Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM Bulletin 2025:18

Part 2 of the risk assessment of nitrate, nitrite and nitrosamines in food: The protocol

Trine Husøy, Monica Andreassen, Annette Bernhard, Monica Hauger Carlsen, Eva Denison, Tove Gulbrandsen Devold, Berit Granum, Inger Therese Laugsand Lillegaard, Gro Haarklou Mathisen, Marcin W. Wojewodzic, Ellen Bruzell

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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The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food and Environment.
13.06.2025

ISSN: 2704-1689
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Cover photo: VKM-made collage from Mostphotos, and Unsplash

Suggested citation: VKM, Trine Husøy, Monica Andreassen, Annette Bernhard, Monica Hauger Carlsen, Eva Denison, Tove Gulbrandsen Devold, Berit Granum, Inger Therese Laugsand Lillegaard, Gro Haarklou Mathisen, Marcin W. Wojewodzic, Ellen Bruzell (2025). Part 2 of the risk assessment of nitrate, nitrite and nitrosamines in food: The protocol. The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food and Environment. VKM Bulletin 2025:18, ISSN: 2704-1689. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

Part 2 of the risk assessment of nitrate, nitrite and nitrosamines in food: The protocol

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, 2024).

Authors of the protocol

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2023). 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

ADME	Absorption, distribution, metabolism, excretion
ADI	Acceptable daily intake
BMD	Benchmark dose
BMDL	The lower bound of the benchmark dose
GI	Gastrointestinal
FAIR	Findability, Accessibility, Interoperability, and Reusability
ICH	International Council for Harmonisation
ISO	International Organization for Standardization
LOD	Limit of detection
LOQ	Limit of quantification
MOE	margin of exposure
NDBA	N-Nitrosodibutylamine
NDiBA	N-Nitrosodiisobutylamine
NDEA	N-Nitrosodiethylamine
NDMA	N-Nitrosodimethylamine
NFC	NutriFoodCalc
NDPA	N-Nitrosodipropylamine
NLP	Natural language processing
NMA	N-nitrosomethylaniline
NMEA	N-Nitrosomethylethylamine
NMOR	N-Nitrosomorpholine
NPIP	N-Nitrosopiperidine
NPYR	N-Nitrosopyrrolidine
NSAR	N-nitrososarcosine

TK Toxicokinetic

QA Quality assurance; QC

QC Quality control

Glossary

ADI
The acceptable daily intake (ADI) is an estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health (EFSA glossary).

Applicability domain
The applicability domain of a model is the suitability and performance of a model for a given question, and it defines the boundaries within which the model's predictions are considered reliable.

BMD/ BMDL
BMD is the minimum level of a substance that produces a clear, low-level health risk. The BMD is estimated from a mathematically fitted dose-response curve. The BMDL is the lower confidence bound of the BMD (EFSA glossary).

Deterministic approach for exposure estimation
The deterministic approach uses single concentration values ("point estimates") representing the central tendency for a food item. The deterministic approach is typically used for screening purposes and when the risk is considered low (EPA, 2023). The deterministic approach is known to overestimate the upper tail of the exposure distribution (Goedhart et al., 2012).

FAIR
Findability, Accessibility, Interoperability, and Reusability of data. "The principle emphasises 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/).

Food code
A numerical identification code that is assigned to food items in the NutriFoodCalc database.

Foods
The term "foods" includes both food items and beverages for human consumption.

FoodEx2
A standardised system for classifying and describing food (The European Food Safety Authority).

Levenshtein distance algorithm
A 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 the degree of dissimilarity between two words. The higher the number, the more different the two words are.

Margin of exposure (MOE)
A tool used in risk assessment to explore safety concerns arising from the presence of a potentially toxic substance in food or animal feed (EFSA glossary)

NFC
The NutriFoodCalc is a food, energy and nutrient database and calculation system (University of Oslo). NFC replaces the food composition and nutrient and food calculation system KBS. The main food groups in NFC are "Cereals and cereal products", "Potatoes and potato products", "Vegetables and vegetable products", "Fruit, berries, nuts and seeds, and products thereof", "Meat and meat products", "Milk and other dairy products", "Fish and fish products", "Egg and egg dishes", "Margarine, oils and products thereof", "Chocolate, sweets", "Other composite dishes and products", "Infant foods and products", "Salt and spices", and "Dietary supplements".

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.

Probabilistic approach for exposure estimation
Probabilistic assessments use more complicated modelling approaches [than the deterministic] that rely on distributions of data as inputs in place of point values for key parameters. This results in a distribution of possible exposure estimates (epa.gov). A probability distribution of concentration values is created by mathematical curve fitting, i.e. a curve is generated that best fits the data points. The probabilistic approach will provide characteristics for the variability and uncertainty in the estimates, and it may be useful for more detailed assessments (EPA, 2023).

TK model
A toxicokinetic model (TK model) is used to mathematically describe the fate of a chemical in the human body, as a function of dose and time, in terms of ADME: absorption, distribution, metabolism and excretion (JRC, 2025):

- The rate of chemical absorption from the site of application into the blood stream
- The rate and extent of chemical movements out of blood into the tissue (distribution)
- The rate and extent of chemical biotransformation into metabolites (metabolism)
- The rate of chemical removal from the body (excretion)

1 Introduction

The purpose of this protocol is to transparently describe how VKM will perform the risk assessment of nitrate, nitrite and nitrosamines in foods consumed by the general Norwegian population.

Nitrate and nitrite are products of the oxidation of nitrogen and are naturally occurring in the environment. Nitrate is the more stable form of oxidised nitrogen, while nitrite is relatively unstable and moderately reactive (WHO, 2016). Sources to human nitrate exposure are exogenous, and sources to nitrite exposure are mainly endogenous via nitrate metabolism (Luetic et al., 2023).

N-nitrosamines (N-NAs) may be spontaneously formed in a variety of foods (EFSA et al., 2023) following nitrosation of secondary amines (Mirvish, 1975).

The following reactions show formation of nitrite and nitrosamine:

$$\text{Nitrate } (NO_3^-) + \text{oxygen } (O_2) \rightarrow \text{nitrite } (NO_2^-)$$

$$\text{Nitrite } (NO_2^-) + \text{secondary amine } (RNH_2) \rightarrow \text{nitrosamine } (R_2N\text{-}N{=}O)$$

Parts of the text in this introduction are identical to the protocol for part 1 of the risk assessment of nitrates and nitrites (VKM et al., 2024b).

1.1 Nitrate, nitrite and nitrosamines in food

Plants take up and metabolise nitrate, and some plants accumulate nitrate in their leaves and other tissues (Luetic et al., 2023). Thus, nitrate is naturally present in many edible plants and products made thereof.

Nitrate and nitrite can enter the food chain as food additives in some selected food products and as environmental contaminants in water from farming, livestock production and sewage (EFSA, 2008; EFSA et al., 2017a; EFSA et al., 2017b; Luetic et al., 2023; WHO, 2016).The formation of nitrite from nitrate and further formation of nitrosamines may occur in consumer products during e.g. food preparation and processing of nitrate- and nitrite-containing foods.

Potassium nitrite (E 249), sodium nitrite (E 250), sodium nitrate (E 251) and potassium nitrate (E 252) are food additives authorised for use in the EU in specific food categories (Regulation (EC) No 1333/2008). Nitrite and nitrate are authorised as food additives primarily for their antimicrobial effect and as such are used as preservatives to enhance microbiological safety. Both food additives are authorised for use in some meat products, while nitrate only are authorised for use in some fish and cheese products. The additives may preserve the red colour of meat and enhance flavour. As an alternative to using food additives, vegetable extracts high in nitrate and nitrite may be added to food products (Bernardo et al., 2021). The use of extracts that serves a

technological function in the final product is deemed deliberate use as food additive; thus, the food additive legislation applies (EC, 2018).

Sources of nitrate and nitrite 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 meat and milk products to be 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 also used in veterinary medicine for farm animals.

1.2 Nitrate, nitrite and nitrosamines in the human body

An overview of sources and metabolism of nitrate, nitrite and nitrosamines in the human body is presented in Figure 1.2-1.

