



Protocol for the mapping of dietary sources of nitrates and nitrites

Part 1 of the risk assessment of nitrates and nitrites in food

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From the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food and Environment

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Preparation of the protocol

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the protocol. The project group consisted of VKM members, external experts, and VKM staff. The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics assessed and approved the final opinion (VKM, 2018).

Authors of the protocol

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics.

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Abbreviations

GI	Gastrointestinal
NLP	Natural language processing

Glossary

FAIR

Findability, Accessibility, Interoperability, and Reuse of data. The principles emphasise machine-actionability (i.e., the capacity of computational systems to find, access, interoperate, and reuse data with none or minimal human intervention) because humans increasingly rely on computational support to deal with data as a result of the increase in volume, complexity, and creation speed of data (<https://www.go-fair.org/fair-principles/>).

Foods

The term “foods” includes food items and beverages.

FoodEx2

A standardised system for classifying and describing food (The European Food Safety Authority).

KBS

A food, energy and nutrient database and calculation system (University of Oslo).

Levenshtein distance algorithm

One technique used in natural language processing that looks for similarities between a word and a given text. The Levenshtein distance algorithm calculates a number that reflects how different two words are. The higher the number, the more different the two words are.

NLP

Natural language processing (NLP) is a subfield of artificial intelligence that uses machine learning to enable computers to process human written and oral language.

1 Introduction

1.1 Nitrates and nitrites in food

Nitrates and nitrites are present in many foods (EFSA, 2008; EFSA et al., 2017a; EFSA et al., 2017b; Luetic et al., 2023), either occurring naturally in plant produce, as contaminants, or they can be deliberately added (Figure 1.1-1).

Plants take up and use nitrate in their metabolism and some plants accumulate nitrate in their leaves (Luetic et al., 2023). Nitrates also occur in specific varieties of seaweed (Martín-León et al., 2021). Both nitrates and nitrites can enter the food chain as environmental contaminants in water (WHO, 2016). The food additives potassium nitrite (E 249), sodium nitrite (E 250), sodium nitrate (E 251) and potassium nitrate (E 252) are authorised for use in the EU in several food categories (Regulation (EC) No 1333/2008). Nitrites and nitrates are authorised as food additives primarily due to their antimicrobial effect and are used as preservatives to enhance microbiological safety. Nitrites are authorised for use in some meat products, while nitrates are authorised in some meat, fish, and cheese products. These additives may also preserve the red colour of meat and enhance flavour. As an alternative to using food additives, vegetable extracts high in nitrates and nitrites may be added to food products for the purpose of simultaneously achieving preservation and reduce the risk of nitrosamine formation, compared to addition of nitrite (Bernardo et al., 2021). However, the use of extracts that perform a technological function in the final product is deemed a deliberate use as a food additive, thus, the food additive legislation applies. (EC, 2018).

Sources of nitrates and nitrites in feed for farm animals include plants and the feed additive sodium nitrite (EC, 2021). Moreover, nitrate can be added to ruminant feed to limit the biosynthesis of methane by the rumen microbiota (EFSA et al., 2020). The European Food Safety Authority (EFSA) assumes the transfer of nitrate and nitrite into food products are small, quote: "*... both the transfer of nitrate and nitrite from feed to food products of animal origin and the nitrate- and nitrite-mediated formation of N-nitrosamines and their transfer into these products are likely to be negligible.*" Potassium nitrate is used in veterinary medicine for farm animals.

Whereas sources to human nitrate exposure are exogenous, sources to nitrite exposure are mainly endogenous via nitrate metabolism in the gastrointestinal tract (Luetic et al., 2023), for instance through reduction of nitrate by bacteria in the mouth (Hord et al., 2009), see further details below.

