort Study. American Journal of Clinical Nutrition, 97, 135–146.

Kilfoy BA, Ward MH, Zheng T, Holford TR, Boyle P, Zhao P, Dai M, Leaderer B and Zhang Y, 2010. Risk of non-Hodgkin lymphoma and nitrate and nitrite from the diet in Connecticut women. Cancer Causes and Control, 21, 889–896.

Kilfoy BA, Zhang Y, Park Y, Holford TR, Schatzkin A, Hollenbeck A and Ward MH, 2011. Dietary nitrate and nitrite and the risk of thyroid cancer in the NIH-AARP Diet and Health Study. International Journal of Cancer, 129, 160–172.

Kim HJ, Lee SS, Choi BY and Kim MK, 2007. Nitrate intake relative to antioxidant vitamin intake affects gastric cancer risk: a case-control study in Korea. Nutrition and Cancer, 59, 185–191.

Kirk-Othmer, 2006a. *Encyclopedia of Chemical Technology*, 5th Edition, Vol. 20. John Wiley and Sons Inc, New York. pp. 939–950.

Kirk-Othmer (2006b). *Encyclopedia of Chemical Technology*, 5th Edition, Vol. 22. John Wiley and Sons, Inc, New York. pp. 843–862.

Knekt P, Järvinen R, Dich J and Hakulinen T, 1999. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow up study. International Journal of Cancer, 80, 852–856.

Korenekova B, Kottferova J and Korenek M, 2000. The fate of added nitrate used in the manufacture of Emmental cheese. Food Additives & Contaminants, 17, 373–377.

Kuo HW, Wu TN and Yang CY, 2007. Nitrates in drinking water and risk of death from rectal cancer in Taiwan. Journal of Toxicology and Environmental Health A, 70, 1717–1722.

Lacroix L, Mian C, Caillou B, Talbot M, Filetti S, Schlumberger M and Bidart JM. Na(+)/I(-) symporter and Pendred syndrome gene and protein expressions in human extra-thyroidal tissues. European Journal of Endocrinology 144, 297–302. Available online: https://www.ncbi.nlm.nih.gov/pubmed/11248751

Lambers AC, Kortboyer JM, Schothorst RC, Sips AJAM, Cleven RFMJ and Meulenbelt J, 2000. The oral bioavailability study of nitrate from vegetables in healthy volunteers. Report No. 235802014 from the National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Law G, Parslow R, McKinney P and Cartwright R, 1999. Non-Hodgkin's lymphoma and nitrate in drinking water: a study in Yorkshire, United Kingdom. Journal of Epidemiology and Community Health, 53, 383–384.

van Leeuwen JA, Waltner-Toew D, Abernathy T, Smit B and Shoukri M, 1999. Association between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987–1991. International Journal of Epidemiology, 28, 836–840.

Ledward DA, 1984. In: Hudson BJF (ed.). *Developments in Food Proteins-3*. Elsevier, London, UK. 33 pp.
Lehman AJ, 1958. Quarterly report to the Editor on Topics of Current Interest. Nitrates and nitrites in meat products. Quarterly Bulletin – Association of Food & Drug Officials, 22, 136–138.
Leszczyńska T, Filipiak-Florkiewicz A, Cieślik E, Sikora E and Pisulewski PM, 2009. Effects of some processing methods on nitrate and nitrite changes in cruciferous vegetables. Journal of Food Composition and Analysis, 22, 315–321.
Leth T, Fagt S, Nielsen S and Andersen R, 2008. Nitrite and nitrate content in meat products and estimated intake in Denmark from 1998 to 2006. Food Additives & Contaminants Part A, 25, 1237–1245.
Li JS, Wang H, Zhang X and Zhang HS, 2003. Spectrofluorimetric determination of total amount of nitrite and nitrate in biological sample with a new fluorescent probe 1,3,5,7-tetramethyl-8-(3′,4′-diaminophenyl)-difluoroboradiaza-s-indacence. Talanta, 61, 797–802.
Liao YH, Chen PS, Chiu HF and Yang CY, 2013. Magnesium in drinking water modifies the association between nitrate ingestion and risk of death from esophageal cancer. Journal of Toxicology and Environmental Health A, 76, 192–200.
Lijinsky W, 1999. N-Nitroso compounds in the diet. Mutation Research, 443, 129–138.
Lijinsky W, Greenblatt M and Kommineni C, 1973. Brief communication: feeding studies of nitrilotriacetic acid and derivatives in rats. Journal of the National Cancer Institute, 50, 1061–1063. as referred to in JECFA 2006.
Lindsay RC, 1996. Food Additives. In: Fennema OW (ed). *Food Chemistry*, 3rd Edition. Marcel Dekker, New York, NY.
Liu C and Russell RM, 2008. Nutrition and gastric cancer risk: an update. Nutrition Review, 66, 237–249.
Luca D, Raileanu L, Luca V and Duda R, 1985. Chromosomal aberrations and micronuclei induced in rat and mouse bone marrow cells by sodium nitrate. Mutation Research, 155, 121–125.
Lundberg JO, Weitzberg E and Gladwin MT, 2008. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nature Reviews Drug Discovery, 7, 156–167.
van Maanen JMS, Van Dijk A, Mulder K, De Baets MH, Menheere PC, Van Der Heide D, Mertens PL and Kleinjans JCS, 1994. Consumption of drinking water with high nitrate levels causes hypertrophy of the thyroid. Toxicology Letters, 72, 365–374.
Maekawa A, Ogiu T, Onodera H, Furuta K, Matsuoka C, Ohno Y and Odashima S, 1982. Carcinogenicity studies of sodium nitrite and sodium nitrate in F-344 rats. Food and Chemical Toxicology, 20, 25–33.
Mascher F and Marth E, 1993. Metabolism and effect of nitrates. Central European Journal of Public Health, 1, 49–52, as referred to in JECFA, 1995 and IARC, 2010.
McElroy JA, Trentham-Dietz A, Gangnon RE, Hampton JM, Bersch AJ, Kanarek MS and Newcomb PA, 2008. Nitrogen-nitrate exposure from drinking water and colorectal cancer risk for rural women in Wisconsin, USA. Journal of Water Health, 6, 399–409.
McMullen SE, Casanova JA, Gross LK and Schenck FJ, 2005. Ion chromatographic determination of nitrate and nitrite in vegetable and fruit baby foods. Journal of AOAC International, 88, 1793–1796.
Menard C, Heraud F, Volatier J-L and Leblanc J-C, 2008. Assessment of dietary exposure of nitrate and nitrite in France. Food Additives & Contaminants, 25, 971–988.
Michaud DS, Holick CN, Batchelor TT, Giovannucci E and Hunter DJ, 2009. Prospective study of meat intake and dietary nitrates, nitrites, and nitrosamines and risk of adult glioma. American Journal of Clinical Nutrition, 90, 570–577.
Millar SJ, Moss BW and Stevenson MH, 1996. Some observations on the absorption spectra of various myoglobin derivatives fond in meat. Meat Science, 42, 277–288.
Miller PE, Lazarus P, Lesko SM, Cross AJ, Sinha R, Laio J, Zhu J, Harper G, Muscat JE and Hartman TJ, 2013. Meat-related compounds and colorectal cancer risk by anatomical subsite. Nutrition and Cancer, 65, 202–226.
Mirvish SM, 1975. Formation of N-nitroso compounds: chemistry, kinetics and in vivo occurrence. Toxicology and Applied Pharmacology, 31, 325–351.
Mirvish SS, 1991. The significance for human health of nitrate, nitrite and N-nitroso compounds. In: Bogárdi I, Kuzelka RD and Ennenga WG (eds.). *Nitrate Contamination: Exposure, Consequence, and Control*. Springer-Verlag, New York, New York. pp. 253–266.
Møller JK and Skibsted LH, 2002. Nitric oxide and myoglobins. Chemical Reviews, 102, 1167–1178.
Montenegro MF, Sundqvist ML, Nihlén C, Hezel M, Carlström M, Weitzberg E and Lundberg JO, 2016. Profound differences between humans and rodents in the ability to concentrate salivary nitrate: implications for translational research. Redox Biology, 10, 206–210.
Morales SVM, Llopis GA, Tejerizo PM and Ferrandiz FJ, 1992. Concentration of nitrates in drinking water and its relationship with bladder cancer. Journal of Environmental Pathology, Toxicology and Oncology, 12, 229–236.
Morales SVM, Llopis GA, Tejerizo PM and Ferrer CE, 1993. Chlorination of drinking water and cancer incidence. Journal of Environmental Pathology, Toxicology and Oncology: Official Organ of the International Society for Environmental Toxicology and Cancer, 13, 39–41.
Mpountoukas P, Vantarakis A, Sivridis E and Lialiaris T, 2008. Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. Food and Chemical Toxicology, 46, 2390–2393.
Mukhopadhyay S, Ghosh D, Chatterjee A, Sinha S, Tripathy S and Chandra AK, 2005. Evaluation of possible goitrogenic and anti-thyroidal effect of nitrate, a potential environmental pollutant. Indian Journal of Physiology and Pharmacology, 49, 284–288.

Munksgaard L and Werner H, 1987. Fate of nitrate in cheese. Milchwissenschaft, 42, 216–219.
Nighat S, Nawae M and Nawae U, 1981. Induced nitrate poisoning in rabbits. Pakistan Veterinary Journal, 1, 10–12.
NMKL (Nordic Committee on Food Analysis), 2013. Nitrate and nitrite. Determination of nitrate and/or nitrite in foodstuffs and water by spectrophotometry after zinc reduction and Griess reaction. Pohjoismainen elintarvikkeiden metodiikkakomitea No 194. Available online: http://www.nmkl.org/dokumenter/fi/194fi2013.pdf
NTP (National Toxicology Program), 2011. *Report on Carcinogens*. 12th Edition. Department of Health and Human Services, USA. 302 pp. Available online: http://ntp.niehs.nih.gov/go/roc12
OECD (Organization for Economic Cooperation and Development), 2007. OECD SIDS dossier for sodium nitrate (CAS No.7631-99-4).
OECD, 2014. OECD Guidelines for the Testing of Chemicals, Section 4 / Test No. 473: In Vitro Mammalian Chromosomal Aberration Test.
Öztekin N, Nutku MS and Erim FB, 2002. Simultaneous determination of nitrite and nitrate in meat products and vegetables by capillary electrophoresis. Food Chemistry, 76, 103–106.
Pannala AS, Mani AR, Spencer JPE, Skinner V, Bruckdorfer KR, Moore KP and Rice-Evans CA, 2003. The effect of dietary nitrate on salivary, plasma and urinary nitrate metabolism in humans. Free Radical Biology & Medicine, 34, 576–584.
Pant N and Srivastava SP, 2002. Testicular and spermatotoxic effect of nitrate in mice. Human and Experimental Toxicology, 21, 37–41.
Pegg RB and Honikel KO, 2015. Principles of curing. Handbook of Fermented Meat and Poultry, 19–30.
Pegg RB and Shahidi F, 2004. *Introduction, in Nitrite Curing of Meat: The N-Nitrosamine Problem and Nitrite Alternatives*. Food & Nutrition Press, Inc., Trumbull, Connecticut, USA. doi: 10.1002/9780470385081.ch1
Pérez-Rodríguez ML, Garcia Mata M and Bosch-Bosch N, 1998. Effect of adding smoke flavouring to frankfurters on nitrite and nitrate levels. Food Chemistry, 62, 201–205.
Rádiková Z, Tajtáková M, Kocan A, Trnovec T, Seböková E, Klimes I and Langer P, 2008. Possible effects of environmental nitrates and toxic organochlorines on human thyroid in highly polluted areas in Slovakia. Thyroid, 18, 353–362.
Ramarathnam N and Rubin LJ, 1994. The flavour of cured meat. In: Shahidi F (ed). *Flavor of Meat and Meat Products*. Blackie, London. pp. 174–198.
Rasheva S, Yantochev I, Boshiolova N and Panova M, 1990. Cytogenetic and pathological changes in mice treated with sodium nitrate: dynamics of the changes for 5 to 15 days. C R Acad Bulg Sci, 43, 93–94.
Riedel C, Dohán O, De la Vieja A, Ginter CS and Carrasco N, 2001. Journey of the iodide transporter NIS: from its molecular identification to its clinical role in cancer. Trends in Biochemical Sciences 26, 490–496. Available online: https://www.ncbi.nlm.nih.gov/pubmed/11504625
RIVM, 1989. *Integrated Criteria Document Nitrate*. Report no. 758473012. Van Duijvenbooden W, Matthijsen AJCM (eds.). National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.
Rogers MA, Vaughan TL, Davis S and Thomas DB, 1995. Consumption of nitrate, nitrite, and nitrosodimethylamine and the risk of upper aerodigestive tract cancer. Cancer Epidemiology, Biomarkers and Prevention, 4, 29–36.
de Roos AJ, Ward MH, Lynch CF and Cantor KP, 2003. Nitrate in public water supplies and the risk of colon and rectum cancers. Epidemiology, 14, 640–649.
Ross JD, 1963. Deficient activity of DPNH-dependent methemoglobin diaphorase in cord blood red blood cells. Blood, 21, 51–62.
Sarikaya R and Cakir S, 2005. Genotoxicity testing of four food preservatives and their combinations in the *Drosophila* wing spot test. Environmental Toxicology and Pharmacology, 20, 424–430.
Scanlan RA, 2003. Nitrosamines. In: Trugo L, Finglas PM (eds.). *Encyclopedia of Food Sciences and Nutrition*. 2nd Edition, Vol. 1–10, pp. 4142–4147.
SCF (Scientific Committee for Food), 1992. Food – science and techniques. Reports of the Scientific Committee for Food (Twenty-sixth series). Nitrates and nitrites (opinion expressed on 19th October 1990).
SCF (Scientific Committee for Food), 1997. Reports of the Scientific Committee for Food (38th series). Opinions of the Scientific Committee for Food on: nitrates and nitrite, draft Commission Directive laying down specific purity criteria on food additives other than colours and sweeteners, cyclamic acid and its sodium and calcium salts, the safety in use of 1,1,1,2-tetrafluoroethane as a solvent for flavour extraction and Bovine Spongiform Encephalopathy. Opinion on nitrates and nitrite expressed on 22 September 1995) report dated 1997.
SCF (Scientific Committee for Food), 2001. Guidance on Submissions for Food Additive Evaluations by the Scientific Committee on Food, opinion expressed on 11 July 2001, SCF/CS/ADD/GEN/26 Final.
Seffner W, 1985. Sum Einfluss von subchronischer Nitratapplikation mit Trinkwasser auf die Schilddriise der Ratte (Morphologische Untersuchungen). Gesundheit und Urnwelt, 4, 16–30, as referred to in JECFA, 1996.
Sen NP, Baddoo PA and Seaman SW, 1986. N-Nitrosothiazolidine and N-Nitrosothiazolidine-4-Carboxylic acid in smoked meats and fish. Journal of Food Science, 51, 821–825.
Shahidi F, Pegg RB and Sen NP, 1994. Absence of volatile N-nitrosamines in cooked nitrite-free cured muscle foods. Meat Science, 37, 327–336.
Shapiro KB, Hotchkiss JH and Roe DA, 1991. Quantitative relationship between oral nitrate-reducing activity and the endogenous formation of N-nitrosoamino acids in humans. Food and Chemical Toxicology, 29, 751–755.