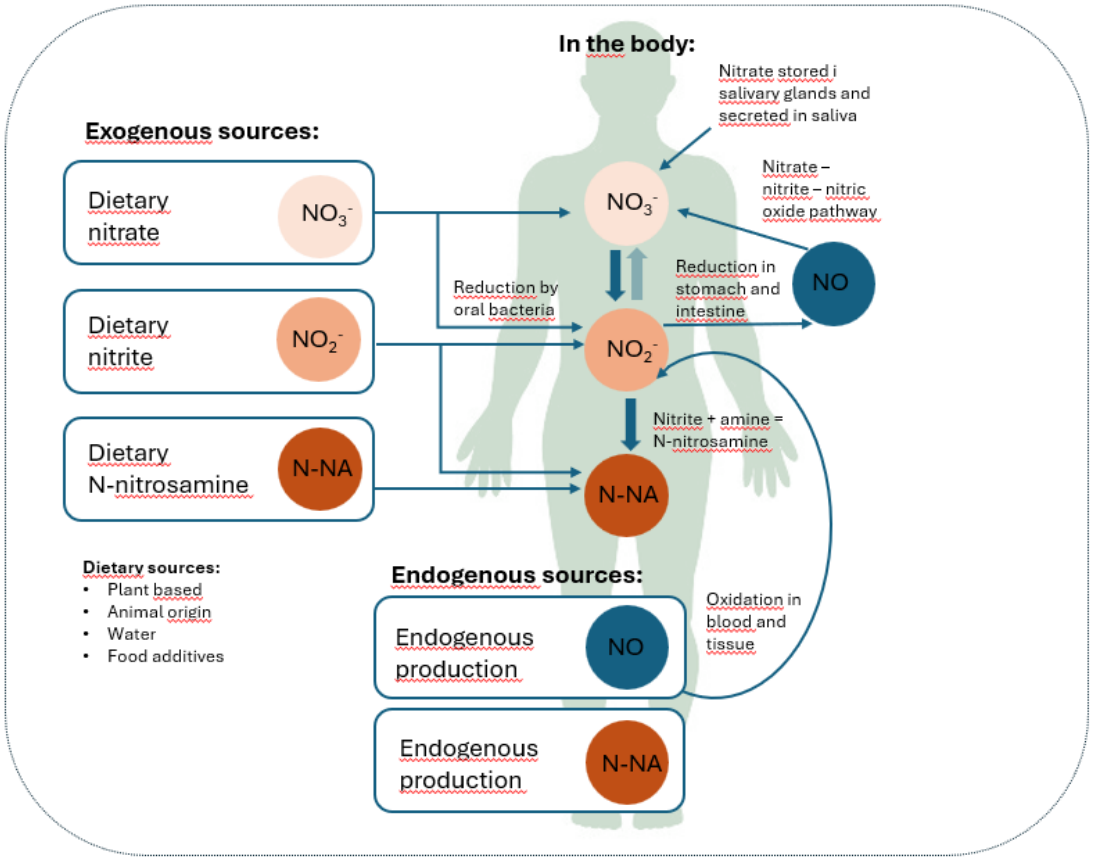

Figure 1.2-1. Simplified schematic overview of sources and formation of nitrate, nitrite and nitrosamines in the human body. NO: Nitric oxide; NO_3-: Nitrate; NO_2-: Nitrite; N-NA: N-nitrosamines.

More than 90 percent of dietary nitrate and nitrite are absorbed from the gastrointestinal tract (EFSA, 2009). EFSA reported 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.

Nitrate is rapidly distributed throughout the body. In the salivary glands, nitrate is secreted by active transport from blood into saliva and thus re-enters the gastrointestinal (GI) tract. Salivary nitrate levels are 10-20 times higher than the plasma level (Lundberg, 2012).

Oral and commensal bacteria, through reductases, convert nitrate to nitrite in the GI tract (Eisenbrand et al., 2024). Nitrite can oxidise the ferrous iron in oxyhemoglobin, converting it into methemoglobin, which is unable to bind and transport oxygen and, thereby, impairing oxygen delivery in the body (EFSA et al., 2017b). Nitrate is also formed in this reaction.

Nitrosamines may be formed in the GI tract following ingestion of food containing nitrate or nitrite. According to EFSA (EFSA et al., 2023), "*very little is known about the fate of N-NAs in humans, and the majority of the available information concerns NDMA*". EFSA characterised the risk of ten carcinogenic nitrosamines applying the margin of exposure approach. They concluded that exposure to these nitrosamines raises health concerns.

Nitric oxide is formed when nitrite is acidified (Benjamin et al., 1994). Nitric oxide is a signaling molecule with various roles in human physiology and pathophysiology. In the cardiovascular system, nitric oxide regulates blood pressure through vasodilation. Additionally, nitric oxide plays a role in the immune and nervous systems (EFSA, 2008; Ma et al., 2018; Stephan et al., 2017). Nitric oxide may be oxidised to nitrate (Lundberg, 2012).

1.3 Established reference points for toxicity of nitrate, nitrite and nitrosamines

Acceptable daily intakes (ADI) have been established as food safety reference points for nitrate and nitrite. (EFSA et al., 2023)The ADI for nitrate was set at 3.7 mg/kg bw per day by the Scientific Committee for Food (SCF, 1997). EFSA considered elevated blood concentrations of methaemoglobin, caused by nitrite converted from nitrate in saliva, to be the most relevant effect for setting the ADI (EFSA et al., 2017a).

The ADI for the nitrite ion, re-established by EFSA, is 0.07 mg/kg bw per day (EFSA et al., 2017b). EFSA concluded that elevated blood concentrations of methaemoglobin following consumption as a food additive was the most relevant effect for setting the ADI.

EFSA derived a benchmark dose lower confidence limit 10% ($BMDL_{10}$) of 10 µg/kg bw per day for increased incidence of liver tumours in rodents caused by N-nitrosodiethylamine (NDEA). This BMDL was established as a reference point for ten carcinogenic nitrosamines (see Section 1.4).

The reference points described above were derived for the external exposure to nitrate, nitrite and nitrosamines.

1.4 Nitrate, nitrite and nitrosamine chemistry

Selected chemical characteristics are described in Table 1.4-1 (nitrate and nitrite) and Table 1.4-2 (N-NAs). Only N-nitrosodiisobutylamine, is not covered by the $BMDL_{10}$ derived by EFSA (EFSA et al., 2023).

Table 1.4-1. Chemical information of nitrate and nitrite ions and their salts in food (data obtained from https://pubchem.ncbi.nlm.nih.gov/, accessed at 05/14/25). CAS RN: CAS (Chemical Abstract Service) registry number.

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

[1]Compound name as used in this report.
[2]Purity specifications for the food additives E 249, E 250, E 251, and E 252 are defined in Commission Regulation (EU) No 231/2012.

Table 1.4-2. Chemical information on N-nitrosamines. CAS RN: CAS (Chemical Abstract Service) registry number. Data obtained from EFSA et al. (2023).

Compound	Acronym	CAS RN	Primary class	Volatile
N-nitrosodimethylamine	NDMA	62-75-9	Acyclic N-NA	Volatile
N-nitrosodiethylamine	NDEA	55-18-5	Acyclic N-NA	Volatile
N-nitrosodipropylamine	NDPA	621-64-7	Acyclic N-NA	Volatile
N-nitrosomethylethylamine	NMEA	10595-95-6	Acyclic N-NA	Volatile

Compound	Acronym	CAS RN	Primary class	Volatile
N-nitrosodibutylamine	NDBA	924-16-3	Acyclic N-NA	Non-volatile
N-nitrosodiisobutylamine	NDiBA	997-95-5	Acyclic N-NA	Non-volatile
N-nitrosomethylaniline	NMA	614-00-6	Acyclic N-NA	Non-volatile
N-nitrososarcosine	NSAR	13256-22-9	Acyclic N-NA	Non-volatile
N-nitrosomorpholine	NMOR	59-89-2	Cyclic N-NA	Volatile
N-nitrosopiperidine	NPIP	100-75-4	Cyclic N-NA	Volatile
N-nitrosopyrrolidine	NPYR	930-55-2	Cyclic N-NA	Volatile

1.5 Regulations

Maximum levels of nitrate are set for specified foods in the Regulation (EC) No 2023/915 on maximum levels for certain contaminants in food (EU, 2023). This regulation is not implemented in Norwegian law (01.05.2025). Regulation (EC) No 1881/2006 still applies in Norway (EU, 2006)).