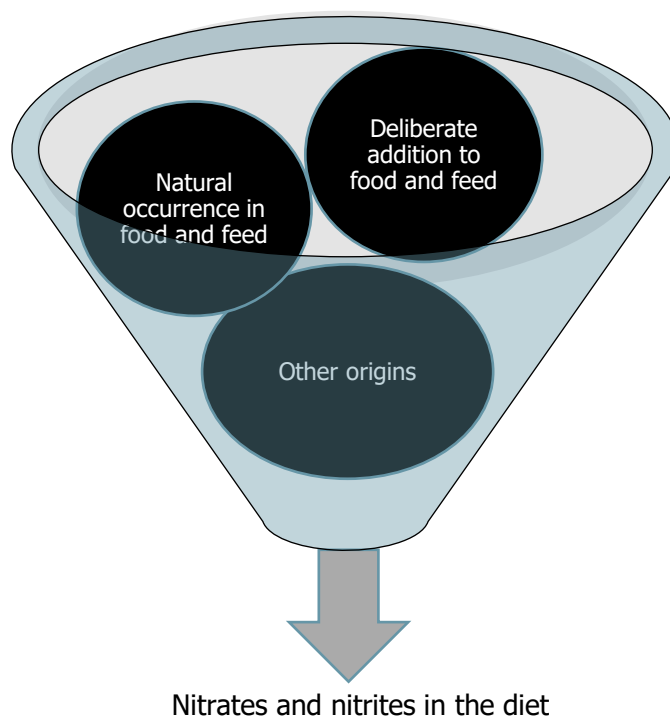


Figure 1.1-1. An overview of the food origins of nitrates and nitrites

1.2 Nitrate and nitrite chemistry

Selected chemical characteristics of nitrates and nitrites are described in Table 1.2-1.

Table 1.2-1. Chemical formulas and molecular weights of nitrate and nitrite ions and their salts in food (data obtained from <https://pubchem.ncbi.nlm.nih.gov/>, accessed at 05/31/24).

Compound ¹	IUPAC name	CAS RN	Molecular formula	Molecular weight (g/mol)
Nitrate	Nitrate	14797-55-8	NO ₃ ⁻	62.005
Nitrite	Nitrite	14797-65-0	NO ₂ ⁻	46.006
Potassium nitrite (E 249)²	Potassium;nitrite	7758-09-0	KNO ₂	85.104
Sodium nitrite (E 250)²	Sodium;nitrite	7632-00-0	NaNO ₂	68.995
Sodium nitrate (E 251)²	Sodium;nitrate	7631-99-4	NaNO ₃	84.995
Potassium nitrate (E 252)²	Potassium;nitrate	7757-79-1	KNO ₃	101.103

¹Compound name as used in this report.

²Purity specifications for the food additives E 249, E 250, E 251, and E 252 are defined in Commission Regulation (EU) No 231/2012.

1.3 Nitrates and nitrites in the human body

Dietary nitrates and nitrites are extensively absorbed through the gastrointestinal tract (EFSA et al., 2017a; EFSA et al., 2017b). Bacteria in the mouth may convert nitrate to nitrite, which re-enters the gastrointestinal (GI) tract. Nitrosamines, a group of which some are potentially genotoxic and carcinogenic substances, may be formed by a reaction between secondary or tertiary amines and nitrates or nitrites. The formation of nitrosamines may occur in consumer products as a result of processing of nitrate-containing foods or in the GI tract following ingestion of food containing nitrates or nitrites. The endogenous formation of nitrosamines from nitrites was considered a concern in EFSA's re-evaluation of the safety of potassium nitrite (E 249) and sodium nitrite (E 250) used as food additives (EFSA et al., 2017b). EFSA also reported some evidence for an association between dietary nitrite and gastric cancers as well as for the combination of nitrate and nitrite from processed meat and colorectal cancers. Nitrite can oxidise oxyhaemoglobin to methaemoglobin, which can reduce the oxygen transport in the body.

The nitrate metabolite nitric oxide (NO) is a signaling molecule with various roles in human physiology and pathophysiology. In the cardiovascular system, NO regulates blood pressure through vasodilation. Additionally, NO plays a role in the immune and nervous systems (EFSA, 2008; Ma et al., 2018; Stephan et al., 2017).

1.4 Regulations

Maximum levels of nitrate are set in specified foods in the Regulation (EC) No 2023/915 on maximum levels for certain contaminants in food (EU, 2023) (not yet implemented in Norwegian law, where Regulation (EC) No 1881/2006 still applies (EU, 2006)).

The use of nitrates and nitrites as food additives is restricted by Regulation (EC) No 1333/2008 on food additives (EU, 2008). The Regulation (EU) No 231/2012 (EU, 2012) lays down specifications for all food additives listed in Annexes II and III to Regulation (EC) No 1333/2008.

Maximum levels are set for both nitrate and nitrite in Directive (EU) 2020/2184 on the quality of drinking water intended for human consumption (EU, 2020) (not yet implemented in Norwegian law, where Directive 98/83/EC still applies (EU, 1998)).

1.5 Assignment

The Norwegian Food Safety Authority asked VKM to prepare an overview of food items containing nitrates and nitrites in the Norwegian diet (Part 1) and to perform an exposure calculation and risk assessment of nitrate and nitrite in foods (Part 2).

1.6 Purpose of the protocol

The purpose of this protocol is to transparently describe how VKM will address Part 1 of the assignment from the Norwegian Food Safety Authority.

