Evaluation of the parametric value for Nitrate in drinking water

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Summary

Background: "In 2019, the *Danish Ministry of Environment and Gender Equality* asked the *DTU National Food Institute* to assess whether the current 50 mg/L drinking-water limit for nitrate sufficiently protects public health in light of emerging evidence linking lower concentrations to increased colorectal cancer risk. The 2019 review concluded that epidemiological evidence was too uncertain to justify revising the limit. Subsequently, additional studies on cancer and adverse birth outcomes, together with an economic analysis, provided further evidence supporting stricter drinking-water standards. In 2024, DTU published an updated report concluding that, although uncertainties persist, the epidemiological evidence for colorectal cancer indicates that "*nitrate in drinking water poses a health risk*". The Ministry therefore convened an international group of experts[1] to evaluate the existing parametric value for nitrate in drinking water and, if warranted, to propose a revised value based on the available evidence.

Assessment: The working group evaluated the two DTU reports as well as the underlying primary studies on cancer and reproductive outcomes. New research published after the 2024 report was also assessed. The working group concluded that while associations with reproductive outcomes, including congenital malformations, are suggestive, they are not sufficiently consistent. For cancers other than colorectal cancer, most studies show no consistent associations, with only bladder cancer and childhood central nervous system cancers providing suggestive evidence. In line with the 2024 DTU report, the working group determined that the strongest evidence for adverse effects below the current parametric value for nitrate in drinking water (of 50mg/L) concerns colorectal cancer and that, for this outcome, "nitrate in drinking water poses a health risk." This conclusion is supported by the current epidemiological evidence, including newer studies not assessed by DTU in 2024, and by clear biological plausibility. Accordingly, the working group concluded that a revision of the current parametric value is justified as a precautionary approach.

Derivation of a parametric value: To derive a revised value, the working group used the large registry-based Danish cohort study by Schullehner et al. (2018). Benchmark dose modelling was performed using EFSA's Bayesian framework with benchmark responses of 5% and 10% relative increases in incidence above background. Sensitivity analyses using alternative modelling approaches were also conducted. Considering both the available data and modelling uncertainties, the working group proposes a parametric value of 6 mg/L for nitrate in drinking water. Based on the broader evidence reviewed, this value is also expected to be protective against other potential adverse health outcomes, including certain malformations observed in some studies. Modelling uncertainties and expected reductions in cancer cases associated with this value were quantified to enable risk managers to evaluate the implications of adopting a limit value.

Conclusion: Based on the totality of evidence, a parametric value of 6 mg/L for nitrate in drinking water should provide protection against potential effects on colorectal cancer and developmental effects associated with chronic exposure to nitrate from drinking water. To further assess the robustness of this proposed value, any revisions to the current drinking-water standard should be accompanied by ongoing evaluation of their implications for disease incidence.

[1] Referred to in this report as 'the working group"

Background

In 2019, the *Ministry of Environment and Gender Equality of Denmark* the *National Food Institute at the Technical University of Denmark (DTU)* to review the scientific evidence on the public health impacts of nitrate exposure. The aim was to determine whether the current drinking water limit of 50 mg/L for nitrate provided adequate protection. This assessment was partly motivated by a large Danish population-based registry study [1] that found associations between nitrate concentrations in drinking water and an increased incidence of colorectal cancer at levels well below the EU limit of 50 mg/L[2].

As a starting point, the *DTU National Food Institute* drew upon previous evaluations conducted by international authorities, including the *International Agency for Research on Cancer (IARC)* [2], the *World Health Organization (WHO)* [3], and the *European Food Safety Authority (EFSA)* [4]. In addition, newly published evidence subsequent to these assessments was identified and critically reviewed. The report also provided an overview of the principal sources of nitrate exposure and the estimated dietary intake in the Danish population. It concluded that *"considerable uncertainty exists with respect to the results of the epidemiological studies; therefore, a firm conclusion could not be drawn."* Consequently, no revision of the current drinking water limit was proposed.