Simko P, 2011. Heat and processing generated contaminants in processed meats. In: Kerry JP, Kerry JF (eds.). *Processed Meats – Improving Safety, Nutrition and Quality*. Woodhead Publishing, Cambridge, UK.
Sindelar JJ and Milkowski AL, 2012. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric Oxide, 26, 259–266.
Sinha R, Park Y, Graubard BI, Leitzmann MF, Hollenbeck A and Schatzkin A, 2009. Meat and meat-related compounds and risk of prostate cancer in a large prospective cohort study in the United States. American Journal of Epidemiology, 170, 1165–1177.
Smiechowska M, Przybylowski P and Kowalski B, 1994. Study of formation of volatile N-nitrosamines in Zulaw, Gouda and Edam cheese. Polish Journal of Food and Nutrition Sciences, 3, 71–80.
Spiegelhalder B, Eisenbrand G and Preussmann R, 1976. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. Food and Cosmetics Toxicology, 14, 545–548.
Spitzweg C, Joba W, Eisenmenger W and Heufelder AE, 1998. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland, and gastric mucosa. The Journal of Clinical Endocrinology and Metabolism, 83, 1746–1751.
Spitzweg C, Joba W, Schriever K, Goellner JR, Morris JC and Heufelder AE, 1999. Analysis of human sodium iodide symporter immunoreactivity in human exocrine glands 1. The Journal of Clinical Endocrinology and Metabolism, 84, 4178–4184.
Stalikas CD, Konidari CN and Nanos CG, 2003. Ion chromatographic method for the simultaneous determination of nitrite and nitrate by post-column indirect fluorescence detection. Journal of Chromatography A, 1002, 237–241.
Stasiuk E and Przybyłowski P, 2006. The impact of the modified method of using KNO3 on the formation of volatile N-nitrosamines in Gouda cheese. Bromatologia i Chemia Toksykologiczna, 39.
Sugiyama K, Tanaka T and Mori H, 1979. Gifu Daigaku Igakubu Kiyo, 27, 1–6, as referenced by JECFA, 1995 and IARC, 2010.
Suh M, Abraham L, Hixon JG and Proctor DM, 2014. The effects of perchlorate, nitrate, and thiocyanate on free thyroxine for potentially sensitive subpopulations of the 2001–2002 and 2007–2008 National Health and Nutrition Examination Surveys. Journal of Exposure Science and Environmental Epidemiology, 24, 579–587.
Tajtáková M, Semanová Z, Tomková Z, Szökeová E, Majoros J, Rádiková Z, Seböková E, Klimes I and Langer P, 2006. Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. Chemosphere, 62, 559–564.
Tamme T, Reinik M, Roasto M, Meremäe K and Kiis A, 2009. Impact of food processing and storage conditions on nitrate content in canned vegetable-based infant foods. Journal of Food Protection, 72, 1764–1768.
Tamme T, Reinik M, Pussa T, Roasto M, Meremae K and Kiis A, 2010. Dynamics of nitrate and nitrite content during storage of home-made and small-scale industrially produced raw vegetable juices and their dietary intake. Food Additives & Contaminants Part A, 27, 487–495.
Tanzi E and Saccani G, 2005. Nitrati in carni e prodotti stagionati. Laboratorio, 2000, 44–47.
TemaNord, 2002. Food additives in Europe 2000 – Status of safety assessments of food additives presently permitted in the EU.
Theiler R, Sato K, Aspelund T and Miller A, 1984. Inhibition of N-Nitrosamine formation in a cured Ground Pork Belly Model System. Journal of Food Science, 49, 341–344.
Thomson B, 2004. Nitrates and nitrites dietary exposure and risk assessment. Prepared as a part of a New Zealand Food Safety Authority contract for scientific services.
Thomson BM, Nokes CJ and Cressey PJ, 2007. Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water. Food Additives and Contaminants, 24, 113–121. https://doi.org/10.1080/02652030600934206
Til HP, Kuper CF and Falke HE, 1985a. Short-term (4 week) oral toxicity in rats with nitrate added to a cereal basal diet. Interim Report V85.288/250458.
Til HP, Kuper CF and Falke HE, 1985b. Short-term (4 week) oral toxicity in rats with nitrate added to a semi-purified basal diet. Interim Report V85.289/250458.
Toldrá F, 2002. *Manufacturing of Dry-cured Ham, in Dry-cured Meat Products*. Food & Nutrition Press, Trumbull, CT, pp. 27–62.
Tompkin RB, 2005. "6 Nitrite". Antimicrobials in Food, 169.
Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K and Gibbs J, 2004. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. Thyroid, 14, 1012–1019.
Tricker AR and Kubacki SJ, 1992. Review of the occurrence and formation of non-volatile N-nitroso compounds in foods. Food Additives and Contaminants, 1, 39.
Tsezou A, Kitsiou-Tzeli S, Galla A, Gourgiotis D, Papageorgiou J, Mitrou S, Molybdas PA and Sinaniotis C, 1996. High nitrate content in drinking water: cytogenetic effects in exposed children. Archives of Environmental Health, 51, 458–461.
Ustyugova IV, Zeman C, Dhanwada K and Beltz LA, 2002. Nitrates/nitrites alter human lymphocyte proliferation and cytokine production. Archives of Environmental Contamination and Toxicology, 43, 270–276.

van Velzen AG, Sips AJ, Schothorst RC, Lambers AC and Meulenbelt J, 2008. The oral bioavailability of nitrate from nitrate-rich vegetables in humans. Toxicology Letters, 181, 177–181.
Vitturi DA and Patel RP, 2011. Current perspectives and challenges in understanding the role of nitrite as an integral player in nitric oxide biology and therapy. Free Radical Biology and Medicine, 51, 805–812.
Volkmer BG, Ernst B, Simon J, Kuefer R, Bartsch G, Bach D and Gschwend JE, 2005. Influence of nitrate levels in drinking water on urological malignancies: a community-based cohort study. BJU International, 95, 972–976.
Wagner DA, Schultz DS, Deen WM, Young VR and Tannenbaum SR, 1983. Metabolic fate of an oral dose of ^{15}N-labeled nitrate in humans: effect of diet supplementation with ascorbic acid. Cancer Research, 43, 1921–1925.
Walters CL, 1992. Reactions of nitrate and nitrite in foods with special reference to the determination of N-nitroso compounds. Food Additives and Contaminants, 9, 441–447.
Ward FW, Coates ME and Walker R, 1986. Nitrate reduction, gastro-intestinal pH and N-nitrosation in gnotobiotic and conventional rats. Food and Chemical Toxicology, 24, 17–22.
Ward MH, Mark SD, Cantor KP, Weisenburger DD, Correa-Villasenor A and Zahm SH, 1996. Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology, 7, 465–471.
Ward MH, Cerhan JR, Colt JS and Hartge P, 2006. Risk of non-Hodgkin lymphoma and nitrate and nitrite from drinking water and diet. Epidemiology, 17, 375–382.
Ward MH, Cross AJ, Divan H, Kulldorff M, Nowell-Kadlubar S, Kadlubar FF and Sinha R, 2007a. Processed meat intake, CYP2A6 activity and risk of colorectal adenoma. Carcinogenesis, 28, 1210–1216.
Ward MH, Rusiecki JA, Lynch CF and Cantor KP, 2007b. Nitrate in public water supplies and the risk of renal cell carcinoma. Cancer Causes and Control, 18, 1141–1151.
Ward MH, Heineman EF, Markin RS and Weisenburger DD, 2008. Adenocarcinoma of the stomach and oesophagus and drinking water and dietary sources of nitrate and nitrite. International Journal of Occupational and Environmental Health, 14, 193–197.
Ward MH, Kilfoy BA, Weyer PJ, Anderson KE, Folsom AR and Cerhan JR, 2010. Nitrate intake and the risk of thyroid cancer and thyroid disease. Epidemiology (Cambridge, Mass.) 21, 389.
Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ and Ahluwalia A, 2008. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension, 51, 784–790.
Weisenburger DD, Schmidt H, Steele SJ, Babbit PR and Armitage JO, 1987. Environmental epidemiology of non-Hodgkins-Lymphoma (NHL) in Eastern Nebraska. Laboratory Investigation, 56, A85.
Weng HH, Tsai SS, Wu TN, Sung FC and Yang CY, 2011. Nitrates in drinking water and the risk of death from childhood brain tumors in Taiwan. Journal of Toxicology and Environmental Health, Part A, 74, 769–778.
Weyer PJ, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, Jones MP, Zheng W and Lynch CF, 2001. Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study. Epidemiology, 12, 327–338.
WHO (World Health Organization), 2007. Nitrate and nitrite in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/07.01/16.
WHO (World Health Organization), 2009. Dietary Exposure Assessment of Chemicals In Food. Available online: http://apps.who.int/iris/bitstream/10665/44065/9/WHO_EHC_240_9_eng_Chapter6.pdf
WHO (World Health Organization), 2011. Guidelines for drinking water quality v4. Available online: http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf
Wind M and Stern A, 1977. Comparison of human adult and fetal hemoglobin: aminophenol-induced methemoglobin formation. Experientia, 33, 1500–1501.
Wisniak J and Garcés I, 2001. The rise and fall of the salitre (sodium nitrate) industry. Indian Journal of Chemical Technology, 8, 427–438.
Witter JP and Balish E, 1979. Distribution and metabolism of ingested NO_3 and NO_2 in germ free and conventional-flora rats. Applied and Environmental Microbiology, 38, 861–869.
Woessner M, Smoliga JM, Tarzia B, Stabler T, Van Bruggen M and Allen JD, 2016. A stepwise reduction in plasma and salivary nitrite with increasing strengths of mouthwash following a dietary nitrate load. Nitric Oxide, 54, 1–7.
Wright RO, Lewander WJ and Woolf AD, 1999. Methemoglobinemia: etiology, pharmacology, and clinical management. Annals of Emergency Medicine, 34, 646–656.
Wu Y, Chen J, Ohshima H, Pignatelli B, Boreham J, Li J, Campbell TC, Peto R and Bartsch H, 1993. Geographic association between urinary-excretion of N-nitroso compounds and esophageal cancer mortality in China. International Journal of Cancer, 54, 713–719.
Wu T, Wang Y, Ho SM and Giovannucci E, 2013. Plasma levels of nitrate and risk of prostate cancer: a prospective study. Cancer Epidemiology, Biomarkers & Prevention, 22, 1210–1218.
Yang CY, Wu DC and Chang CC, 2007. Nitrate in drinking water and risk of death from colon cancer in Taiwan. Environment International, 33, 649–653.
Yang CY, Tsai SS and Chiu HF, 2009. Nitrate in drinking water and risk of death from pancreatic cancer in Taiwan. Journal of Toxicology and Environmental Health, Part A, 72, 397–401.
Yang YJ, Hwang SH, Kim HJ, Nam SJ, Kong G and Kim MK, 2010. Dietary intake of nitrate relative to antioxidant vitamin in relation to breast cancer risk: a case-control study. Nutritionand Cancer, 62, 555–566.