The use of nitrate and nitrite as food additives is restricted by Regulation (EC) No 1333/2008 on food additives (Regulation (EC) No 1333/2008). 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 implemented in Norwegian law per 01.05.2025, where Directive 98/83/EC still applies (EU, 1998)).

There is no EU legislation regulating the presence of N-NAs in food or drinking water.

1.6 Research questions

The Norwegian Food Safety Authority asked VKM to perform a risk assessment of nitrate and nitrite in foods, including different exposure assessment scenarios (Part 2). Since nitrite is a N-NAs source, the present risk assessment will also include a selection of N-NAs. To perform this assessment, the research questions listed in Table 1.6-1 will be answered.

Table 1.6-1. Research questions that will be answered in the risk assessment.

Part of the assessment	Research questions

Conversion of nitrate, nitrite, and nitrosamines in foods	1. What is the fraction and conversion between nitrate, nitrite, and nitrosamines in different foods? 2. Are there any existing models to predict conversion of nitrate to nitrite, and nitrite to nitrosamine in foods?
Absorption, distribution, metabolism and excretion (ADME)	3. What is the level of endogenous nitrite and nitrosamine production in the body? 4. What are the ADME characteristics of nitrate, nitrite, and nitrosamines in humans? 5. What is the fraction and conversion between nitrate, nitrite, and nitrosamines in the body? 6. Are there established models for nitrate, nitrite, and nitrosamine metabolism and conversion in humans? 7. Are the ADME characteristics of nitrate, nitrite, and nitrosamines in research animals similar to that in humans? If not, which adjustment factor should be applied?
Hazard identification and characterisation	8. Should the ADI for nitrite be updated?
Exposure assessment	9. What is the concentration of nitrate, nitrite, and nitrosamines in foods? 10. What is the consumption of nitrate, nitrite, and nitrosamine containing foods? 11. What is the nitrate, nitrite, and nitrosamine exposure in the Norwegian population? 12. What is the nitrate, nitrite, and nitrosamine exposure if the Norwegian population follow the national food-based dietary guidelines?
Risk characterisation	13. Does the dietary exposure to nitrate, nitrite and nitrosamines represent a risk for negative health effects for the Norwegian population?

1.7 Delimitations

The ADI for nitrate is based on the conversion of nitrate to nitrite. For the risk characterisation of nitrate, the ADI for nitrite will be used to evaluate whether the exposure to nitrate through estimated conversion to nitrite constitutes a health risk. Therefore, VKM will not answer the research question "Should the ADI for nitrate be updated?".

Since EFSA recently derived a reference point ($BMDL_{10}$) for ten carcinogenic N-NAs (EFSA et al., 2023), VKM will use the same $BMDL_{10}$ for the risk characterisation of N-NAs.

The food items on the Norwegian market that may contain nitrate and/or nitrite are limited to those that 1) naturally contain nitrate and/or nitrite, 2) are added potassium nitrite (E 249), sodium nitrite (E 250), sodium nitrate (E 251) and/or potassium nitrate (E 252), and 3) are added ingredients that naturally contain nitrate and nitrite (VKM et al., 2024a).

2 Methods

2.1 Conversion of nitrate, nitrite, and nitrosamines in foods

Since factors such as food processing and the presence of antioxidants (EFSA, 2008; EFSA et al., 2017a; EFSA et al., 2017b; EFSA et al., 2023) (see Section 1.1) may affect the conversion between nitrate, nitrite, and N-NAs in foods, the concentration of these substances in ready-to-eat food may differ from the concentration in food raw materials. VKM therefore aims to answer the research question "What is the fraction and conversion between nitrate, nitrite, and nitrosamines in foods?".

To make the exposure estimations as precise as possible for the Norwegian population, concentration values of nitrate, nitrite, and N-NAs in ready-to-eat foods are needed. When such data are not available, information on the conversion rates between nitrate, nitrite and N-NAs can be used to predict the actual concentrations of these substances in ready-to-eat foods.

2.1.1 Search for prediction models

VKM will search for publicly available models that predict the conversion rates and partition coefficients between nitrate, nitrite, and N-NAs in foods to answer the research question "Are there any existing models to predict conversion of nitrate to nitrite, and nitrite to nitrosamine in foods?". Rather than performing a systematic literature search to identify all relevant models, the project group will search for and curate existing models. VKM considers this approach to be a more efficient way of identifying models, since we expect that few models exist. Identified models will be checked for suitability for our purpose and performance (the applicability domain) to identify the parameters with highest impact on the uncertainty of the model output. The applicability domain of a model defines the boundaries within which the model's predictions are considered reliable.

Based on uncertainty analysis of the identified parameters (uncertainty quantification), literature searches may be performed to identify data for parameters that have a major impact on the uncertainty.

2.1.2 Conversion modelling

If no prediction models are identified, concentration values for nitrate, nitrite and N-NAs in the same food item together with information of substances that may impact the conversion, can be used to build simple conversion models in the programming language R. The predicted concentrations will be dependent on the food item in question, the concentration of precursors in the food item and the processing it undergoes.

The building of such conversion models is possible only if sufficient concentration values are available. The following data sources will be used to identify such values:

- EFSA opinions on nitrate, nitrite and N-NAs (EFSA, 2008; EFSA et al., 2017a; EFSA et al., 2017b; EFSA et al., 2023).
- A call for data on concentration values for nitrate and/or nitrite and/or N-NAs analysed in the same food item has been sent from the EFSA contact point (Norway) to the contact points in all EFSA member countries.

The models may be specific for different food categories, depending on the available data. VKM aims to implement information on factors that may influence the rate of conversion between nitrate/nitrite and N-NAs in a conversion model. We will prioritise to build models for food categories with a high impact on the dietary exposure.

2.2 Absorption, distribution, metabolism and excretion (ADME)

2.2.1 The fate of nitrate, nitrite and nitrosamines in humans

Several factors affect the fate of nitrate, nitrite and N-NAs in the body (see Section 1.2) and should, therefore, be considered in the exposure estimations. We aim to answer the following four research questions:

- What are the ADME characteristics of nitrate, nitrite, and nitrosamines in humans?
- What is the level of endogenous nitrite and nitrosamine production in the body?
- What is the fraction and conversion between nitrate, nitrite, and nitrosamines in the body?

The EFSA re-evaluations of nitrate (EFSA et al., 2017a) and nitrite (EFSA et al., 2017b) and the EFSA risk assessment of N-NAs (EFSA et al., 2023) will be used to answer the questions above. Literature searches will be performed if we do not obtain sufficient information on ADME for any of the substances. If additional information is needed, narrative literature searches designed to fill the information gaps, will be performed.

2.2.2 Models for nitrate, nitrite, and nitrosamine metabolism and conversion in humans

Knowledge about conversion rates of nitrate to nitrite and further to N-NAs in the human body is needed for evaluating health effects of exposure to nitrate and nitrite from food. Mathematical models that predict the formation of nitrite from nitrate and N-NAs from nitrite can aid in the evaluation. The research question to be answered is "Are there established models for nitrate, nitrite, nitrosamine metabolism and conversion in humans?".

In the EFSA opinion on nitrite (EFSA 2017), two toxicokinetic (TK) models on endogenous N-NA formation was discussed. One model was published by Zeilmaker et al. (2010) in which the simulation of N-nitrosodimethylamine (NDMA) formation in an in vitro model of the GI tract was presented. Another simple mathematical model on endogenous N-NA formation was described in "The Guidelines for Canadian Drinking Water Quality" (Health Canada, 2013). The model by Zeilmaker et al. (2010) was further updated by Lin et al. (2020) and Cheng et al. (2021), by including the reaction

of nitrite to methaemoglobin (see Figure 2.2.2-1). However, the conversion rate of nitrite to N-NAs was not included in that model. The code for the TK model by Lin et al. (2020) was published in Berkeley Madonna software. We will implement the model by Health Canada into R and translate the model by Zeilmaker et al. (2010)/Lin et al. (2020) from Berkeley Madonna into R. These models will be employed to test their predictive value of conversion of nitrate to nitrite and of nitrite to N-NAs. We will calibrate these models with human data found in the literature to furthervalidate the applicability of these models for our purpose. One appropriate data set is sufficient to validate the model, thus no systematic literature search will be performed. Simultaneously, we will search the literature for alternative models to predict endogenous conversion of nitrate to nitrite, and nitrite to N-NAs in the human body, including BioModels, GitHub and Zenodo.