1.7 Aim of the mapping of dietary sources of nitrates and nitrites

The mapping of dietary sources of nitrates and nitrites will be the first part of a risk assessment of nitrates and nitrites in food. The aim is to prepare an overview of food items containing nitrates and nitrites in the Norwegian diet resulting from natural occurrence, use of food additives or other sources, that can be used by the Norwegian Food Safety Authority (NFSA) to select foods for analysis of nitrate and/or nitrite content. Exposure assessments of the selected food items will be undertaken in the second part. Information that will be relevant for NFSA to prioritise foods for analysis includes e.g. identification of existing analytical data and identification of data on consumption (amount, frequency, population groups).

1.8 Delimitations

The overview (see 1.7) of food items will be limited to items that naturally contain nitrates and/or nitrites, food items with added potassium nitrite (E 249), sodium nitrite (E 250), sodium nitrate (E 251) and/or potassium nitrate (E 252), and food items with added ingredients that naturally contain nitrates and nitrites.

The data sources used to identify the food items will be limited to those described in Chapter 2.

2 Methods

A selection of databases and publications will be used to identify foods containing nitrates and/or nitrites (Section 2.1). Norwegian national food consumption surveys will be used to identify the amount and frequency of the consumption of these foods by the Norwegian population (Section 2.2). An overview of the information to be charted for foods containing nitrates and/or nitrites is presented in Section 2.3.

2.1 Identification of foods containing nitrate and/or nitrite

Food items containing nitrates and/or nitrites will be identified. The occurrence of nitrate and nitrite in food items could be due to 1) natural presence, 2) addition of food additives, or 3) addition of ingredients that naturally contain nitrates and/or nitrites, such as vegetable broths and plant extracts.

Some databases and publications containing information on nitrites and/or nitrates in food items have been selected to identify this information (see the overview below). This is not an exhaustive list of databases and publications that may contain relevant information, however, VKM considers that this selection will be sufficient to identify food items containing nitrates and/or nitrites.

- Databases
 - [VetDuAt.no](#). A Norwegian database with brand level food product information, including their ingredients.
 - EuroFIR. A database containing food composition information from 26 research institutions and organisations in Europe, New Zealand, Australia, South Africa, USA, and Thailand.
- Publications
 - Re-evaluation of potassium nitrite (E 249) and sodium nitrite (E 250) as food additives (EFSA et al., 2017b). The opinion includes supplementary materials with reported use levels provided by industry and analytical results for E 249 and E 250 provided by the member states.
 - Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives (EFSA et al., 2017a). The opinion includes supplementary materials with reported use levels provided by industry and analytical results for naturally occurring nitrates, E 251 and E 252 provided by the member states.
 - Survey of nitrates and nitrites in food and beverages in Australia (FSANZ, 2011). The report contains nitrate and nitrite analysis results for 52 selected food items.

- Nitrate in vegetables – Scientific Opinion of the Panel on Contaminants in the Food chain (EFSA, 2008). Summary data for the content of nitrates in different food items are included in the report.
- Food items in the Commission Regulation (EC) No 2023/915 with maximum levels for nitrates (EU, 2023). Maximum levels are included for food items that may have a high nitrate content.

An ontology of terms that will be used to identify foods containing nitrates and/or nitrites in the databases is presented in Table 2.1-1.

Table 2.1-1. Terms that will be used to identify foods containing nitrates and/or nitrites.

Substance	Search terms in English	Search terms in Norwegian
Nitrates	<ul style="list-style-type: none"> - Ammonium nitrate bicarbonate - Chile saltpeter - Chilean nitrate - E 251 - E 252 - Niter - Nitrate - Nitrate of potash - Potassium nitrate - Saltpeter - Soda niter - Sodium nitrate 	<ul style="list-style-type: none"> - Ammoniumnitrat bikarbonat - E 251 - E 252 - Kaliumnitrat - Niter - Nitrat - Nitrat av kaliumklorid - Salpeter - Natriumnitrat
Nitrites	<ul style="list-style-type: none"> - E 249 - E 250 - Nitrite - Nitrous acid, potassium salt - Nitrous acid, sodium salt - Potassium nitrite - Sodium nitrite 	<ul style="list-style-type: none"> - E 249 - E 250 - Nitritt - Kaliumnitritt - Natriumnitritt
Nitrates and/or nitrites	<p>Search terms for identification of potential sources of nitrates and nitrites:</p> <ul style="list-style-type: none"> - Vegetable extract - Plant extract - Botanical extract - Herbal extract - Veggie extract - Vegetable broth - Phyto extract - Plant essence - Vegetable essence 	<p>Søkeord for å identifisere potensielle kilder til nitrat og nitritt:</p> <ul style="list-style-type: none"> - Grønnsaksekstrakt - Planteekstrakt - Urteekstrakt - Grønnsaksbuljong - Fytoekstrakt - Planteessens - Grønnsaksessens - Plantekonsentrat - Grønnsakskonsentrat - Grønnsakstinktur