Subsequently, additional epidemiological studies were published indicating potential associations between nitrate exposure in drinking water and adverse health outcomes, including low birth weight, congenital malformations, and cancer. In addition, an economic analysis that incorporated the previously reported association with colorectal cancer suggested substantial health and economic benefits from lowering the nitrate standard for drinking water [5]. In light of this emerging evidence, the *Ministry of Environment and Gender Equality of Denmark* once again commissioned the DTU National Food Institute, in 2023, to update its previous evaluation. The updated assessment, completed in 2024, concluded that:

> *"…exposure to nitrate in drinking water is positively associated with risk of developing colorectal cancer based on the overall knowledge. DTU therefore finds that nitrate in drinking water constitutes a health risk. This assessment also applies to exposure to nitrate in drinking water below the existing parametric value of 50 mg/L. DTU notes, however, that the assessment of the parametric value in relation to the risk of developing colorectal cancer is subject to some uncertainty. In addition, DTU finds that it cannot be excluded that nitrate in drinking water is a risk factor for certain other types of cancer. DTU also notes that beneficial effects of nitrate were not included in the assessment"*

Based on this conclusion, the *Ministry of Environment and Gender Equality of Denmark* appointed an international expert group to review the scientific evidence supporting a potential reduction of the current nitrate limit of 50 mg/L in drinking water.

[2] https://www.eea.europa.eu/en/analysis/indicators/nitrate-in-groundwater-8th-eap

Terms of reference

The Scope and Tasks of this work as provided the *Ministry of Environment and Gender Equality of Denmark* are presented here below. The full terms of reference, including background and further specification of work are provided in **appendix I**.

"Scope

The scope of the expert group is to provide scientific expertise in areas of relevance for evaluating and determining health-based reference values, in particular when the available data are from epidemiological studies.

Using a multidisciplinary approach, the expert group will contribute to the protection of human health by evaluating the existing parametric value for nitrate in drinking water and potentially devise a proposal for a revised parametric value based on the best available scientific knowledge and evidence. The evaluation by the expert group could be based on the two evaluations from the DTU National Food Institute and should be supplied with other relevant information including any new scientific literature of relevance for the scope until two months before the termination of this mandate.

Tasks

The tasks of the expert group are:

- *to evaluate the parametric value for nitrate in drinking water in order to determine if it is sufficiently protective for the public with respect to the risk of developing cancer, in particular colorectal cancer.*

- *if required, to devise a health-based proposal for a revised parametric value for nitrate in drinking water based on the overall evidence.*

    - *This should consider also the overall exposure to nitrate and its transformation product nitrite from all sources.*

    - *If relevant, it may include a meta-analysis and modelling of exposure/response relationships based on data from high-quality epidemiologic studies.*

    - *If possible, the feasibility in terms of technologic/economic possibilities for obtaining a revised parametric value for nitrate in drinking water should be considered.*

    - *If possible, to estimate the decrease of risk to the Danish public in terms of the development of cancer, in particular colorectal cancer, if the parametric value for nitrate in drinking water is changed to a certain lower level.*

- *to propose additional studies to be performed if a lack of data is identified in order to evaluate if the existing parametric value of nitrate in drinking water is sufficiently protective of human health."*

Assessment

The assessment was conducted in three stages.

First, the working group evaluated the 2019 and 2024 reports on nitrate prepared by the *DTU National Food Institute*. This step included a critical review of several key primary research studies cited in those reports, focusing on associations between nitrate concentrations in drinking water and health outcomes such as cancer, low birth weight, and congenital malformations. To obtain additional clarification and contextual information, an online consultation was held with Dr. Schullehner, whose publication [1] was considered highly relevant to the assessment. A targeted literature search was also performed to identify any new publications that might materially challenge the main conclusions of the 2024 DTU report.

Second, the working group assessed the biological plausibility of the reported associations between nitrate exposure and various health outcomes, with particular emphasis on cancer. In addition to the DTU reports, this step drew upon evaluations by international agencies, including the *Joint FAO/WHO Expert Committee on Food Additives* (JECFA), IARC, and EFSA.

Finally, based on the findings and conclusions from the first two stages, the working group concluded that the evidence for an association between nitrate exposure in drinking water and colorectal cancer was considered sufficiently strong to warrant, as a precautionary measure, a revision of the current parametric value of nitrate in drinking water. This step involved a bench-mark dose–response modelling based on results from the study by Schullehner et al. [1] and evaluation of the associated uncertainties.