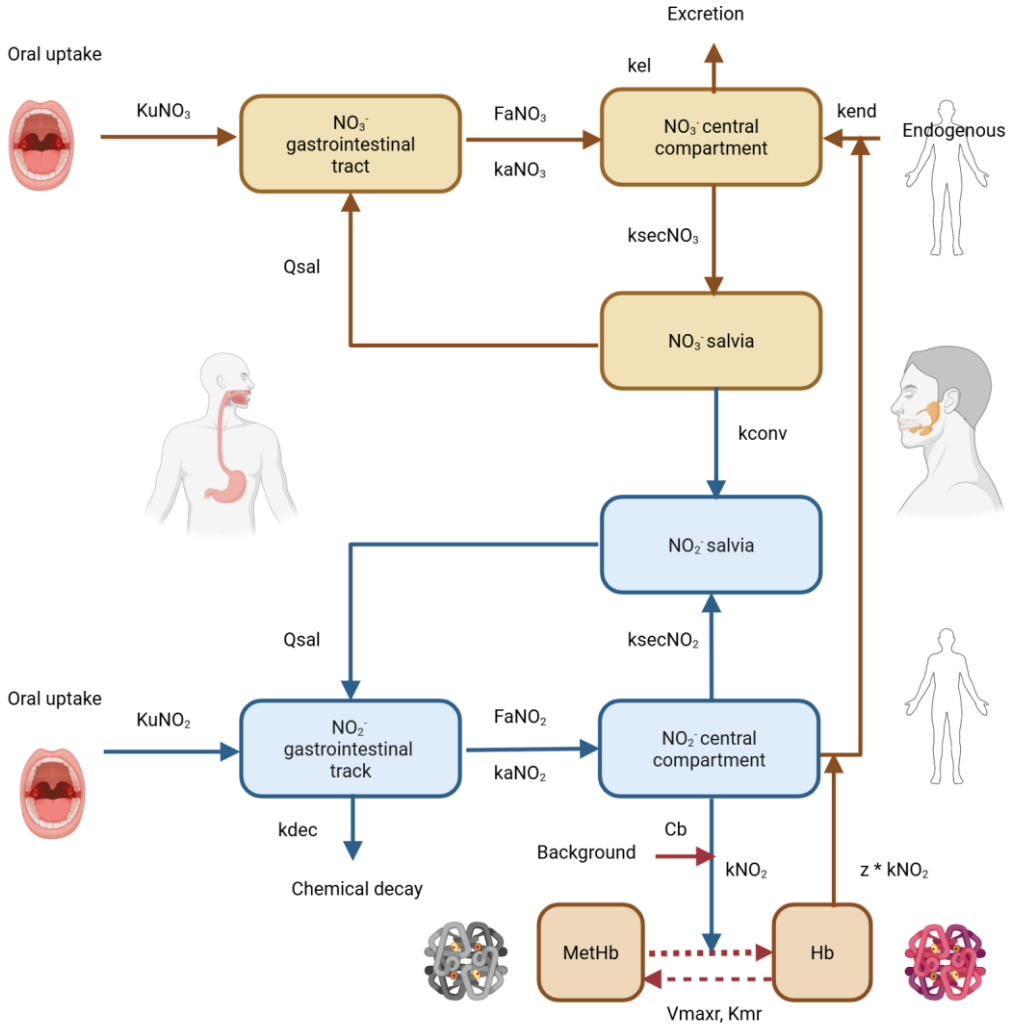

Figure 2.2.2-1. Schematic presentation of the human toxicokinetic model for nitrate and nitrite following oral intake. This model structure was modified from Cheng et al. (2021), which was based on Lin et al. (2020)), and the model first published by Zeilmaker et al. (2010). $KuNO_3$ /$KuNO_2$: dietary intake of NO_3/NO_2; $FaNO_3$/$FaNO_2$: gastrointestinal absorption fraction for NO_3/NO_2; $kaNO_3$/$kaNO_2$: gastrointestinal absorption rate constant for NO_3/NO_2; ksecNO3/ksecNO_2: blood-to-saliva secretion rate for NO_3/NO_2; Qsal: salivary flow rate; kel:

overall elimination rate constant of nitrate from the central compartment; kend: rate constant of endogenous nitrate synthesis; kconv: conversion rate of nitrate to nitrite in saliva; kdec: rate constant of nitrite gastrointestinal decay to other; Cb: background concentration of hemoglobin oxidation; kNO_2: nitrite reaction rate constant with hemoglobin; z: stoichiometric constant for regeneration of nitrate from methemoglobin; Vmaxr: methemoglobin reductase maximum metabolic rate; Kmr: Michaelis-Menten constant of methemoglobin reductase activity; Hb: hemoglobin; MetHb: methemoglobin. Figure created with BioRender (https://app.biorender.com/illustrations/682e26025c63bb47db37c4b3).

2.2.3 The fate of nitrate, nitrite and nitrosamines in research animals

Adjustment for possible differences in conversion rates of nitrate to nitrite and further to N-NAs in experimental animals (mammals) and humans is important when using experimental animal data in exposure modelling and toxicity studies.

We aim to answer the research question "Are the ADME characteristics of nitrate, nitrite, and nitrosamines in research animals similar to that in humans? If not, which adjustment factor should be applied?".

The data sources that will be used to answer these questions are the EFSA re-evaluations of nitrate (EFSA et al., 2017a; EFSA et al., 2017b; EFSA et al., 2023) and nitrite (EFSA et al., 2017b), and the EFSA risk assessment of N-NAs (EFSA et al., 2023). If additional information is needed literature searches designed to fill the information gaps will be performed.

2.3 Hazard identification and characterisation

VKM will prepare an overview of systematic reviews on nitrite exposure and negative health effects to answer the research question "Should the ADI for nitrite be updated?". We will conduct the hazard identification and characterisation for nitrite according to the Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration (OHAT handbook; (NTP, 2019)). This handbook describes methods to determine association of a given exposure with one or more health outcomes, and eventually whether the exposure is deemed to be a hazard for humans.

The methods for conducting an overview of reviews are not described in the OHAT handbook, but are described elsewhere, e.g. in The Cochrane Handbook for Systematic Reviews of Interventions, Chapter V (Pollock et al., 2024). We aim to follow the OHAT handbook because the core question underlying our research question is one of association of exposure with health outcomes. In contrast, the core question addressed in the Cochrane handbook is one of effects of interventions on health outcomes. Further, the OHAT handbook allows for inclusion of experimental animal studies and mechanistic studies, which the Cochrane handbook does not. We describe amendments to the methods in the OHAT handbook in sections 2.3.6 (Risk of bias assessment) and 2.3.7 (Analysis and results).

We anticipate that experimental animal studies investigating effects of nitrite exposure on outcomes may be eligible for inclusion (Table 2.3.1-1). Therefore, we use the expression "estimates of association/effect" to accommodate for this possibility in sections 2.3.5 and 2.3.7.

The Rayyan software (Ouzzani et al., 2016) or the EPPI-Reviewer software (Thomas, 2022) will be used for the screening, EPPI-reviewer will be used for the full-text assessment, data extraction, and the risk of bias evaluation.

2.3.1 Eligibility criteria

The eligibility criteria for systematic reviews on nitrite and negative effects are given in Table 2.3.1-1.

Table 2.3.1-1. Eligibility criteria for studies on negative health outcomes of nitrite.