Substance	Search terms in English	Search terms in Norwegian
	<ul style="list-style-type: none"> - Plant concentrate - Vegetable concentrate - Vegetable tincture - Plant-derived extract 	
Nitrates and/or nitrites	<ul style="list-style-type: none"> - Spinach - Celery - Rucola - Lettuce - Cabbage - Beetroot - Seaweed 	<ul style="list-style-type: none"> - Spinat - Selleri - Rucola - Salat - Kål - Rødbete - Tare

Relevant products will be retrieved from databases using the defined terms by applying the programming languages R 4.1 or Python 3.0. If necessary, Natural Language Processing (NLP) will also be used for this task. For instance, during the retrieval process, we will extract misspelled items or differences in capitalisation of letters using Levenshtein distance algorithm (Berger et al., 2021), followed by manual curation by experts. For reproducibility, all analyses will be documented in GitHub repository.

From "Vannverksregisteret" VKM have access to concentrations of nitrate and nitrite from drinking water sources in Norway. This information will be used in the exposure assessment in Part 2. Nitrate and nitrite from drinking water will not be addressed in Part 1.

2.2 Identification of consumption data for food containing nitrate and/or nitrite

The Norwegian national food consumption surveys Norkost 3 (Totland et al., 2012), Ungkost 3 (Hansen et al., 2015), Småbarnskost 3 (Astrup et al., 2020), and Spedkost 3 (Myhre et al., 2020) will be used to identify data on consumption of food items containing nitrates and/or nitrites.

2.3 Information that will be extracted for food containing nitrate and/or nitrite

Information that will be included in the database, if it is available, are the food name and category, substance(s) analysed in the food, means of processing, identified concentration data, method used for the analysis, sampling season, type of cultivation (for plant products), and consumption by the Norwegian population. For each food item identified to contain nitrate and/or nitrite (Section 2.1), we will chart the abovementioned information on one or more levels as shown in Table 2.3-1 (a non-exhaustive overview of the types of information to be included in a database if

available). This will be piloted before we decide the final design of the database. We will use Excel for data charting.

All foods will be categorised according to the food composition and nutrient and food calculation system at the University of Oslo (KBS). For data from EFSA, food categories according to the FoodEx2 standardised system for classifying and describing foods will also be included.

Table 2.3-1. Examples (non-exhaustive list) of types of information to be included in the database at two information levels. KBS: Food composition and nutrient and food calculation system (University of Oslo); FoodEx2: a standardised system for classifying and describing food (The European Food Safety Authority).

Type of information	Information level 1	Information level 2
Reference	Year	
Food name (as reported) and category	Name Category <ul style="list-style-type: none"> - KBS-code - FoodEx2-code, if given in the reference 	
Substance analysed	<ul style="list-style-type: none"> - Nitrates - Nitrites - Nitrosamines 	
Origin of substance	<ul style="list-style-type: none"> - Naturally occurring - Food additive - Addition of nitrite-containing ingredients - Other sources 	
Means of food processing	<ul style="list-style-type: none"> - None - Cooked - Dried - Fermented/smoked - Other 	
Concentration data	Identified <ul style="list-style-type: none"> - Yes - No 	If yes <ul style="list-style-type: none"> - Name of substance(s) - Number of analyses per substance - Country of origin of the samples - Year of sampling - Year of publication - Concentration value
Method of analysis	Available <ul style="list-style-type: none"> - Yes 	If yes <ul style="list-style-type: none"> - Type of method

Type of information	Information level 1	Information level 2
	- No	- LOD and/or LOQ - Other
Sampling season	- Fall/winter - Spring/summer	If data on season are available, include the month(s) of sampling
Type of cultivation (plant products only)	- Open field - Under film/glass	
Consumption data	Identified - Yes - No	If yes <ul style="list-style-type: none"> • Amount (g/day) • Frequency of intake • Household or other units • Population (age-group, gender)

Consumption data will be categorised as regular or occasional consumption based on available data.

Column titles will be based on information from the different food data bases. Each database will provide different information, and all information relevant for the purpose will be collected. We will follow the principle of “one cell, one piece of information” for input in the Excel file.

2.4 Synthesis of findings

A comprehensive database including all charted data will be prepared and archived in the form of an Excel file, as described in Section 2.3. In addition, the entire process of the synthesis will be documented according to the FAIR principles.

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