Zaki A, Ait Chaoui A, Talibi A, Derouiche AF, Aboussaouira T, Zarrouck K, Chait A and Himmi T, 2004. Impact of nitrate intake in drinking water on the thyroid gland activity in male rat. Toxicology Letters, 147, 27–33.
Zeegers MP, Selen RF, Kleinjans JC, Goldbohm RA and van den Brandt PA, 2006. Nitrate intake does not influence bladder cancer risk: the Netherlands cohort study. Environmental Health Perspectives, 114, 1527–1531.
Zeilmaker MJ, Bakker MI, Schothorst R and Slob W, 2010. Risk assessment of *N*-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. Toxicological Sciences, 116, 323–335.
Zeman CL, Kross B and Vlad M, 2002. A nested case–control study of methemoglobinemia risk factors in children of Transylvania, Romania. Environmental Health Perspectives, 110, 817–822.
Zhang N, Yu C, Wen D, Chen J, Ling Y and Terajima K, 2012. Association of nitrogen compounds in drinking water with incidence of oesophageal squamous cell carcinoma in Shexian, China. The Tohoku Journal of Experimental Medicine, 226, 11–17.
Zhu Y, Wang PP, Zhao J, Green R, Sun Z, Roebothan B, Squires J, Buehler S, Dicks E, Zhao J, Cotterchio M, Campbell P, Jain M, Parfrey P and Cotterchio M, 2014. Dietary N-nitroso compounds and risk of colorectal cancer: a case-control study in Newfoundland and Labrador and Ontario, Canada. British Journal of Nutrition, 111, 1109–1117.

Abbreviations

17-βHSD	17-β-hydroxysteroid dehydrogenase
γ-GT	γ-glutamyl transpeptidase
AARP	American Association of Retired Persons Diet and Health Study
ADI	acceptable daily intake
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
AUC	area under the curve
BaP	benzo(a)pyrene
BMD	benchmark dose
BMI	body mass index
BP	blood pressure
bw	body weight
CAS	Chemical Abstract Service
CEN	European Committee for Standardization
CI	confidence interval
CONTAM	The EFSA Scientific Panel on Contaminants in the Food chain
CRC	colorectal cancer
DiMeIQx	2-amino-3,4,8 trimethylimidazo[4,5-f]quinoxaline
DM	dry matter
EAC	oesophageal adenocarcinoma
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Commercial Chemical Substances
ENOC	Endogenous *N*-nitroso compounds
EPIC	European Prospective Investigation of Cancer
ESCC	oesophageal squamous cell carcinoma
FAO	Food and Agriculture Organization
FCS	Food Classification System
FFQ	food frequency questionnaire
γ-GT	γ-glutamyl transpeptidase
GCA	gastric adenocarcinoma
GD	gestational day
GI	gastrointestinal
GLP	good laboratory practice
GNCA	gastric non-cardia adenocarcinoma
GNPD	Global New Products Database
GSH	glutathione
HCA	heterocyclic amine
HCC	hepatocellular carcinoma
HPLC	high performance liquid chromatography
HR	hazard ratio
I/R	ischaemia–reperfusion
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases

JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal dose, 50% (i.e. dose that causes death among 50% of treated animals)
IL	interleukin
ISO	International Organization for Standardization
LOD	limit of detection
LOQ	limit of quantification
MB	middle-bound
Mb	myoglobin
MeIQx	2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
MetMb	metmyoglobin
MoE	margin of exposure
MPL	maximum permitted level
NDBA	*N*-nitroso-di-n-propylamine
NDEA	*N-nitrosodiethylamine*
NDMA	*N*-nitrosodimethylamine
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NHPRO	*N*-nitrosohydroxyproline
NIH	National Institutes of Health
NIS	Na^+/I^- symporter
NMKL	Nordic Committee of Analysis of Food
NMTCA	*N*-nitroso-2-methyl-thiazolidine 4-carboxylic acid
NOAEL	no-observed-adverse-effect level
NOC	*N*-nitroso compound
NOEL	no-observed-effect level
NOMb	nitric oxide myoglobin
NOMetMb	*nitrosometmyoglobin*
NPIC	*N*-nitrosopipecolic acid
NPIP	*N*-nitrosopiperidine
NPRO	*N*-nitrosoproline
NPYR	*N*-nitrosopyrrolidine
NSAR	*N*-nitrososarcosine
NTCA	*N*-nitroso-thiazolidine-4-carboxylic acid
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PBMC	peripheral blood mononuclear cell
PHIP	2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine
RCC	renal cell carcinoma
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals
RH	relative humidity
RR	relative risk
SCE	sister chromatic exchange
SCF	Scientific Committee for Food
SIDS	Substance Information Data Set
SMART	somatic mutation and recombination test
TemaNord	Nordic Council of Ministers
TPO	thyroid peroxidase
TSH	thyroid-stimulating hormone
UDS	unscheduled DNA synthesis
UV	ultraviolet
VIS	visible
WHO	World Health Organization

Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of food additive nitrates (251–252) provided by industry and of analytical results (mg/kg) of nitrates (E 251–252) and nitrates from other sources (natural presence or contamination) in foods as reported by Member States

Appendix A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4787

Appendix B – Number and percentage of food products labelled with nitrates (E 251–252) out of the total number of food products present in Mintel GNPD per food sub-category between 2011 and 2015

Appendix B can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4787

Appendix C – Concentration levels of sodium and potassium nitrate (E 251–252) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

Appendix C can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4787

Appendix D – Summary of total estimated exposure of sodium and potassium nitrate (E 251–252) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix D can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4787

Appendix E – Toxicological studies and human studies on thyroid reviewed

Table E.1: List of the toxicological studies reviewed

Compound tested	Strain experimental animals	Duration	Equivalent doses	NOAEL	Main observed effects	Reference
Subchronic toxicity studies						
Sodium nitrate and potassium nitrate	Rats, strain not specified	4 weeks	Sodium nitrite = 4,500 mg/kg bw per day Potassium nitrite = 900, 1,800, 2,700, 3,600, 5,400 mg/kg bw per day	NI	♂ ↗ Methaemoglobin from 2,700 onwards; ↗ kidney relative weights from 2,700 ♀+♂↘ Mean body weights from 2,700 onwards ♀ ↗ Methaemoglobin at 3,600; ↗ kidney relative weights from 1,800	Til et al. (1985a,b)
Sodium nitrate	F-344 rats	6 weeks	1,125, 2,250, 4,500, 9,000, 18,000 mg/kg bw per day	Maximum tolerable dose by authors = 4,500 mg/kg bw per day	All ♀ and 7♂ died at 18,000 ♂↘ body weight gain at 18,000; ♀ at 9,000 ♀+♂ Abnormal colour of blood and spleen 'due to metheamoglobin'	Maekawa et al. (1982)
Potassium nitrate	Wistar male rats	6 weeks	1,800 mg/kg bw per day alone or + 900 mg ascorbic acid/kg bw/ day	NI	↗ serum glucose, cholesterol, ALAT, ASAT. Effects reversed upon ascorbic acid added	Azeez et al. (2011)
Sodium nitrate	Wistar rats	2 weeks	25 mg/kg not clear if it is per diet or per bw alone or + 10 mg/kg day vit C or 100 and 200 mg/day ethanolic extract of *Hibiscus sabdariffa* Linn.	NI	↘ Total serum protein, white blood cells, haemoglobin	Bako et al. (2009)
Potassium nitrate	Rabbits, strain not specified	4 weeks	200, 400, 600 mg/kg bw per day by doses in capsules	NI	↗ Weight reduction, tachycardia, weakness, polyuria	Nighat et al. (1981)
Chronic toxicity and carcinogenicity studies						
Sodium nitrate	Mice (strain not specified)	1 year	3,750, 7,500 mg/kg bw per day	NI	No effects	Greenblatt and Mirvish (1973); Sugiyama et al. (1979)
Sodium nitrate	Mice, strain A	25 weeks + 13 weeks recovery follow-up	1,267 mg/kg bw per day	NI	No effects	Greenblatt and Mirvish (1973)
Sodium nitrate	Mice ICR	2 years	3,750, 7,500 mg/kg bw per day	NI	No effects	Sugiyama et al. (1979), and as described by IARC (2010)
Calcium nitrate	Mice ♀	18 months	30, 300 mg/kg bw per day	NI	↗ Weight loss and death at 30; not at 300	Mascher and Marth (1993)
Sodium nitrate	Rats, strain not specified	14 months	400 mg/kg bw per day	NI	No effects	Chow et al. (1980)
Sodium nitrate	Wistar rats	6 months	Equal to 1,333 mg/kg bw per day + 0.1 N HCl applied into laryngopharyngeal mucosa	NI	↗ Inflammatory histopathological changes	Del Negro et al. (2008)

Compound tested	Strain experimental animals	Duration	Equivalent doses	NOAEL	Main observed effects	Reference
Sodium nitrate	F344 rats	2 years (104 weeks treatment + 19 weeks follow-up)	1,250, 2,500 mg/kg bw per day	NI	↗ Tumours incidences in all groups including controls	Maekawa et al. (1982)
Sodium nitrate	Rats (strain not specified)	2 years	Equal to 250, 500, 2,500, 5,000 mg/kg bw per day	500 mg/kg bw per day	No effects	Lehman (1958) and as described by JECFA (1996)
Sodium nitrate	Rats (strain not specified)	104 weeks (84 weeks treatment + 20 weeks follow-up)	Equal to 2,500 mg/kg bw per day	NI	No effects	Lijinsky et al. (1973)
Reproductive and developmental toxicity studies						
Potassium nitrite	Wistar rats	2 weeks premating, mating, gestation and lactation period until day 4	0, 250, 750 or 1,500 mg/kg bw per day	1,500 mg/kg bw per day	No effects	(OECD, 2007)
Sodium nitrate	CD-1 mice	Gestational day (GD) 6–15	0, 4, 20, 100 or 400 mg/kg bw per day	400 mg/kg bw per day	No effects	FDRL (1972a)
Potassium nitrate	CD-1 mice	GD 6–15	0, 4, 20, 100 or 400 mg/kg bw per day	400 mg/kg bw per day	No effects	FDRL (1972b)
Sodium nitrate	Wistar rats	GD 6–15	0, 2.5, 12, 54 or 250 mg/kg bw per day	250 mg/kg bw per day	No effects	FDRL (1972a)
Potassium nitrate	Wistar rats	GD 6–15	0, 2, 9, 20, 180 mg/kg bw per day	180 mg/kg bw per day	No effects	FDRL (1972b)
Sodium nitrate	Golden Hamsters	GD 6–10	0, 4, 20, 100 or 400 mg/kg bw per day	400 mg/kg bw per day	No effects	FDRL (1972a)
Potassium nitrate	Golden Hamsters	GD 6–10	0, 3, 20, 70 or 280 mg/kg bw per day	280 mg/kg bw per day	No effects	FDRL (1972b)
Sodium nitrate	Dutch belted rabbits	GD 6–18	0, 2.5, 12, 90, 250 mg/kg bw per day	NI	No effects	FDRL (1972a)
Potassium nitrate	Dutch belted rabbits	GD 6–18	20, 2, 10, 50, 206 mg/kg bw per day	NI	No effects	FDRL (1972b)
Potassium nitrate	Swiss mice	35 days	15.7, 34.8, 87, 121.8, 156.6 mg/kg bw per day	NI	♂↘ Sperm count, sperm mobility, ↗ Sperm abnormalities, histopathological changes, 17-β-HSD; ↘ γ-GT at 156.6 7♀	Pant and Srivastava (2002)

NI: not indicated.