Population	Humans of all age groups, males, and females Non-human mammals
Exposure	Oral exposure to nitrite in foods
Comparison	No intake Placebo Dose comparison
Outcomes	Any negative health effect, including, but not restricted to: <u>Humans</u> • Carcinogenicity • Genotoxicity • Cardiovascular effects • Endocrine effects • Haematotoxicity • Immunotoxicity • Reproductive and developmental effects <u>Animals</u> • Body weight changes, • Carcinogenicity (non-genotoxic/genotoxic) • Cardiovascular effects • Clinical signs of toxicity • Endocrine toxicity • Food and drink consumption • Haematotoxicity • Immunotoxicity • Neurotoxicity • Organ weight changes and histopathological changes • Reproductive and developmental toxicity
Study design	Systematic reviews* * A publication qualifies as a systematic review if 1) it describes a specific research question and the specific criteria used for selecting studies, 2) the

	authors have performed a systematic literature search, and 3) it includes a quality assessment of the selected studies (Cochrane Glossary, 2020). We will exclude systematic reviews that does not contain one or more of the specified study designs
Publication year	No restriction
Country	No restriction
Language	Danish, English, German, Norwegian, Polish, and Swedish

2.3.2 Information sources

We will search the electronic databases CAB abstracts, Cochrane, Embase, Epistemonikos, Medline, Scopus, and Web of Science. A research librarian will conduct the searches after drafting the search strategies with the project group.

We will also check reference lists in included systematic reviews and search web sites of relevant organisations such as EFSA.

2.3.3 Literature search

A research librarian will search the electronic databases from inception to search date. Appendix 1 shows the planned search strategy for all databases.

2.3.4 Study selection

We will independently, in pairs of two, check 1) titles and abstracts for relevance, and 2) full text articles against eligibility criteria (Table 2.3.1-1). Disagreements in step 2 will be solved by consensus or by consulting a third reviewer.

2.3.5 Data extraction

We will extract the following data from each included systematic review, using a pre-prepared, structured data extraction form:

- Authors
- Title
- Journal
- Year of publication
- Funding
- Reported conflict of interest
- Main objective(s), including PECOs (Population, Exposure, Comparison, Outcome)
- Number of primary studies included, and their study designs
- Publication years (range) of the included primary studies
- List of the main outcomes/endpoints considered
- List of key findings that relate to the systematic review questions
- The risk of bias tool used
- Method of synthesis (meta-analysis, narrative)

- Measures of association/effect (e.g. relative risk, odds ratio, mean difference)
- Estimates of association/effect with 95% confidence intervals (95% CI)

We will extract the following data on the primary studies included in each systematic review (information will be extracted from the systematic review):

- The country where the study was conducted
- The year the study was conducted
- Population, including number of participants or animals
- Exposure, including administration of the substance (in food or tested separately), dose, duration of exposure, and follow-up time
- Comparison, including placebo, no treatment, dose comparison
- Outcomes measured
- Reported estimates of association/effect

One author will extract the data, and another author will verify the data extraction against the full text articles.

2.3.6 Risk of bias assessment

We will use the ROBIS tool for the assessment of risk of bias in the systematic reviews fulfilling the eligibility criteria (University of Bristol, 2025; Whiting et al., 2016). This tool is divided into three phases, phase 1 is the assessment of relevance of the systematic reviews, phase 2 is the identification of concerns with the review process, and phase 3 is the judging risk of bias. The following four domains are included in the phase 2 assessment:

- Study eligibility criteria
- Identification and selection of studies
- Data collection and study appraisal
- Synthesis and findings

Each of the four domains includes signalling questions for the assessment of the risk for introduction of bias. An overall judgement of high, unclear, or low risk of bias is made based on the assessments in each domain. Appendix 2 shows the signalling questions in the ROBIS tool.

Two reviewers will independently assess the risk of bias. Disagreements will be solved by consensus or by consulting a third reviewer.

2.3.7 Analysis and results

We will present a) characteristics of the included studies according to populations, exposures, comparisons, and outcomes b) risk of bias assessments, and c) estimates of association/effect (95% CI) in text, tables and figures. If feasible, we will further sort estimates of association/effect (95% CI) according to outcomes across systematic reviews.

Regarding c), we will preferably present pooled estimates of association/effect extracted from meta-analyses reported in included systematic reviews.

2.3.8 Assessment of confidence in the evidence

We will follow the OHAT handbook, although the handbook concerns the conduct of systematic reviews, i.e., by synthesising results from primary studies. However, we anticipate that the methods are amenable to overviews of systematic reviews as well, expecting that sufficient data are reported in included systematic reviews to complete the assessment.

We will rate initial confidence in the body of evidence separately for human and animal experimental data. Confidence for each outcome is assessed by the following key features of study designs included in the systematic reviews: controlled exposure, exposure prior to outcome, individual outcome data, and comparison group used. A "High" rating is given when studies include four features; three features will give a "Moderate" rating; two features will give a "Low" rating; one feature will give a "Very low" rating.

Following our initial rating we will consider factors that decrease confidence, i.e., risk of bias across studies, unexplained inconsistency, indirectness, imprecision, and publication bias. If none of these factors were downgraded, we will consider factors that increase confidence (applicable to outcomes with initial confidence ratings lower than "High"), i.e., large magnitude of effect, dose response relationship, residual confounding, consistency across species or population, and "other" (e.g., particularly rare outcomes). We will present our judgements for each outcome and each evidence stream (human or animal) in an Evidence profile table. Final ratings will be described as follows:

- High confidence (++++) in the association between exposure to the substance and the outcome. The true effect is highly likely to be reflected in the apparent relationship.

- Moderate confidence (+++) in the association between exposure to the substance and the outcome. The true effect may be reflected in the apparent relationship.

- Low confidence (++) in the association between exposure to the substance and the outcome. The true effect may be different from the apparent relationship.

- Very low confidence (+) association between exposure to the substance and the outcome. The true effect is highly likely to be different from the apparent relationship.

2.3.9 Translation of confidence ratings into level of evidence for health effect

Conclusions for the level of evidence for health effects will be based on the ratings of overall confidence in the association between exposure to the substance (high,

moderate, low, or very low) and the nature of the effect (health effect or no health effect). The descriptors used to categorize the level of evidence are high, moderate, low, evidence of no effect, and inadequate evidence. The descriptors "high", "moderate", and "low" translate directly from the Evidence profile table. A rating of very low translates to the descriptor inadequate evidence. The descriptor evidence of no health effect indicates that there is no association between the substance and the health outcome. This descriptor is used only when there is high confidence in the body of evidence.

2.3.10 Integration of evidence to develop hazard conclusions

We will base conclusions of hazard identification for each health outcome on integration of evidence streams for human and animal studies as described in the OHAT handbook (NTP, 2019). The hazard identification conclusions are:

a) Known to be a hazard to humans
b) Presumed to be a hazard to humans
c) Suspected to be a hazard to humans
d) Not classifiable as a hazard to humans
e) Not identified as a hazard to humans

Evidence receiving the hazard conclusions a, b or c will be used to answer the research question "Should the ADI for nitrite be updated?".

2.3.11 Toxicity reference points for internal exposure

The toxicity reference points for nitrite and N-NAs were derived for external exposure (EFSA et al., 2017b; EFSA et al., 2023). In the VKM risk assessment of nitrate, nitrite and N-NAs, both external and internal exposure will be estimated (Section 2.4.3) and toxicity reference points for internal exposure are therefore needed. VKM will derive internal reference points based on the reference points for external exposure (Section 1.3) and ADME data, such as absorption and metabolism for nitrite and N-NAs (Section 2.2).

2.4 Exposure assessment

The exposure assessment will answer the research question "What is the nitrate, nitrite, and nitrosamine exposure in the Norwegian population?".

Intake data from national dietary surveys (Section 2.4.1) and concentration values for nitrate, nitrite and N-NAs (Section 2.4.2) will be used for the exposure estimation (Section 2.4.3). Both the external and the internal exposure to nitrate, nitrite and N-NAs for the age groups included in the national dietary surveys will be estimated by applying the traditional deterministic approach (Section 2.4.3.1) and a probabilistic approach (2.4.3.2). Information on the conversion between nitrate, nitrite and N-NAs in foods (Section 2.1) and the ADME characteristics of these substances (Section 2.2) will be taken into consideration and accounted for when possible.