The methodology, main conclusions, and justifications for each of these three stages are described in the following sections.

Evaluations of previous DTU reports

The working group evaluated the content of both the 2019 and 2024 DTU reports on nitrate. Among these, the 2024 report was considered the most relevant, as the studies reviewed in 2019 were also included in the 2024 evaluation. Moreover, the 2024 report was more comprehensive, incorporating a structured literature search and a risk-of-bias assessment for colorectal cancer, which was identified by the report's authors as a critical outcome. The working groups conclusion on the studies and findings of the 2024 DTU report are briefly summarized below.

Outcomes other than cancer

As noted in the DTU report the associations between nitrate exposure and methemoglobinemia and inhibition of iodine uptake are well established based on both human and animal studies at high exposures. Associations with these two outcomes are also widely recognized by other international agencies [3, 4] and form the basis for the current parametric value for nitrate in drinking water of 50 mg/L [3].

The 2024 DTU report additionally reviewed several new Danish studies examining associations between nitrate concentrations in drinking water and reproductive outcomes [6-9], including reduced fetal growth, preterm birth, stillbirth. These studies shared a similar design to the nationwide study on colorectal cancer by Schullehner et al. [1], in which nitrate exposure was estimated by linking residential addresses with nitrate concentrations from local water suppliers. Several studies, including new Danish investigations [9, 10], have also suggested possible associations between nitrate exposure (including from nitrosatable drugs) and congenital malformations. However, findings have not been consistent across malformation types, leaving some uncertainty.

Overall, the working group agreed with DTU's conclusion that the reported associations with reduced birth weight, preterm delivery are *suggestive*[3] and that for preterm birth and certain malformations the *"risk cannot be excluded"*. Associations with other reproductive outcomes were inconclusive. Despite indications of risk for preterm birth and certain malformations the working group considered the current evidence not sufficiently robust, in terms of consistency and dose–response, to support derivation of a parametric value for nitrate in drinking water.

Studies on cancer other than colorectal cancer

The 2024 DTU report provided a detailed review of studies examining the association between nitrate in drinking water and various cancer outcomes. This analysis is not directly comparable to other evaluations, such as the EFSA 2017 assessment [4], which considered studies on both dietary and drinking-water nitrate exposure together. As outlined in the 2024 DTU report and further discussed below, the presence of dietary antioxidants that inhibit the formation of N-nitroso compounds (NOCs) provides a strong scientific basis for considering dietary and drinking-water exposures separately.

Based on the studies reviewed in the 2024 DTU report, no consistent association was observed between nitrate in drinking water and cancers of the lung [11], oesophagus [12, 13], adult brain [14, 15], uterus [11], skin [11], small intestine [12], liver [12], bile ducts [12], pancreas [11, 16, 17], stomach [11-13, 18][4], or leukaemia in adults [11] and children [19, 20].

Similarly, the working group considers that studies on lymphoma [11, 20-23], adult brain tumors [14, 15], prostate [24], ovary [11], [25][5], and thyroid [26] cancers are inconclusive, as indications of increased risk were reported in single studies only and not replicated independently in different populations. Findings for kidney cancer [11, 27, 28] were likewise inconsistent and inconclusive across studies.

For bladder cancer [11, 23, 29-32], the results were more suggestive with three studies [11, 30, 32] observing statistically significant or indicative positive associations, while three did not. Regarding childhood brain and central nervous system (CNS) cancers, studies of early-life exposure (prenatal and postnatal) to nitrate or nitrite in drinking water [20, 33-35] tended to show elevated risks at higher exposure levels [20, 33, 35], though not always statistically significant. Notably, a large Danish registry study [20] reported odds ratios (OR) of approximately 1.5–1.8 for CNS cancer at nitrate concentrations above 25 mg/L with no evidence of elevated risk or clear dose–response relationship below that concentration.

A key limitation of the above-mentioned studies is that, for most cancer sites, the number of available studies is small. In addition, differences in study design and exposure assessment introduce considerable uncertainty. In line with the DTU report the working group concluded that the evidence for bladder cancer and childhood CNS cancers is suggestive but not sufficiently strong to support derivation of a parametric value for nitrate in drinking water. Evidence for other types of cancer was considered inconclusive.