Table E.2: Animal studies with nitrate exposure and effects on the thyroid

Animal study	Type	Duration of study	Nitrate intake	Nitrate in drinking water	NOAEL	Reported effect
Höring (1985), Höring et al. (1988), Seffner (1985) all as referred by JECFA (1996)	Rats		3.6–360 mg/kg bw per day	40–4,000 mg/L	NI	Minor changes in ^{131}I uptake in thyroid, thyroid weight, thyroid histology seen at all doses

Animal study	Type	Duration of study	Nitrate intake	Nitrate in drinking water	NOAEL	Reported effect
Jahreis et al. (1987) as referred by JECFA (1996)	Pigs	2 days or 6 weeks	730 mg potassium nitrate/kg bw per day		NI	After 6 weeks ↘ T4 levels, serum somatomedin, body weight gain
Mukhopadhyay et al. (2005)	Wistar rats	28 days	1,500 mg/kg bw per day		NI	↗ Thyroidal weight, urinary iodine concentration, TPO activity, TSH hormone; ↘ plasma T4 and T3 levels
Zaki et al. (2004)	Wistar male rats	5 months	4.5, 9, 13.5, 45 mg potassium nitrate/ kg bw per day; controls 1.3 mg potassium nitrate/ kg bw per day		NI	↘ Body weight gain at two highest doses; plasma urea; thyroid weight statistical significant at 13.5; plasma T3 statistical significant at 13.5 and 45; plasma T4 statistical significant at 45. No histopathological changes at two lowest doses
Eskiocak et al. (2005)	Female rats	30 weeks	2.5, 5, 12.5, 25 mg sodium nitrate/kg bw per day		NI	↗ Thyroid weights at all doses; iodine ↗ iodine uptake at 12.5 and 25; ↘ Iodine uptake at 2.5 and 5; TSH at 2.5, 12.5, 25
Chaoui et al. (2004)	Wistar rats	5 months	2.5, 7.5, 25 mg/kg bw per day	0.7 mg/kg bw per day	NI	↗ Thyroid absolute weight, plasma T3 at 7.5 and 25, plasma T4 at 25; size thyroid follicles at 7.5 and 25 and flat epithelium shape reported

Table E.3: Human studies with nitrate exposure and effects on the thyroid

Human Studies	Type	Nitrate intake	Nitrate in drinking water	Nitrate intake in food restricted	Nitrate in urine	Reported effect
Hunault et al. (2007)	Interventional, 28 days (n = 20)	15 mg/kg bw per day	Low	Yes	Not measured	None
van Maanen et al. (1994)	Cross-sectional (n = 70)		Nitrate water levels	No, measured, total nitrate intake mean 245 ± 92 mg/day	Not measured	Enlarged thyroid if water 109.5 ± 68.1 mg/L and total intake 245 ± 92 mg/day
Tajtáková et al. (2006)	Cross-sectional (n = 1,088) children			No	Not measured	Enlarged thyroid in high exposure group, TSH level in blood elevated
Below et al. (2008)	Cross-sectional (n = 3,772) adults		Not known	No	High (> 69.0 mg/L) and low (≤ 69.0 mg/L	No effect
Gatseva and Argirova (2008)	Cross-sectional (n = 319) children		High: 75 mg/L low: 8 mg/L	No	Not measured	High: 13.9% goitre, low: 4.9% goitre
Rádiková et al. (2008)	Cross-sectional Pregnant women (n = 48)		High: 93 mg/L low: 8 mg/L	No	Not measured	Goitre grade 1 more frequent in high exposed women

Human Studies	Type	Nitrate intake	Nitrate in drinking water	Nitrate intake in food restricted	Nitrate in urine	Reported effect
Suh et al. (2014)	Cross-sectional		Not known	No	Measured	Urine nitrate was a significant predictor of free T4 for both non-pregnant (n = 307) with urinary iodine of less than 100 μg/L and non-pregnant women (n = 564) with urinary iodine of 100 μg/L or more
Ward et al., 2010	Cohort study		Not known	41.1 mg nitrate-N per day vs Q1 ≤ 17.4 mg nitrate-N per day	Not measured	Associated with increased prevalence of hypothyroidism

TSH: thyroid-stimulating hormone; NI: not indicated.

Appendix F – Report on the systematic review on the types and levels of nitrosamines and nitrosamides produced in food products from the use of nitrates and nitrites as food additives

F.1. Objective of the Report

This report illustrates the results obtained from the Systematic Review conducted in order to gather evidence on nitrosamines and nitrosamides types and levels in the EU authorised products containing nitrates and/or nitrites.

The systematic review process has been already described in detail in the Protocol (see Annex A of this report).

F.2. Review question

The objective of this systematic review was to select reliable studies performed to identify the type of nitrosamines and nitrosamides and to measure their respective levels in food products found in the European market to which specified amounts of nitrates/nitrites have been added with the aim to investigate any quantitative relation between such nitrosamine and nitrosamides formation and the levels of nitrate and nitrite added.

F.3. Search strings

Two different search strings were developed for the systematic review (please see details in the Protocol in Annex A and in Table F.1).

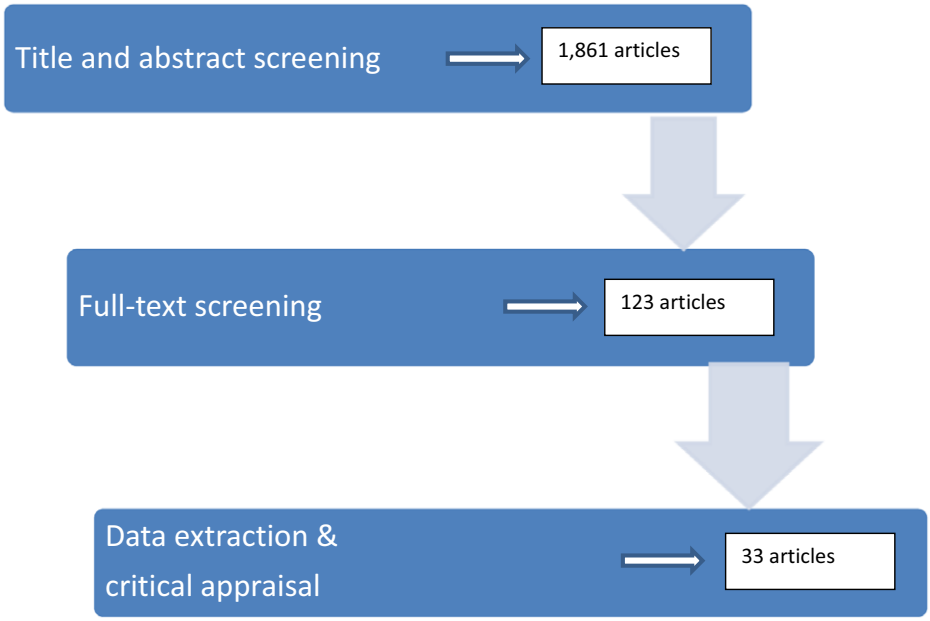
Table F.1: Search strings for conducting the systematic review

N°	Search strategy details
(1)	NDMA OR DMNA OR "N-nitrosodimethylamine$" OR "Dimethylnitrosamine$" OR NMOR OR "N-nitrosomorpholine$" OR NMEA OR "N-nitrosomethylethylamine$" OR "N-Methyl-N-nitrosoethanamin$" OR "1-Ethyl-1-methyl-2-oxohydrazine$" OR "Methylaethylnitrosamin$" OR "N-ethyl-N-methyl-nitrous$" OR NPYR OR "N-nitrosopyrrolidine$" OR NDEA OR DENA OR "N-nitrosodiethylamine$" OR "diethyl-2-oxohydrazine$" OR "N-Ethyl-N-nitrosoethanamin$" OR NPIP OR "N-nitrosopiperidine$" OR NDPA OR "N-nitrosodi-n-propylamine$" OR "N-Nitroso-N-propyl-1-propanamin" OR Oryzalin OR NHPRO OR "N-nitrosohydroxyproline$" OR NPRO OR "N-nitrosoproline$" OR NSAR OR "N-nitrososarcosine$" OR "N-Nitrosomethylglycine" OR NMA OR "N-nitrosomethylaniline$" OR "Phenylmethylnitrosamine" OR NDBA OR DBNA OR "N-nitrosodibutylamine$" OR NDiBA OR "N-nitrosodiisobutylamine$" OR NDBzA OR "N-nitrosodibenzylamine$" OR NHMTCA OR "N-nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic$" OR NTCA OR "N-Nitroso-thiazolidine-4-carboxylic$" OR NMTCA OR "N-Nitroso-2-methyl-thiazolidine-4-carboxylic$" OR NDPhA OR "N-nitrosodiphenylamine$" OR NPIC OR "N-nitrosopipecolic$" OR nitrosamine$ OR nitrosamide$ OR NOC$ AND (Lomo OR "cerdo adobado" OR "Pincho moruno" OR "Careta" OR "cerdo adobada" OR "Castilla" OR "cerdo adobada" OR Kasseler OR Bräte OR Surfleisch OR Toorvorst OR Šašlõkk OR Ahjupraad OR "Kiełbasa surowa biała" OR "Kiełbasa surowa metka" OR "Tatar wołowy" OR "danie tatarskie" OR "Dried ham" OR "dried sausage" OR salami OR "Cooked ham" OR "Emulsified sausages" OR "Filet d Ardenne" OR "Swedish Christmas Ham" OR Papillotes OR "Blinde vink" OR meat OR fish OR cheese OR "non-heat treated processed meat" OR "heat-treated processed meat" OR "Filet d Ardenne" OR "ripened cheese" OR "whey cheese" OR "beverage whiteners" OR "Wiltshire bacon" OR "Wiltshire ham" OR Entremeada OR entrecosto OR chispe OR orelheira OR cabeca OR salgados OR "toucinho fumado" OR "cured tongue" OR kylmâsavustettu poronliha OR "kallrökt renkött" OR bacon OR "filet" OR "bacon" OR rohschinken OR nassgepökelt OR "dry cured bacon" OR "dry cured ham" OR "jamon curado" OR "paleta curada" OR "lomo embuchado" OR cecina OR presunto OR "jambon" OR rohschinken OR trockengepökelt OR saucisson* OR salchichon OR chorizo OR curacion OR rohwürste OR salami OR kantwurst OR brisket OR rohschinken OR trockennassgepökelt OR nassgepökelt OR meat OR cheese OR fish OR dairy OR "pickled herring" OR sprat OR offal OR pâtés OR terrine OR poultry OR game animals OR milk OR whey)
(2)	(Nitrate$ OR Nitrite$) AND (nitrosamine$) OR (nitrosamide$) OR (NOC$) AND (meat OR cheese OR fish OR dairy OR milk OR whey)