2.4.1 Dietary intake data for food containing nitrate, nitrite and/or nitrosamines

Four national dietary surveys are planned to be used to estimate the intake of nitrate, nitrite and N-NAs to answer the research question "What is the consumption of nitrate, nitrite, and nitrosamine containing foods?". These surveys include Norkost 4 (Myhre et al., 2024), Ungkost 3 (Hansen et al., 2015), Småbarnskost 3 (Astrup et al., 2020), and Spedkost 3 (Myhre et al., 2020). Some main characteristics for the surveys are presented in Table 2.4.1-1.

Table 2.4.1-1. The Norwegian dietary surveys planned to be used to estimate the intake of nitrate, nitrite and nitrosamines.

Survey	Year	Age (years)	Methods	Estimation of food portions
Norkost 4 **Myhre et al., 2024**	2022-2023	18 - 80	Two 24-hour recalls, by telephone	Natural units (e.g. apples, eggs), household measures, or food portion photographs
Ungkost 3 **Hansen et al., 2015**	2015	4, 9, 13	Four-days web-based food diary	Natural units (e.g. apples, eggs), household measures, or food portion photographs
Småbarnskost 3 **Astrup et al., 2020**	2019	2	Semi-quantitative food frequency questionnaires[1]	Predefined household units (eggs, slice of bread, decilitres etc.), or food portion photographs
Spedkost 3 **Myhre et al., 2020**	2019	1	Semi-quantitative food frequency questionnaires[1]	Predefined household units (eggs, slice of bread, decilitres etc.), or food portion photographs

[1]The caretaker was instructed to consider the previous two weeks as a timeframe when completing the questionnaire.

All individual food items reported to be consumed in the national dietary surveys are included in the NutriFoodCalc system (NFC; the University of Oslo). NFC is a food composition and nutrient calculation system combining dietary assessment data with food composition data. Within each of the 14 main group foods of NFC, different levels of subgroups are organised (examples are provided in Table 2.4.1-2). NFC is planned to be used for the exposure assessment (Section 2.4.3).

Table 2.4.1-2. Examples of main food groups (Level 1) and subgroups (Levels 2, 3, and 4) in the NutriFoodCalc system.

Level 1 (examples)	Level 2 (examples)	Level 3 (examples)	Level 4 (examples)
Cereals and cereal products	Flours; bread; crisp bread; breakfast cereals; traditional soft and flat bread	**Flours:** flours dry; grains cooked **Bread:** 0.25% wholegrain; 25-50% wholegrain; 50-75% wholegrain	**Flours dry:** flours, white and wholegrain, of barley, wheat, oat, maize, rye, etc**.** **Bread:** homemade; store baked; industrially produced ***(Next level:*** *brand specific data if applicable)*
Potatoes and potato products	Raw; boiled; oven cooked; potato stews; potato salads	**Raw:** Norwegian potatoes; imported potatoes; winter-potatoes; summer-potatoes; etc. **Potato salads:** with creme fraiche; with dressing; home-made; with full fat mayonnaise; with light fat mayonnaise	-

2.4.2 Concentration values for nitrate, nitrite, and nitrosamines

In the report:" Mapping of dietary sources of nitrates and nitrites. "Part 1 of the risk assessment of nitrates and nitrites in food" (VKM et al., 2024a), VKM identified foods on the Norwegian market containing nitrates and/or nitrites and their concentrations. The data were compiled in an Excel spreadsheet to create a database. In Part 2 of the request, concentration values from recent analyses of products on the Norwegian market provided by the Norwegian Food Safety Authority and data used in the EFSA risk assessment of N-NAs (EFSA et al., 2023), will be compiled and included in the database to answer the research question "What is the concentration of nitrate, nitrite, and nitrosamines in foods?". In addition, a systematic literature search will be performed to identify additional concentration values on N-NAs for food categories for which EFSA et al. (2023) reported lack of data (see Section 2.4.2.1).

In the exposure estimation (Section 2.4.3), concentration values for products on the Norwegian market will be prioritised over other concentration values when this is considered to provide more precise exposure assessments for the general Norwegian population. However, if the concentration values are in the same order of magnitude, data will be pooled. This will be based on expert judgement and documented in the risk assessment workflow.

Concentration values for foods not reported to be consumed by participants in the national dietary surveys (see Section 2.4.2) will not be included in the exposure estimates.

If the concentration values for a specific food item are uncertain due to limited or lack of data, concentration values for comparable foods may be assigned the food item in question. For example, if concentration values for certain types of cabbages are not available, we will assign them concentration values from, primarily, other cabbages or, secondarily, from a similar food group, e.g. other cruciferous vegetables. The assigning of concentration values from similar groups will be based on expert judgement and confer with the grouping of foods in NFC. If the concentrations values of any food groups or specific food items are deviating from those of similar food items, they will not be assigned to food items lacking concentration values. The process of assigning concentration values will be documented in the risk assessment. The assigned concentrations will be used as the basis for the deterministic exposure estimation (Section 2.4.3.1). The assignment of concentration values will be taken into consideration for the grouping of concentration values for the probabilistic exposure estimation (Section 2.4.3.2).

2.4.2.1 Systematic literature search for concentration values

The systematic literature search will be performed to identify concentration values for the following N-NAs and food categories:

- N-NAs: NDEA, NDPA, NMEA, NDBA, NDiBA, NMA, NSAR, NMOR, NPIP, and NPYR (see Abbreviations).

- Food categories as named in FoodEx2 (the EFSA standardised system for classifying and describing food): "fruit and fruit products", "fruit and vegetable juices and nectars (including concentrates)", "grains and grain-based products", "legumes, nuts, oilseeds and spices", "milk and dairy products", "starchy roots or tubers and products thereof, sugar plants" "vegetables and vegetable products", and "water and water-based beverages".

We will independently, in pairs of two, check titles and abstracts for relevance, and full text articles against eligibility criteria (Table 2.4.2.1-1). Disagreements in the full text assessment will be solved by consensus or by consulting a third reviewer. The Rayyan software (Ouzzani et al., 2016) will be used for the screening and the full text assessment.

Table 2.4.2.1-1. Eligibility criteria for concentration values for nitrosamines.

Substances	NDEA, NDPA, NMEA, NDBA, NDiBA, NMA, NSAR, NMOR, NPIP, and NPYR
Study design	Analytical studies Exposure studies (reporting concentration values in foods and drinks)
Outcome of interest	Concentration values for nitrosamines in food
Publication year	No restriction

Countries	Europe
Language	Danish, English, German, Norwegian, Polish, and Swedish

In an initial screening step, it will be evaluated whether the retrieved studies meet minimum methodological requirements that allow an assessment of the methodological reliability and reporting quality. Studies not reporting original data on N-NAs in food will be excluded. Studies in which the analytical method, including analytical technique target analytes, sample matrix and either limit of detection (LOD) or limit of quantification (LOQ), are not sufficiently described within the publication itself will be excluded. An exception is if the method is clearly Cited and traceable to a previously published source in which all relevant methodological details are provided.

For studies fulfilling the eligibility criteria, the quality of the analysis will be evaluated using an evaluation framework based on seven quality indicators. The indicators are scored between 0 and 2 according to the criteria provided in Table 2.4.2.1-2.

Table 2.4.2.1-2. Approach for evaluation of methodological quality. ICH: International Council for Harmonisation; ISO: International Organization for Standardization; LOD: Limit of detection; LOQ: Limit of quantification; QC: Quality control; RSD/CV: Relative Standard Deviation/Coefficient of Variation.