Studies on colorectal cancer

The DTU (2024) report identified 13 primary studies investigating the association between nitrate concentrations in drinking water and colorectal cancer. Five studies that examined the association

[3] DTU concluded that a risk for reduced fetal growth and preterm birth "*cannot be excluded"*.
[4] The studies by Weyer et al 2001 and Buller et al 2021 are based on the same Iowa Women's Health Study
[5] Both publications are based on the same Iowa Women's Health Study

between nitrate exposure and mortality from colorectal cancer were excluded[6] from further analysis [36-39].

Of the eight remaining studies, two publications were based on the same cohort study [11, 40], and another two on the same case–control study [41, 42]. To avoid duplication, only the cohort study with the longest follow-up period [40] and the case–control study with the largest number of cases and controls [41] were included for assessment, leaving a total of six primary studies. Of these, two were cohort studies [1, 40] and four were case–control studies [41, 43-45], all of which were evaluated by DTU for reliability (i.e. risk of bias).

The conclusions of the DTU evaluation in terms of study reliability and main findings are shown in Table 1. To facilitate interpretation, the percentage of participants (in cohort studies) or controls (in case–control studies) with nitrate exposure levels above the cutoff of 10 mg/L in drinking water have been added to the table, where such information was reported or could be estimated. When such data were unavailable, the proportion of participants or controls in the highest reported exposure category of that study is shown. The rationale for selecting a value of 10 mg/L is that this value corresponds approximately to the cutoff used for the highest exposure category in the study by Schullehner et al [1] where a clear indication of risk (as reflected by significant hazard ratio (HR) of ~1.15) was observed. As such this cutoff can be used to compare the exposure distribution of the other studies relative to Schullehner et al. A summary of each of the six studies assessed in the 2024 DTU report is provided below.

Table 1. Summary of results, reliability and nitrate exposure for the six primary research papers used to evaluate the relationship between nitrate exposure in drinking water and the development of colorectal cancer as reported in the DTU (2024) report[1].

	Cancer site					
	colorectal	***colon***	***rectum***	***Reliability[2]***	***Nitrate Cutoff***	***% of population above cutoff***
Cohort						
Schullehner 2018	Positive	Positive	Positive	High	>10 mg/L	~19%
Jones 2019	Not reported	None	None	Medium	>3.5 mg/L	~19%
Case-control						
De Roos 2003	Not reported	None	None	Medium	>10 mg/L	<1%
McElroy 2008	None	Positive/none[4]	None	Low	>10 mg/L	~6%
Espejo-Herrera 2016[3]	positive	positive	Positive/none[5]	High	>10 mg/L	~23%
Fathmawati 2017	Positive	Not reported	Not reported	Low	>50 mg/L	~89%

[1]The two right most columns have been added to the table (not included in the 2024 DTU report)
[2] In the DTU report "reliability of each study is assessed based on a risk of bias assessment that included seven quality parameters addressing study design and execution, population, exposure assessment, outcome assessment, control for confounding, statistical analysis and reporting". However, the exact instrument used for that assessment is not referenced.
[3] In the 2024 DTU report the results for males and females were reported separately for this study but here the two columns are combined as findings were significant for both sexes except for rectal cancer
[4] Significant for proximal but not distal colon cancer
[5]Not significant for females

Cohort studies

- **Schullehner et al. (Denmark) [1]:** "In this large registry-based cohort study, the association between nitrate concentrations in drinking water and colorectal cancer (overall and by subsite) was examined among 1,742,093 individuals who were alive on their 35th birthday between 1978 and 2011, and subsequently followed until cancer onset. Over a total of 23 million person-years, 6,008 colorectal cancer cases were identified, of which 3,700 were colon cancers.

[6] Although the reasons were not stated in the 2024 DTU report, studies using cancer-specific mortality rather than incidence as the outcome are limited in that observed associations reflect both cancer risk and post-diagnosis survival, thereby complicating interpretation and potentially introducing bias.