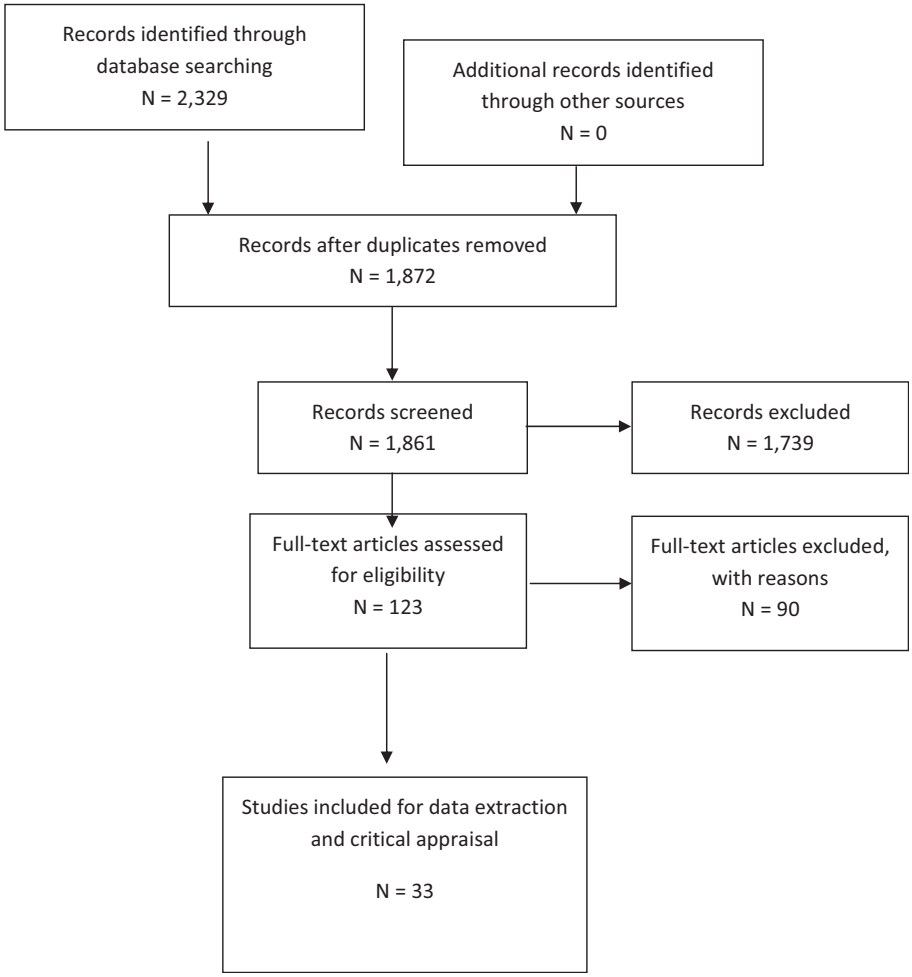
F.4. Results from literature searches and screening of papers

Inclusion and exclusion criteria pre-defined in the Protocol were used to select and screen papers. In total, 1,861 papers were retrieved from literature searches. After screening process, 33 papers were selected for data extraction and critical appraisal.

(a): Results from the different steps of literature searches and screening



(b): Results from the different steps of literature searches and screening including the number of excluding papers



F.5. Results from data extraction and critical appraisal of studies

The data extraction form contained 124 questions (see Protocol Annex A). The results from the critical appraisal of studies are presented in Table F.2.

The system for the classification of selected papers into 'tiers of reliability' (presented in the Protocol) was designed as follows:

Tier 1– 'good quality' papers – the answers to the first three questions were yes. These questions have been considered as crucial in order to retrieve studies of good quality.

Tier 2- 'low quality' papers – one or more of the answers to the first three questions was/were no.

Out of 33 articles, 23 of them were considered of 'low quality' (tier 2) and 10 papers were considered 'good quality' papers (tier 1). The papers included in tier 1 were used to produce data synthesis and conclusions for the Opinion.

Table F.2: Results from the implementation of a Critical Appraisal Tool

Refid	Does the treatment described in the text reflect the recent practises used to prepare meat products as available on the European market?[(a)]	Were appropriate control samples used?[(b)]	Were the methods used for the measurement of nitrates/ nitrites and nitrosamines appropriate?[(c)]	Was the design of experiments and the methodology adequately reported?	Were important additional factors considered?	Was the variability reported?
3672	Yes	Yes	Yes	Yes	Yes	Yes
3722	No	Yes	Yes	No	Yes	Yes
3784	Yes	Yes	Yes	Yes	Yes	Yes
3816	No	No	Yes	Yes	Yes	Yes
3843	No	No	Yes	No	Yes	Yes
3919	No	No	Yes	Yes	Yes	Yes
3936	Yes	Yes	Yes	Yes	Yes	Yes
4080	Yes	Yes	Yes	Yes	Yes	Yes
4089	No	No	No	No	Yes	Yes
4170	No	Yes	Yes	Yes	Yes	No
4220	Yes	Yes	YES	Yes	No	No
4272	No	No	Yes	Yes	Yes	No
4283	No	No	Yes	Yes	Yes	No
4333	No	No	Yes	Yes	Yes	No
4355	No	Yes	No	No	Yes	No
4378	Yes	No	Yes	Yes	Yes	No
4385	No	No	No	No	Yes	No
4386	Yes	Yes	No	No	Yes	Yes
4440	Yes	Yes	no	No	Yes	Yes
4516	Yes	Yes	Yes	Yes	Yes	Yes
4574	Yes	Yes	Yes	Yes	No	Yes
4637	No	No	Yes	No	Yes	No
4666	No	No	No	Yes	Yes	No
4727	Yes	Yes	Yes	Yes	Yes	No
4731	Yes	Yes	Yes	No	Yes	Yes
4750	No	No	No	No	Yes	Yes
4776	No	No	Yes	Yes	No	Yes
4796	No	No	Yes	No	Yes	Yes
4820	No	No	Yes	No	No	Yes
4864	No	No	No	No	Yes	Yes
4870	Yes	No	Yes	No	No	No
4872	Yes	No	No	Yes	Yes	Yes
5809	Yes	Yes	Yes	Yes	Yes	Yes

(a): In this question, 'treatment/s' refer to sample preparation appropriateness including sample manipulation and addition of compounds such as nitrates, salt and others.
(b): Although the use of controls in the studies was not an inclusion criteria, the use of controls in experimental studies was considered important for the quality assessment of the studies included in the review.
(c): Although the reference to LOD in the studies was an inclusion criterion, studies suggesting the availability of LOD were also considered.
(a),(b),(c): These criteria have been considered important for the selection of acceptable quality studies.

F.6. Studies selected for narrative appraisal

Meat products

Regarding studies on meat products six articles were identified fulfilling the criteria a, b and c, e.g. using appropriate meat products, including appropriate controls and presenting limit of quantitation (LOQ) values. Below there is the detailed narrative appraisal of these studies. In these studies, added amounts of nitrites are reported. None of them included the addition of nitrates in the meat products.

De Mey et al. (2014)

This study was carried out in raw fermented sausage, for the determination of the *N*-nitrosopiperidine (NPIP) formation, in relation to the addition of nitrite, ascorbic acid, biogenic amine (cadaverine) and its precursor (piperidine). The effect of pH on NPIP formation has been also investigated.

The ingredients were pork meat, pepper, salt and nitrite, ascorbic acid, piperidine and cadaverine depending on the experiment. The raw fermented sausages were produced with a standard process in a fermentation during 3 days at 24°C and 94% relative humidity (RH) of the mixed ingredients stuffed in casings, followed by a ripening during 21 days at 15°C and 85% RH. During fermentation, the sausages were smoked. The reported limit of detection (LOD) and LOQ were 0.9 μg/kg dry matter (DM) and 2.7 μg/kg DM, respectively, for NPIP analysis, and 2 mg/kg and 10 mg/kg for nitrite determination. The amount of nitrite added was 150 mg/kg. When sodium ascorbate (500 mg/kg) was added, the concentration of nitrite decreased immediately after stuffing under the LOQ. When ascorbic acid was not added, an average concentration of nitrite of 37 mg/kg was detected after stuffing, meaning that only a 10% of the nitrite added was left in this initial step before fermentation. It was also demonstrated that NPIP formation was not affected by pH difference and that cadaverine, the biogenic amine added as a precursor for NPIP formation, did not have any effect. Only for the formulation with added PIP, the maximum level of NPIP reported was 2.9. It was also observed that the formation of nitrosamines occur mainly at the beginning of the production before fermentation.

The Panel noted that for control samples with or without nitrite added, NPIP was not detected except for the samples with nitrite added with maximum level of 1.2 μg/kg that is below the quantification level. It is also noted that the basic formulation contained white pepper (2 g/kg) and eventually PIP is also present. The levels of NPIP in the final product are very low and in most cases below the LOQ even when piperidine is added. The Panel indicates that only minimum and maximum data are reported. No mean values are provided and the smoking process is not described. Given the properties of the smoke as an antioxidant due to its phenolic content, it is not clear if the observed effect of nitrate reduction at the beginning, could be due in part to this treatment.

Drabik-Markievicz et al. (2011)

The study has been made in a meat model corresponding to the raw cooked meat product type. The nitrosamines analysed were *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR). The effect of temperature and the amount of nitrate and some biogenic amines (putrescine, cadaverine, spermidine and spermine) in the nitrosamines formation have been studied. The LOD and LOQ were 0.125–0.136 μg/mL (add LOD in μg/kg) and 0.375–0.408 μg/mL, respectively. This shows that there is a clear increasing effect of temperature only for NDMA, when the amount of nitrite is > 120 mg/kg and temperature > 120°C. The maximum level of NDMA reported is 0.375 μg/mL for 220°C when 120 mg/kg of nitrite is added. The Panel noted that when the amount of nitrite added is 300% higher (480 mg/kg), there is no proportional increase in NDMA because there is an increase of only 18%. The type of biogenic amine present in food has an influence on the final amount of nitrosamines. Among several biogenic amines added, only the addition of spermidine, resulted in higher amounts of NDMA formed, under conditions of amounts above the legal limits of nitrite is added.

In the case of NPIP, the nitrite and high temperatures had no role in the formation of this nitrosamine, with levels found below the LOQ with the higher temperatures. However, biogenic amines caused a significant increase in the concentration of NPIP reaching values of 0.491 and 0.747 μg/mL when cadaverine and spermidine were added, respectively.

De Mey et al. (2009)

The study reports the impact of heating on nitrosamine formation in a lean meat model corresponding to the 'raw cooked meat products' category. The impact of a biogenic amine,

cadaverine, has been also studied. Cadaverine can be present in fresh meat and could be very high in low quality meat due to the growth of bacteria.

The heat treatments simulated were pasteurisation (85°C), sterilisation (120°C), baking and roasting (160 and 220°C). The levels of NDMA, *N*-nitrosodiethylamine (NDEA), *N*-nitroso-di-*n*-propylamine (NDBA), NPIP and NPYR have been measured.

The amounts of nitrite used in the experiments were 120 and 480 mg/kg, with appropriate controls included without nitrite.

The LODs and LOQs are reported for each of the nitrosamines analysed (Table provided). NDEA and NDBA were not detected, and NPIP was only found when cadaverine was added. NPYR was detected in meat products processed at 220°C. Cadaverine had no impact on NPYR. NDMA was only detected above LOQ (0.2 μg/kg) when amounts of nitrite above the legal limits were added (480 mg/kg) and meat samples processed at 220°C.

Shahidi and Pegg (1994)

In this research, an alternative to the use of nitrite is tested to avoid the formation of nitrosamines. The raw materials used were pork, cod and cod surimi, and mixtures of pork with 15% or 50% of cod or surimi, and were tested with (156 mg/kg $NaNO_2$) and without the addition on nitrites. The samples were heat treated (85°C).

The only nitrosamine measured was NDMA, and it was not found in any meat sample even those with 156 mg/kg $NaNO_2$ added. Only in cod, levels of NDMA were reported from 0 to 9 μg/kg when mixtures were used, but levels were 0–3 μg/kg when nitrite was added to pork and cod mixtures and for the others 0–2 μg/kg. The LOD reported was 2 μg/mL. LOQ is not provided.