Indicator	Criteria	Score (0–2)
1. Method Description	Full details (technique, analytes, matrix, sample preparation), appropriate method[1] is used	2
	Partial detail (some steps missing), appropriate method is used	1
	Inappropriate method is used, or method description is insufficient: A priori exclusion, study does not meet the minimum inclusion criteria	0
2. Sensitivity	LOD and/or LOQ reported, appropriately low	2
	LOD and/or LOQ reported, but close to or above thresholds	1
	LOD and LOQ unknown: A priori exclusion, study does not meet the minimum inclusion criteria	0
3. Method Validation	Fully validated (ICH/ISO, interlaboratory comparison, peer-reviewed validation)	2
	Internal or partial validation	1
	Not validated / unknown method	0
4. Internal standard used	Stable isotope-labelled internal standard used and clearly described	2
	Possibly used, unclear	1

	Not used or not mentioned	0
5. Quality Control Measures	Full QC documentation (blanks, spikes, replicates, calibration checks)	2
	Limited QC reported	1
	Not reported	0
6. Performance parameters	Recovery, accuracy, RSD/CV, matrix effects provided	2
	Only some reported	1
	Not reported	0
7. Reporting transparency	Full disclosure of sample size, detection frequency, non-detect handling	2
	Partial information	1
	Incomplete or unclear	0

[1]GC-MS; GC-TEA; GC-MS/MS; LC-MS/MS are appropriate methods. Methods that lack sensitivity or selectivity for trace-level nitrosamine analysis in food matrices which are not appropriate: UV-Vis Spectrophotometry, Calorimetric assays, TLC, GC or LC methods without mass spectrometry, indirect estimation approaches.

The summed scores will be used to assign one of three confidence levels for the study. Reaching an overall score between 11-14, studies meeting all or most criteria on all quality indicators will be assigned a "high confidence level" and prioritised for extraction and synthesis of concentration values of N-NAs in food. Studies reaching a score range between 7-10 will be assigned a "medium confidence level". Studies assigned "medium confidence level" show gaps, but are overall reliable, and may be considered for data extraction. Studies scored between 0-6 will be assigned a "low confidence level" and will not be considered due to major deficiencies in method quality description. A pilot study will be carried out, and the criteria in Table 2.4.2.1-2, and/or the score range for assignment of the confidence levels may be refined as needed based on initial findings and feedback from the pilot.

An overview of the data that will be extracted from studies assigned a high or moderate confidence level is given in Table 2.4.2.1-3. The concentration values will also be included in the database and used in the exposure estimation (see Section 2.4.3). Excel will be used as spreadsheet for evaluation of the methodological quality and the data extraction from these studies. The Excel file will be published as Supplementary materials to the risk assessment.

Table 2.4.2.1-3. Data items to be extracted.

Study characteristics	Title Author(s) Publication year

	Year of sampling and/or analyses Country Funding Reported conflict of interest
Methods for analyses	Analytical technique used Analytes included Limit of detection Limit of quantification Method validation parameters Use of internal standard Method performance parameters Quality control measures (blanks and replicates included, calibration method)
Results	Sample matrix (food) Edible part Processing Number of samples Single or pooled samples Concentration values Detection frequency
Comments	Laboratory accreditation

2.4.3 Exposure estimation

The approach for the application of concentration values differs for the deterministic and probabilistic approaches. The deterministic approach applies a single value representing the central tendency of all concentration values for a food item. Probabilistic assessments use more complicated modelling approaches [than the deterministic] that rely on distributions of data as inputs in place of point values for key parameters. This results in a distribution of possible exposure estimates (epa.gov). A probability distribution of concentration data is created by mathematical curve fitting, i.e. a curve is generated that best fits the data points. The probabilistic approach will provide characteristics for the variability and uncertainty in the estimates (EPA, 2023). As a result of this difference, the grouping of foods for the exposure estimation may differ. We will pursue similar use of the concentration values across different food groups for both methods to the greatest extent feasible

2.4.3.1 Deterministic estimation of external exposure

Exposure to nitrate, nitrite and N-NAs will be estimated based on the individual intakes reported by the participants in the dietary surveys and the central tendency such as the median or the mean of the concentration values for each reported consumed food item. Some food groups may have a number of values below the LOD/LOQ, which can significantly impact downstream calculations. For concentration values below LOQ and LOD (left-censored data), VKM will either apply the same approach as for the probabilistic exposure estimation (see Section 2.4.3.2), or we will replace all data below LOQ with ½ LOQ. The choice of method is dependent on the number of analyses, and the number of analyses below LOD/LOQ.

The internal exposure will be estimated using TK models describing endogenous conversion and partition of nitrate/nitrite/ N-NAs (Section 2.2).

The mean, median, minimum, maximum and the 95th percentile will be reported.

2.4.3.2 The probabilistic estimation of external and internal exposure

The exposure assessment will be performed using custom made methods in R. The probabilistic exposure estimation draws on data distributions to select multiple input values for concentration in food for the exposure equation across numerous simulations. The individual intake reported in the dietary surveys will be used and is therefore not sampled. This process generates a distribution of potential exposure outcomes for each participant in the survey.

For concentration values below LOQ and LOD (left-censored data), we will use one of the methods suggested by Bolks et al. (2014) depending on the proportion of left-censored data for a food category (Bolks et al., 2014; EFSA, 2010). The R package NADA will model the data distribution accounting for data points below LOQ/LOD and estimate the distributional summary metrics (i.e. mean, median, standard deviation) of concentration values for each food category. The internal exposure will be estimated using TK models describing endogenous conversion of nitrate/nitrite/ N-NAs (Section 2.2).

Probabilistic exposure estimation of some contaminants has previously been published using intake data from another survey (EuroMix (Husøy et al., 2019)), and the codes are available at https://github.com/TrineHusoy/Acrylamide_exposure.git, https://github.com/TrineHusoy/PBPK_PFOA.git. Since the EuroMix survey has similarities to the national dietary surveys planned to be used in the current assessment, the codes applied in it will be the starting point for the development of new codes for the exposure estimation in the current assessment. The codes will be adapted to fit the assessed substances (nitrate, nitrite and N-NAs) and the intake data (the national dietary surveys).

The results generated using the probabilistic approach will be presented as a distribution of possible exposure estimates and will represent a more comprehensive characterisation of variability and uncertainty around these estimates than the deterministic approach. In addition, the mean, median, minimum, maximum and the 95th percentile will be reported. A global sensitivity analyses of all the parameters in

the models will be performed and the impact of the most sensitive parameters on the uncertainty in the exposure assessment will be described.

All R codes of the exposure modelling will be published open access on repositories such as GitHub and archived on Zenodo with stable DOI. Where data are used that cannot be freely shared (such as individual data from the dietary surveys), dummy data files will be created for testing the code.

2.4.3.3 Scenarios

To answer the research question "What is the nitrate, nitrite, and nitrosamine exposure if the Norwegian population follow the food based dietary guidelines?", two scenarios representing the lower and upper intervals of the amounts specified in the dietary guidelines will be designed (VKM et al., 2024c). The selection of foods for the scenarios will be based on the wording in the dietary guidelines and the foods reported to be consumed in the national dietary surveys.

In the lower interval scenario, the amounts for all food groups will be set to the lower level specified in the dietary guidelines. In the higher interval scenario, all food groups will be set to the upper level, except for certain food groups which are set to the same amount in both scenarios in line with the guidelines. The dietary guidelines do not specify quantity intervals for all food groups. For these food groups, quantities previously derived in consultation with the Norwegian Directorate of Health will be used (VKM et al., 2024c). The selection of foods from the food groups that are included in low and high dietary guidance scenarios is based on the wording in the dietary guidelines (e.g. low fat, low sugar, high fibre, etc.).

Both the deterministic and the probabilistic approach will be applied to estimate the exposure from these scenarios. The mean, median, minimum, maximum and the 95th percentile will be reported for both approaches. In addition, the results generated using the probabilistic approach will be presented as a distribution of possible exposure estimates.

2.4.3.4 Data management

We will perform our analysis in TSD "Services for sensitive data (TSD)" hosted by University of Oslo, service for high security data storage and computing. No individual data will be exported from the TDS server and only aggregated data will be presented. While waiting for the access to the data from the consumption surveys in Norway, we will start to develop methods using the small biomonitoring study EuroMix, since this study have dietary records from two separate study days similar to Norkost 4 as well as dummy data. At the completion of the project, the TSD machine will be destroyed, and the code will be exported to Zenodo and GitHub for archiving.

A comprehensive data management plan will be created at https://ds-wizard.org/ and will be publicly available for maintaining FAIR principles and to trace reproducibility.