The Panel commented that

- fish products have higher contents of biogenic amines that are necessary as substrate for the formation of nitrosamines.
- ascorbate is present in all samples as an additive. This means that ascorbate could have an influence as nitrite or *N*-nitrosamine scavenger modifying the results.
- the levels of nitrate or nitrites in control samples should have been measured. Also the residual levels in the final product.
- comminuted mixtures of meat and fish are used for the production of some types of sausages, in which the nitrites might be present due to their permission in meat. In these products, the risk of formation of nitrosamines is higher due to the higher content of biogenic amines that are the *N*-nitrosamines precursors.
- the temperature of treatment (85°C) is very low for *N*-nitrosamines formation. In other studies, temperatures up to 220°C are required for the formation of NOCs, even with very high amounts of nitrite added (ca 400 mg/kg), for raw cooked products.
- biogenic amines are formed from amino acids. Surimi can be a source of biogenic amines.
- if LOQ is considered three times the LOD, then only the levels of 9 μg/kg reported are above the LOQ.

Drabik-Markievicz et al. (2010)

In this research, the influence of pyrrolidine on *N*-nitrosamine formation in a raw cooked meat product and the effect of processing temperatures were studied, and controls with the presence and absence of nitrites were included. The LOD for a solution containing NAs was 0.125 μg/mL and the LOQ was 0.375 μg/mL. The volatile nitrosamines measured were NDMA and NPYR.

Different amounts (0, 120 and 480 mg/kg) of nitrate were added. Proline or hydroxyproline (1,000 mg/kg) or pyrrolidine (10 mg/kg) were also been added.

Temperatures applied were 85°C (pasteurisation), 120°C (sterilisation) and 120–220°C (baking and roasting).

For levels of nitrite of 480 mg/kg and temperatures up to 220°C, NDMA and NPYR were detected in the highest amounts (0.441 and 0.523 μg/mL for NDMA and NPYR, respectively).

It was found that addition of proline did not affect the formation of NDMA, but had a significant influence in the formation of NPYR. This is not the case of hydroxyproline, which is one of the main constituents of collagen.

Herrmann et al. (2015)

The research in this study is focused on the effect of the amount of nitrite, storage conditions (after preparation, after drying and refrigerated at 5°C for 24 h) and frying (10 min, internal temperature of 100°C) on the formation of volatile and non-volatile nitrosamines in raw cooked sausages. The influence of water-soluble (erythorbic acid) and fat-soluble (ascorbyl palmitate) antioxidants, fat content, tripolyphosphate, black pepper, haem iron and calcium carbonate were also reported.

The cooked pork sausages were prepared with 67% of meat, potato flour (4%), black pepper (0.125% or 0.5%), paprika (0.5%), sodium chloride (2%) and nitrite (0, 60, 100, 150, 250 and 350 mg/kg), filled into sheep casings and dried for 50 min at 70°C in an oven or drying cabinet. For other experimental setups, the sausages were prepared with 150 mg/kg of sodium nitrite, erythorbic acid (250 or 1,000 mg/kg), ascorbyl palmitate (0 or 250 mg/kg), fat content (12% or 25%), black pepper (1.25 or 5 g/kg), myoglobin (0 or 1.5 mg/kg), iron (III) sulfate hydrate (0 or 36 mg/kg), calcium carbonate (0 or 6 g/kg) and tripolyphosphate (0 or 5 g/kg). Different combinations of the two antioxidants were also tested.

The levels of non-volatile (*N*-nitrosohydroxyproline (NHPRO), *N*-nitrosoproline (NPRO), *N*-nitroso-thiazolidine-4-carboxylic acid (NTCA), *N*-nitroso-2-methyl-thiazolidine 4-carboxylic acid (NMTCA)) and volatile (*N*-nitrososarcosine (NSAR), NDMA, NPYR, *N*-nitrosopipecolic acid (NPIC), NPIP) nitrosamines were measured. An increase in the level of non-volatile nitrosamines was reported with increasing ingoing amounts of nitrite, and particularly the levels of NTMCA for concentrations of nitrite > 150 mg/kg. Regarding the volatile nitrosamines, NSAR was only detected for nitrite > 150 mg/kg, and the levels of NDMA and NPYR were practically not affected by the level of nitrite and remained at or below the LOQ.

For the other factors studied, the increasing levels of antioxidants diminished the levels of nitrosamines, except for the volatile nitrosamines (NSAR, NDMA and NPYR) that were at the LOD and NPIP that was also not reduced. When both antioxidants are added, erythorbic acid presented the higher effect on the reduction of nitrosamine levels, with an increase in the inhibition of nitrosamine formation with increasing levels of erythorbic acid. This effect has been found to be counteracted with the addition of Fe(III) that could act as a prooxidant. For different fat contents, no significant differences were found in the levels of nitrosamines. The level of NPIP increased from 0.1 to 0.4 μg/kg with increasing amount of black pepper, and the difference was significant when the products were stored four days at 5°C (data not shown). This study indicates that NPIP could partly originate from black pepper and that the levels of NPIP when no antioxidants were added were from 2 to 2.7 μg/kg. The use of tripolyphosphate had no significant effect. No significant effect was observed for the supplementation with myoglobin indicating that haem is not a catalyst for the formation of nitrosamines in meat products.

The Panel noted that there is a clear positive correlation between the amount of nitrite added in raw cooked sausages and the levels of non-volatile nitrosamines.

The Panel further noted that with thermal treatments > 120°C to the meat products, there was an increase in the levels of NPIP and NMTCA (2, 6 and 80 μg/kg, respectively), while only a slight increase was observed for NDMA and NPYR.

Dairy products

Regarding studies on dairy products four articles were identified fulfilling the criteria a, b and c, e.g. using appropriate dairy products (cheeses), including appropriate controls and presenting LOQ values. Below there is the detailed narrative appraisal of these studies. In these studies, added amounts of nitrates are reported.

Stasiuk and Prybylowski (2006)

In this study, the effect of ingoing amounts of potassium nitrate on the formation of volatile nitrosamines in Gouda cheese has been reported. The experimental approach consisted on cheese prepared without the addition of KNO_3 and immersed in brine for 48 h in different concentrations (0.05%, 0.10% and 0.15%) of nitrate after pressing step, and cheese made with milk containing 0.02% KNO_3. The cheeses were ripened for 6 weeks at a 10–12°C and 80–90% RH. The levels of NDMA and NDEA were measured. The LOD was 0.01 μg/kg. The results demonstrated that the addition of nitrate to brine instead of adding to milk did not have any impact on the levels of nitrosamines in cheese, with values up to 0.55 μg/kg for NDMA and 1.44 μg/kg for NDEA.

It was found that there was no relationship between the level of nitrate and the amount of NDMA and NDEA formed in Gouda cheese after ripening.

Bouchikhi et al. (1999)

The effect of the addition of nitrate to milk for the production of fresh semihard cheese (Saint-Paulin) on the formation of volatile nitrosamines has been reported. The cheese was made with pasteurised milk with rennet and 20 g KNO_3/100 mL. A mixture of lactobacilli and streptococci was added for the elaboration of the cheese. The period of ripening was 8 weeks and samples were taken at 20, 40 and 60 days. It was observed that a significant decrease (56%) in the levels of NO_3 during the first 20 days of ripening, this could be explained by the reduction of some NO_3^- to NO_2^-. All the cheeses with and without added nitrate had the same nitrate levels at the end of ripening. The levels of NDMA were very low during the ripening period in controls and experimental cheeses, and therefore, any influence of the amount of nitrate added could not be demonstrated.

Gloria et al. (1997)

The influence of nitrate added on volatile nitrosamine content (NDMA and NDEA) in Gruyère cheese has been studied. It should be noted that nitrate was detected in cheese where not nitrate was added and that nitrite was in much smaller amounts than nitrate. Certain strains of lactobacilli can have a reducing activity. There was no correlation between residual nitrite levels and nitrate levels added or nitrate detected in Gruyère.

Even though the nitrate levels used in the experiments are much higher than the permitted legal levels, the levels of NDMA and NDEA formed are very low.

Smiechowska et al. (1994)

The formation of volatile nitrosamines (NDMA, NDEA, NDPA, NDBA, NPYR, NPIP and NMOR) in different types of cheese (Zulaw, Gouda and Edam) has been investigated. Cheeses were made with pasteurised milk. Different amounts of KNO_3 were added to milk (0%, 0.01% and 0.02%). The cheeses were ripened for 14, 28 and 42 days. NDMA was found in almost all samples, with amounts ranging from 18.94 to 168.80 μg/kg in some samples of Gouda and Edam cheeses. It should be noted that volatile nitrosamines can be formed in cheese produced without addition of KNO_3, but containing native nitrates. The results showed a significant influence of ripening time on the amount of NDMA in Zulaw cheese. However, the nitrate added to milk did not influence the level of NDMA (0.04–3.79 μg/kg). NDPA was not found in all the samples. NDEA was found only in some samples after 4 or 6 weeks of ripening in Zulaw cheese and it was occasionally found in Gouda and Edam cheeses.

It should be noted that the formation of volatile nitrosamines is not related with KNO_3 but with the degree of ripeness.

Fish products

Regarding studies on fish products, no articles were identified fulfilling the criteria a, b and c, e.g. using appropriate fish products, including appropriate controls and presenting LOQ values.

Annex A – Protocol for the systematic review on the types and levels of nitrosamines and nitrosamides produced in food products from the use of nitrates and nitrites as food additives

Background

Nitrate/nitrites are naturally occurring compounds in foods, especially foods of plant origin and vegetables, and are also used as food additives, mainly in meat products. Nitrites amounts are reduced rapidly in meat products and monitoring residual levels of nitrite in the final product are much lower that the nitrites amount initially added.

Under the appropriate conditions (pH, concentration of reactants), nitrites have been shown to form *N*-nitroso compounds (nitrosamines and nitrosamides) from constituents in the food. Some nitrosamines are among important potential carcinogens found in the usual diet of Western populations. Diet is a main source of exposure to these compounds, although there are other main sources of exposure, such as smoking and environmental pollution. Although there is extensive evidence of their carcinogenic effects in experimental studies in animals, there are inadequate data of their concentrations in food and of exposure in human populations. The concentration of these compounds in foods is associated with preparation, preservation and cooking methods.

Therefore, during the re-evaluation of the nitrites and nitrates as food additives, it was considered important to address the question of which nitrosamines and nitrosamides are produced in food products from the use of nitrates and nitrites as food additives and at which levels they can be found in those food products.

Review question

Which nitrosamines and nitrosamides are produced in food products from the use of nitrates and nitrites as food additives and at which levels they can be found in those food products?

Objectives of the review

The objective of this systematic review is to select reliable studies performed to identify the type of nitrosamines and nitrosamides and to measure their respective levels in food products found in the European market to which nitrates/nitrites have been added with the aim to investigate any quantitative relation between such nitrosamine and nitrosamides formation and the levels of nitrate and nitrite added.

Eligibility Criteria for the selection of relevant studies

The criteria that will be applied to select the studies that are to be included in or excluded from the review are described in Table 1.

Table 1: Eligibility (inclusion) criteria for studies

#	Studies will be included in the review if presenting the following characteristics
1.	Time frame 1.1.1990 until 31.12.2015
2.	Type of studies Experimental studies assessing the levels of nitrosamines and nitrosamides in food products to which nitrites or nitrates have been added. Survey and reviews will be saved for comparison and source of additional literature
3.	Languages Studies in English, French, Italian, Spanish, Portuguese, Dutch and German for papers extracted from electronic databases
4.	Population Studies that include food products to which nitrates and nitrites have been added in specified amounts consumed in the European market (food could originate from EU or outside EU), including fish, cheese, whey, meat preparations, non-heat treated processed meat, heat treated processed meat sterilised and non-sterilised, other meat products)

#	Studies will be included in the review if presenting the following characteristics
5.	Intervention Studies where nitrates and nitrites have been added to the products in specified amounts
6.	Outcome Studies reporting the types of nitrosamines and nitrosamides and their measured levels (nitrosamines and nitrosamides), individually or as a combination in food products found in the European market

Main exclusion criteria

Studies before 1.1.1990

Languages excluded: non-European Union languages and European languages which cannot be dealt with by the reviewers.

Compounds excluded: *N*-nitroso compounds other than nitrosamines and nitrosamides.

Studies lacking data on quality of the measurements, e.g. LOD or LOQ will be excluded.

Nitrates/nitrites amounts added per unit of weight not specified.

Method foreseen for performing the systematic review

Searching for relevant studies

The search process will aim at retrieving primary and secondary research studies relevant to the review question as described above.