2.5 Risk characterisation

With the risk characterization, the research question "Does the dietary exposure to nitrate, nitrite, and nitrosamines represent a risk for negative health outcomes for the Norwegian population?" will be answered.

For nitrite, VKM will assess whether the exposure is below or above the ADI. For nitrate, the conversion to nitrite in the body will be estimated, and the nitrite level from nitrate will be compared to the ADI for nitrite.

The risk characterisation of N-NAs will use the margin of exposure (MOE) approach, and the estimated N-NAs exposure will be compared to the $BMDL_{10}$ based on the incidence of tumors in rats. A MOE at or above 10 000 for carcinogenic and genotoxic substances will be considered as low risk. This is in accordance with the statement from EFSA (EFSA, 2012): "*The Scientific Committee reiterates its view expressed in 2005 that in general a margin of exposure of 10,000 or higher, if it is based on the BMDL10 from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view; the magnitude of an MOE however only indicates a level of concern and does not quantify risk*".

3 Discussion

We consider precise exposure estimations for the Norwegian population to be of high importance in this risk assessment. However, given the limited timeframe, some pragmatic solutions were chosen for the following: identification of concentration data and models for the transformation of nitrate, nitrite and N-NA-nitrosamines in food and body, identification of ADME of the same substances, as well as hazard identification and characterization.

Models for conversion of nitrate, nitrite, and N-NAs in food

Hand search will be substituted for a systematic search, since identifying all models may not improve the quality of the risk assessment. Performing a systematic literature search has been identified as being a particularly time intensive task.

ADME of nitrate, nitrite, and N-NAs

Information on ADME has been comprehensively summarised by EFSA (EFSA et al., 2017a; EFSA et al., 2017b; EFSA et al., 2023). Thus, we will apply their information instead of performing a systematic search. We consider this approach not to be an important source of uncertainty in the assessment.

Hazard identification and characterisation

The previously derived ADI for nitrate and the $BMDL_{10}$ for N-NAs will be used as reference point for toxicity instead of performing literature searches for hazard data and assessing whether these values should be updated.

A systematic literature search for toxicity data for nitrite will be performed but will be limited to systematic reviews. Toxicity studies not included in systematic reviews will therefore not be included.

Concentration values

A systematic literature search will only be performed for N-NA concentration values in food categories for which such data were reported to be missing in the EFSA assessment (EFSA et al., 2023).

Concentration values for nitrate and nitrite will be identified using a limited number of selected data sources. VKM assume that uncertainty in the risk assessment will not be increased despite omission of searches given the high number of concentration values for a wide array of foods.

Exposure assessment

The deterministic approach is known to overestimate the upper tail of the exposure distribution (Goedhart et al., 2012) whereas the probabilistic approach will solve this issue by sampling from the distribution for all concentration values. The deterministic

approach can be used to validate the central estimate from the probabilistic approach, since the central estimates from both approaches are expected to be in the same range. See EPA (2023) for comprehensive information of deterministic and probabilistic exposure assessment. Both exposure assessment approaches will be limited by the availability and quality of the input data, and both will require expert judgement regarding grouping of foods and use of the concentration values for similar foods when concentration values are lacking.

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Appendix 1

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to May 07, 2025>

1	Nitrites/
2	(nitrite? or sodiumnitrite? or "nitrous acid salt?" or "E 249" or "E 250" or "7758-09-0" or "7632-00-0").tw,kf.
3	1 or 2
4	limit 3 to "reviews (maximizes specificity)"
5	Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.
6	4 or (3 and 5)

Database: Embase <1974 to 2025 May 07> via Ovid

1	nitrite/
2	(nitrite? or sodiumnitrite? or "nitrous acid salt?" or "E 249" or "E 250" or "7758-09-0" or "7632-00-0").tw,kf.
3	1 or 2
4	limit 3 to "reviews (maximizes specificity)"
5	exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.
6	4 or (3 and 5)
7	limit 6 to "remove medline records"

Database: CAB Abstracts <1973 to 2025 Week 19> via Ovid

1	Nitrite/ or exp nitrites/
2	(nitrite? or sodiumnitrite? or "nitrous acid salt?" or "E 249" or "E 250" or "7758-09-0" or "7632-00-0").tw.
3	1 or 2
4	limit 3 to "systematic review"
5	(meta-analysis or systematic reviews).sh. or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw.
6	4 or (3 and 5)

Database: Cochrane Database of Systematic Reviews

1	[mh ^Nitrites]
#2	(nitrite? or sodiumnitrite? or ("nitrous acid" NEXT salt?) or "E 249" or "E 250" or "7758-09-0" or "7632-00-0"):ti,ab
#3	#1 or #2
#4	#3 in Cochrane Reviews

Database: Web of Science Core Collection: Science Citation Index Expanded (SCI-EXPANDED) --1987-present, Social Sciences Citation Index (SSCI) --1987-present, Arts & Humanities Citation Index (A&HCI) --1987-present, Emerging Sources Citation Index (ESCI) --2015-present

3	#1 AND #2
2	TS=(("systematic*" NEAR/1 "review*") or ("review" and (("structured" or "database*" or "systematic*") NEAR/1 "search*")) or "integrative review*" or ("evidence" NEAR/1 "review*")) OR TI=("metaanal*" or "meta anal*") OR AB=("metaanal*" or "meta anal*")
1	TS=(nitrite$ or sodiumnitrite$ or "nitrous acid salt$" or "E 249" or "E 250" or "7758-09-0" or "7632-00-0")

Database: Scopus

1	TITLE-ABS(nitrite or nitrites or sodiumnitrite or sodiumnitrites or "nitrous acid salt" or "nitrous acid salts" or "E 249" or "E 250" or "7758-09-0" or "7632-00-0") OR AUTHKEY(nitrite or nitrites or sodiumnitrite or sodiumnitrites or "nitrous acid salt" or "nitrous acid salts" or "E 249" or "E 250" or "7758-09-0" or "7632-00-0")
2	TITLE-ABS((systematic* W/1 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) W/1 search*)) or "integrative review*" or (evidence W/1 review*)) OR AUTHKEY((systematic* W/1 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) W/1 search*)) or "integrative review*" or (evidence W/1 review*))
3	1 and 2

Database: Epistemonikos

(title:((nitrite OR nitrites OR sodiumnitrite OR sodiumnitrites OR "nitrous acid salt" OR "nitrous acid salts" OR "E 249" OR "E 250" OR "7758-09-0" OR "7632-00-0")) OR abstract:((nitrite OR nitrites OR sodiumnitrite OR sodiumnitrites OR "nitrous acid salt" OR "nitrous acid salts" OR "E 249" OR "E 250" OR "7758-09-0" OR "7632-00-0")))

Publication type: Systematic Review

Appendix 2

The ROBIS tool: signalling questions for phase 2 (https://www.bristol.ac.uk/media-library/sites/social-community-medicine/robis/ROBIS%201.2%20Clean.pdf).

Domain 1

- Did the review adhere to pre-defined objectives and eligibility criteria?
- Were the eligibility criteria appropriate for the review question?
- Were eligibility criteria unambiguous?
- Were any restrictions in eligibility criteria based on study characteristics appropriate (e.g. date, sample size, study quality, outcomes measured)?
- Were any restrictions in eligibility criteria based on sources of information appropriate (e.g. publication status or format, language, availability of data)?

Domain 2

- Did the search include an appropriate range of databases/electronic sources for published and unpublished reports?
- Were methods additional to database searching used to identify relevant reports?
- Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?
- Were restrictions based on date, publication format, or language appropriate?
- Were efforts made to minimise error in selection of studies?

Domain 3

- Were efforts made to minimise error in data collection?
- Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?
- Were all relevant study results collected for use in the synthesis?
- Was risk of bias (or methodological quality) formally assessed using appropriate criteria?
- Were efforts made to minimise error in risk of bias assessment?

Domain 4

- Did the synthesis include all studies that it should?
- Were all pre-defined analyses reported or departures explained?
- Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?
- Was between-study variation (heterogeneity) minimal or addressed in the synthesis?
- Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?
- Were biases in primary studies minimal or addressed in the synthesis?