Search strategy: The search strategy is displayed in Table 2.

Information sources:

- Published reviewed scientific literature will be searched using the following two bibliographic databases: Web of Science (WoS) and PubMed.
- Grey literature will be searched using the following databases: Système Universitaire de Documentation (SUDOC), Trove, Global ETD search and OpenGREY.

Due to the limited time and resources, information sources other than the above mentioned will not be searched.

The search will be performed by the EFSA staff that will then merge the search results using appropriate reference management software (i.e. EndNote®) and remove duplicate records.

The possible studies retrieved from grey literature or received from the EU MS will be inserted into the reference management software by the EFSA staff (FIP unit).

Table 2: Bibliographic databases and search strategy to be applied

Name	Timespan of the database	Search strategy to be applied*
Web of Science™ Core Collection (Editions=SCI-EXPANDED, SSCI. Interface= Web of Science)	1975–present	(1)
Current Contents Connect® (Editions= all. Interface= Web of Science)	1998–present	(1)
CABI: CAB Abstracts® (Interface= Web of Science)	1910–present	(1)
FSTA® - the food science resource (Interface= Web of Science)	1969–present	(1)
Medline® - (Interface= Web of Science)	1946–present	(1)
Système Universitaire de Documentation (SUDOC) http://www.sudoc.abes.fr	From inception–present	(2)

Name	Timespan of the database	Search strategy to be applied*
Trove http://trove.nla.gov.au	Not specified	(2)
Global ETD search http://search.ndltd.org/	1900–present	(2)
OpenGREY http://www.opengrey.eu/	1990–present	(2)
N°	***Search strategy details**	
(1)	NDMA OR DMNA OR "N-nitrosodimethylamine$" OR "Dimethylnitrosamine$" OR NMOR OR "N-nitrosomorpholine$" OR NMEA OR "N-nitrosomethylethylamine$" OR "N-Methyl-N-nitrosoethanamin$" OR "1-Ethyl-1-methyl-2-oxohydrazine$" OR "Methylaethylnitrosamin$" OR "N-ethyl-N-methyl-nitrous$" OR NPYR OR "N-nitrosopyrrolidine$" OR NDEA OR DENA OR "N-nitrosodiethylamine$" OR "diethyl-2-oxohydrazine$" OR "N-Ethyl-N-nitrosoethanamin$" OR NPIP OR "N-nitrosopiperidine$" OR NDPA OR "N-nitrosodi-n-propylamine$" OR "N-Nitroso-N-propyl-1-propanamin" OR Oryzalin OR NHPRO OR "N-nitrosohydroxyproline$" OR NPRO OR "N-nitrosoproline$" OR NSAR OR "N-nitrososarcosine$" OR "N-Nitrosomethylglycine" OR NMA OR "N-nitrosomethylaniline$" OR "Phenylmethylnitrosamine" OR NDBA OR DBNA OR "N-nitrosodibutylamine$" OR NDiBA OR "N-nitrosodiisobutylamine$" OR NDBzA OR "N-nitrosodibenzylamine$" OR NHMTCA OR "N-nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic$" OR NTCA OR "N-Nitroso-thiazolidine-4-carboxylic$" OR NMTCA OR "N-Nitroso-2-methyl-thiazolidine-4-carboxylic$" OR NDPhA OR "N-nitrosodiphenylamine$" OR NPIC OR "N-nitrosopipecolic$" OR nitrosamine$ OR nitrosamide$ OR NOC$ AND (Lomo OR "cerdo adobado" OR "Pincho moruno" OR "Careta" OR "cerdo adobada" OR "Castilla" OR "cerdo adobada" OR Kasseler OR Bräte OR Surfleisch OR Toorvorst OR Šašlõkk OR Ahjupraad OR "Kiełbasa surowa biała" OR "Kiełbasa surowa metka" OR "Tatar wołowy" OR "danie tatarskie" OR "Dried ham" OR "dried sausage" OR salami OR "Cooked ham" OR "Emulsified sausages" OR "Filet d Ardenne" OR "Swedish Christmas Ham" OR Papillotes OR "Blinde vink" OR meat OR fish OR cheese OR "non-heat treated processed meat" OR "heat-treated processed meat" OR "Filet d Ardenne" OR "ripened cheese" OR "whey cheese" OR "beverage whiteners" OR "Wiltshire bacon" OR "Wiltshire ham" OR Entremeada OR entrecosto OR chispe OR orelheira OR cabeca OR salgados OR "toucinho fumado" OR "cured tongue" OR kylmäsavustettu poronliha OR "kallrökt renkött" OR bacon OR "filet" OR "bacon" OR rohschinken OR nassgepökelt OR "dry cured bacon" OR "dry cured ham" OR "jamon curado" OR "paleta curada" OR "lomo embuchado" OR cecina OR presunto OR "jambon" OR rohschinken OR trockengepökelt OR saucisson* OR salchichon OR chorizo OR curacion OR rohwürste OR salami OR kantwurst OR brisket OR rohschinken OR trockennassgepökelt OR nassgepökelt OR meat OR cheese OR fish OR dairy OR "pickled herring" OR sprat OR offal OR pâtés OR terrine OR poultry OR game animals OR milk OR whey)	
(2)	(Nitrate$ OR Nitrite$) AND (nitrosamine$) OR (nitrosamide$) OR (NOC$) AND (meat OR cheese OR fish OR dairy OR milk OR whey)	

Selecting the studies

The study selection/screening process, data extraction and appraisal of the selected studies will be performed using the systematic review system DistillerSR (Evidence Partners, Ottawa, Canada).

The stepwise selection process and related responsibilities are described in Table 3 here below.

Additional information on how the study selection process will be undertaken:

Table 3: Study selection process

#	WHAT	WHO
First screening step (title and abstract screening)	Examine titles and abstracts to remove obviously irrelevant citations (reviewers must be over-inclusive at this stage). In cases when the reviewer cannot make a decision ("Cannot tell" in the Distiller form) and/or in case of disagreements, the paper will proceed to full text screening	In parallel by two mutually independent reviewers per reference (by two EFSA staff)
Second screening step (full text screening)	Retrieve full-text documents of the potentially relevant citations. If within 15 days from the beginning of the search, the full text is not found the paper will be marked as not available The full text documents will be screened for exclusion criteria, relevance and for nitrate and nitrite measurements and estimations in food products consumed in the EU Disagreements will be solved by discussion between the reviewers. In the case where an agreement between reviewers cannot be reached, the opinion of one or more experts from the WG will be sought	FIP staff, in case the paper is not available under current EFSA subscription, a request will be sent to EFSA Library to obtain the article Screening done by two reviewers (two FIP staff)

1) Reviewers will be domain experts and experts with broader expertise in food safety.
2) The records will not be blinded for, e.g. authors names, journal, etc. The study selection will be performed in parallel by two mutually independent reviewers per paper (two FIP EFSA staff).

Extracting data from included studies

The methodology that will be applied for the data extraction process is summarised as follows:

3.1) The data that will be extracted from the included studies are illustrated in Table 4.
3.2) The data extraction will be performed in parallel by three mutually independent reviewers (three EFSA staff, one of them acting as overall reviewer).
3.3) Disagreements will be solved by discussion between the reviewers. In case of doubts, the paper will be put to the attention of the ANS Panel working group in charge of the assessment that will decide on the data to be extracted.
3.4) Full-text documents written in a language not readable by the reviewers will be excluded.
3.5) Studies published more than once in multiple reports will be identified and included only once in the final review.

Table 4: Data to extract from the included studies

Question	Type
1	Experiment setting (laboratory or other type)
2	Food/s category/ies?
3	Meat categories
4	Meat product origin
5	Fish categories
6	Fish treatment
7	Dairy product
8	Whey product
9	Whey product description
10	Meat product name
11	Meat product description
12	Cheese product name
13	Cheese product description
14	Method of nitrate addition in cheese
15	Time in brine
16	Time units
17	Cheese ripening

Question	Type
18	Humidity
19	Temperature of ripening°C
20	Time of ripening
21	Time units
22	Curing of the meat product
23	Description of the salt preparation
24	Humidity
25	Temperature of curing°C
26	Time of curing
27	Time units
28	Precooking of the meat product
29	Temperature of precooking°C
30	Time of precooking
31	Time units
32	Heat processing of the meat product
33	Temperature of heating°C
34	Type of heating
35	Time of heating
36	Time units
37	Fermentation of the meat product
38	Fermentation mix
39	Humidity
40	Temperature of fermentation°C
41	Time of fermentation
42	Time units
43	Smoking of the meat product
44	Smoking mix
45	Temperature of smoking°C
46	Time of smoking
47	Time units
48	Drying of meat product
49	Humidity
50	Temperature of drying°C
51	Time of drying
52	Time units
53	Ripening of meat product
54	Humidity
55	Temperature of ripening°C
56	Time of ripening
57	Time units
58	Other type of processing of meat product
59	Humidity
60	Temperature of other process°C
61	Time of other process
62	Time units
63	Preservation before analysis
64	pH of the product
65	Packaging
66	Time of storage in hours
67	Temperature of storage in°C

Question	Type
68	Other conditions of storage
69	Amount of ascorbates
70	Units as reported in the paper
71	Amount of erythorbates
72	Units as reported in the paper
73	Amount of polyphosphates
74	Units as reported in the paper
75	Amount of pepper
76	Units as reported in the paper
77	Amount of paprika
78	Units as reported in the paper
79	Amount of tocopherol
80	Units as reported in the paper
81	Amount of other_old
82	Amount of other_old
83	Units as reported in the paper
84	Other
85	Amount of other
86	Units as reported in the paper
87	Amount of other
88	Units as reported in the paper
89	Amount of nitrates added
90	Units as reported in the paper
91	Amount of nitrites added
92	Units as reported in the paper
93	Were residual nitrates/nitrites levels measured?
94	Extraction method used for nitrates/nitrites
95	Detection method used for nitrates/nitrites
96	LOD for nitrates/nitrites
97	LOQ for nitrates and nitrites
98	Levels of nitrates
99	Please indicate if this is the mean, median, other for the level of nitrates
100	Variability levels of nitrates
101	Measurement of variability levels of nitrates
102	Units as reported in the paper
103	Levels of nitrites
104	Please indicate if this is the mean, median, other for the level of nitrites
105	Variability level of nitrites
106	Measurement of variability levels of nitrites
107	Units as reported in the paper
108	Number of NOCs measured
109	Extraction method used for NOC
110	Detection method for NOC
111	LOD for NOC
112	LOQ for NOC
113	Number of NOC
114	Outcome: type of NOCs
115	Outcome: type of NOC SHORT NAME
116	Outcome: level of NOCs
117	Please indicate if this is the mean, median, other for the level of NOCs

Question	Type
118	Min value for NOCs
119	Max value for NOCs
120	Units as reported in the paper
121	Variability levels of NOCs
122	Measurement of variability levels of NOCs
123	Number of repetitions from sample/s
124	Comments

Assessing the methodological quality of the included studies

The aim of this step is to appraise the internal validity (i.e. risk of bias) of the studies included in the review.

The criteria that will be applied for the assessment of the methodological quality of selected papers is summarised as follows:

1) Full-text documents that pass the eligibility criteria will be assessed using the Critical Appraisal Form illustrated in Figure 1.
2) The methodological quality assessment will be performed by three mutually independent reviewers (three FIP unit staff) one of them acting as overall reviewer and a WG expert will supervise the work.
3) Disagreements will be solved by discussion between the reviewers. In case of doubts the paper will be put to the attention of the ANS Panel working group in charge of the assessment that will decide on the appraisal.
4) A system for categorising selected studies based on the final quality assessment will be in place. Studies will be assigned to one of two 'tiers of reliability'. The decision regarding which studies to include for data synthesis (all or some of them e.g. considered of higher quality) will be taken by the WG.

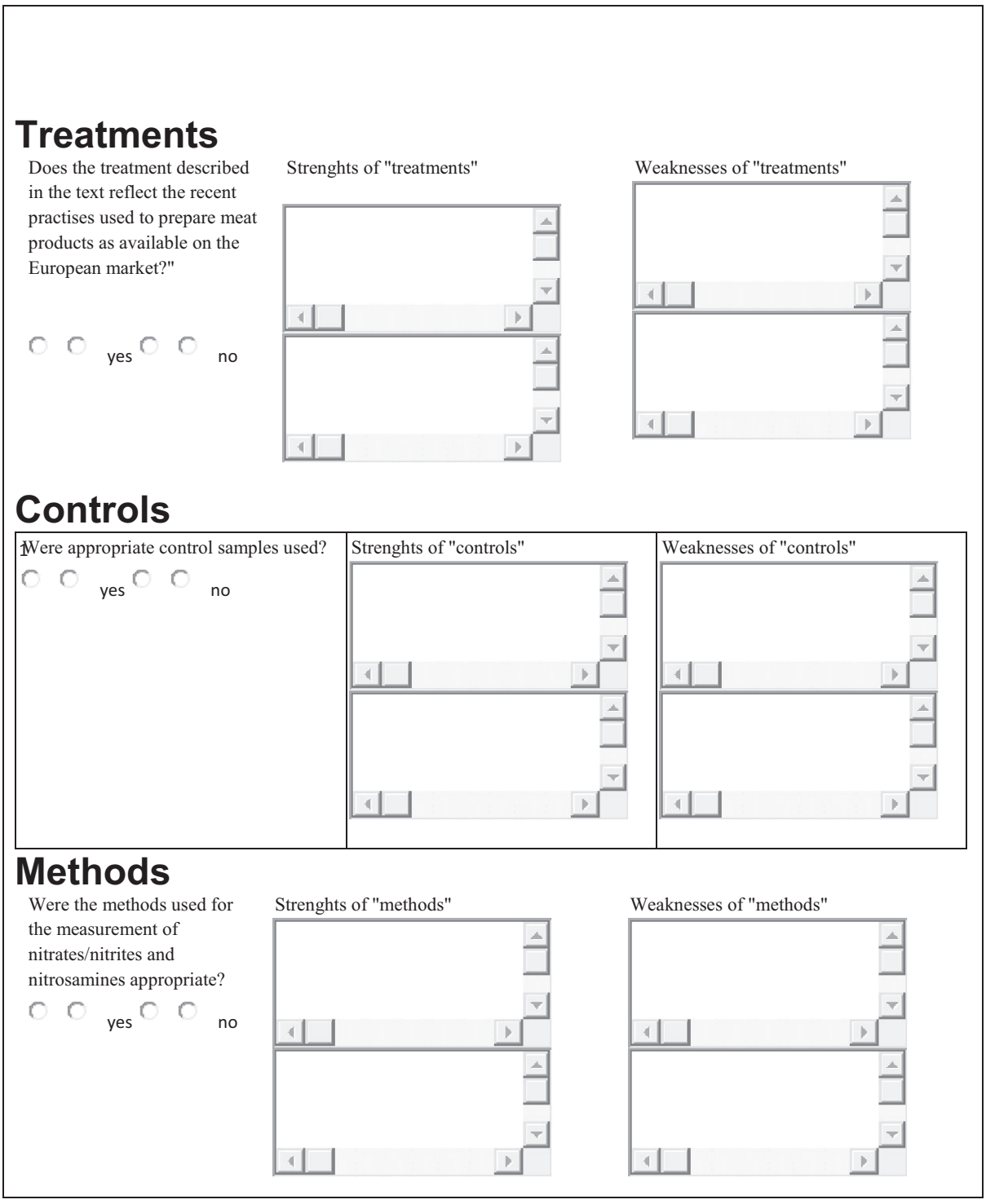


Figure 1: Critical Appraisal Form which includes key questions to critically appraise and to assess the internal validity of the studies included in the systematic review

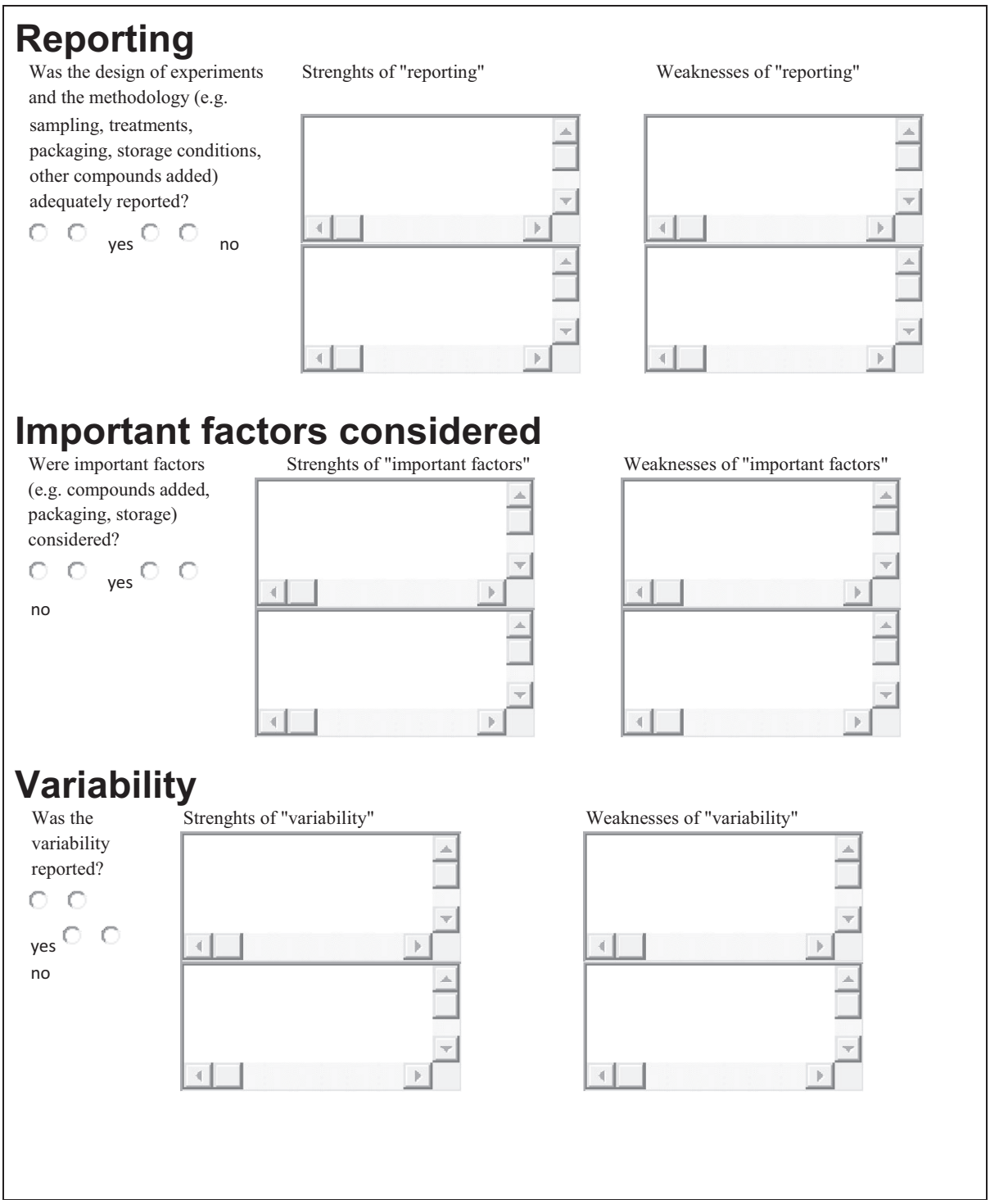


Figure 1: Continued

System for the classification of selected publications into 'tiers of reliability'

Selected papers will be classified into different 'tiers of reliability' as follows:

Tier 1 – 'acceptable quality' papers — the answers to the first three questions were yes. These questions have been considered as crucial in order to retrieve studies of good quality.

Tier 2 – 'low quality' papers – one or more of the answers to the first three questions was/were no.

Synthesising the data from the included studies and weight of evidence

For this systematic review, a meta-analysis of the results of the experimental studies might be feasible, and therefore, the decision for a meta-analysis or a narrative synthesis of the evidence will not be taken *a priori*. In the case that a meta-analysis will not be feasible, the results will be synthesised using tables, graphical methods (forest plot) and/or textual description. The following are proposed outcomes for analysis: types of *N*-nitroso compounds most likely to be formed in the different food products and their estimated levels reported in the literature.

The studies could be grouped (for data synthesis) based on study designs, type of compounds and/or 'tiers of reliability'.

Appendix G – Report on the selection of epidemiological studies

Objective

To assess, if any, the association between nitrates, nitrites and NO compounds and cancer.

Methods

Types of studies and participants

All observational studies (cohort, case–control and ecological) that investigated the association between nitrates, nitrites and their compounds in diet (including drinking water) and cancer were included published up to December 2014. Studies conducted before 2005 were excluded because they were already included in the IARC report of 2010. Studies that were not included in the IARC report but were considered informative were included. Additional articles were identified after December 2014 by PubMed and by searching in the reference lists from recent reviews (last search April 2016).

Types of outcome measures included

Primary outcome:

Incidence

Search Strategy and Data Extraction

Electronic searches

Relevant studies were located by searching PubMed. PRISMA flow diagram (Moher, Liberati, Tetzlaff, Altman, & Group, 2009) helped managing search strategy and data extraction. Systematic searches from 1980 to December 2014 were performed. No language restriction was applied.

("nitrates"[MeSH Terms] OR "nitrates"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields])

("nitrates"[MeSH Terms] OR "nitrates"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND cohort [All Fields]

("nitrates"[MeSH Terms] OR "nitrates"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND case-control [All Fields]

("nitrates"[MeSH Terms] OR "nitrates"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND ecological [All Fields]

AND

("nitrites"[MeSH Terms] OR "nitrites"[All Fields] OR "nitrite"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields])

("nitrites"[MeSH Terms] OR "nitrites"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND cohort [All Fields]

AND

("nitrites"[MeSH Terms] OR "nitrites"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND case-control [All Fields]

AND

("nitrites"[MeSH Terms] OR "nitrites"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND ecological[All Fields]

AND

noc[All Fields] AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND cohort[All Fields]

AND

noc [All Fields] AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND case-control[All Fields]

noc [All Fields] AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) AND ecological[All Fields]

Study selection, data extraction and assessment of methodology quality (bias)

Two epidemiologists identified potential studies to be added in the opinion provided by EFSA. Full-text study reports/publications were provided by EFSA. The two epidemiologists screened the full-texts and identified studies for inclusion, and identified and recorded reasons for exclusion of the ineligible

studies. Disagreement were solved through discussion or, if required, through consultation of the working group. Duplicate records were identified and excluded.

The reviewers assessed the quality of a study in a narrative way. Any discrepancies were addressed by a joint re-evaluation of the original article by the epidemiologist group.

All studies were described and appeared in the text following the ICD-10 coding ordering. Description of studies was initiated by the name of the first author and year of publication.

The following items were included while describing each study:

1) Type of study (case–control/cohort/ecological)
2) Characteristics of the population and setting (e.g. age, sex, sample size, cases, controls)
3) Objective of the study
4) Exposure (type of dietary questionnaire and mode of assessment)
5) Type of outcome (incidence/ mortality)
6) Number of cases identified during the follow-up (cohort)
7) Time of follow-up and number of lost to follow-up
8) Results of the main findings:

8.1) ORs or hazard ratios, with their 95% confidence intervals and p_{trend} if present and cut-off values associated with the risk of cancer.

8.2) Confounding factors considered by the authors (main risk factors for the specific cancer) and included in the multivariate analysis (e.g. age, sex, smoking, *helicobacter pylori* (for gastric cancer), BMI, total energy intake)

9) Subgroup analysis if conducted (e.g. sex and factors that may potentially affect nitrosation such as vitamin C, vitamin E)
10) Strength and limitation of each study.

References

1. IARC 2010. Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14–21 June 2006.

2. Higgins JPT, Green S, & Cochrane Collaboration, 2008. *Cochrane handbook for systematic reviews of interventions*. Chichester, England; Hoboken, NJ: Wiley-Blackwell.

3. Moher D, Liberati A, Tetzlaff J, Altman DG, & Group P, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ, 339, b2535. https://doi.org/10.1136/bmj.b2535

4. http://apps.who.int/classifications/icd10/browse/2015/en#/INeoplasms (C00-D48).

Annex 1: Article selection and studies described