Individual exposure to nitrate was estimated by linking participants' residential addresses over time to water supply areas, allowing calculation of average nitrate exposure from ages 20 to 35. When comparing the highest (≥9.3 mg/L) to the lowest (< 1.3 mg/L) quintile of nitrate in drinking water, the HR for colorectal cancer was 1.15 (95% CI: 1.07–1.24), with similar estimates reported for colon and rectal cancer. Across exposure quintiles, there was a relatively clear indication of a dose–response relationship.

- **Jones et al. (US) [40]:** In a subset of 15,910 participants from the Iowa Women's Health Study who reported using the same public water supply for more than 10 years, Jones et al. identified 624 colon and 158 rectal cancer cases between 1986 (recruitment) and 2010 (end of follow-up). No significant association was observed with HRs of 0.97 (95% CI: 0.75–1.26) being observed for colon cancer and 0.64 (95% CI: 0.38–1.07) for rectal cancer when comparing ≤0.36 mg/L to > 3.5 mg/L nitrate in drinking water.

DTU rated the exposure assessment in the study by Jones et al. as low quality, whereas the corresponding assessment for the Schullehner et al. study was rated as medium. It is difficult to determine how the different bias domains could have contributed to the divergent findings. However, based on the nitrate concentrations, participants in the Jones et al. study were exposed to substantially lower concentrations, as reflected by the fact that only about 19 % of participants had exposures above 3.5 mg/L, whereas approximately 19 % of participants in the Schullehner et al. study were exposed to levels exceeding 10 mg/L. As such, the estimates from the two studies are not directly comparable.

Case-control studies

- **De Roos et al. (US) [43]** examined the association between nitrate concentrations in drinking water and the risk of colon (n = 376) and rectal cancer (n = 338) compared with 1,244 healthy controls from Iowa, matched by age and sex. Mean nitrate concentrations in drinking water were assigned to participants based on self-reported residential history. Individuals who primarily consumed bottled water were assigned a low exposure value. Fewer than 1 % of the study population had nitrate concentrations above 10 mg/L. When comparing low (≤ 1 mg/L) versus higher (> 5 mg/L) exposure categories, the odds ratios (ORs) for colon and rectal cancer were 1.2 (95 % CI: 0.8–1.7) and 1.2 (95 % CI: 0.9–1.8), respectively.

- **McElroy et al (US) [45]** examined the association between nitrate in drinking water and colorectal cancer, including subsites of proximal colon, distal colon, and rectum, among 475 cases aged 20–74 years (recruited during 1990–1992 and 1999–2001) and 1,447 controls from rural Wisconsin. Nitrate exposure was assessed by linking participants' residential addresses with 1994 measurements of nitrate in drinking water, corresponding approximately to the period of case identification, which represents a clear limitation due to temporal ambiguity. Comparing those with higher (≥ 10 mg/L) versus lower (< 0.5 mg/L) exposure, the OR for colorectal cancer was 1.57 (95 % CI: 0.97–2.52). However, there was no clear dose–response relationship, as elevated effect estimates were also observed at lower exposure levels. The corresponding ORs were 2.76 (95 % CI: 1.42–5.38) for proximal colon cancer, 1.23 (95 % CI: 0.59–2.56) for distal colon cancer, and 1.26 (95 % CI: 0.47–3.43) for rectal cancer.

  Espejo-Herrera 2016 (Spain and Italy) [41] examined the association between nitrate in drinking water and colorectal cancer in a study including 1,869 cases (1,285 colon and 557 rectal cancer) and 3,530 controls from Spain and Italy. The Italian controls (n = 396) were hospital-based[7], while the Spanish controls were population-based. All controls were matched by age, sex, and area of residence. Average nitrate intake from drinking water during the "main

[7] admitted to the same hospital as cases for acute, non-chronic disease

exposure period" covering 30 to 2 years before the interview was estimated from participants' residential history, nitrate measurements from local water suppliers, and self-reported water source (tap, bottled, well) and consumption quantity. Comparing those with lower (≤ 5 mg/L) versus higher (> 10 mg/L) exposure, the ORs for colorectal, colon, and rectal cancer were 1.49 (95 % CI: 1.24–1.78), 1.52 (95 % CI: 1.24–1.86), and 1.62 (95 % CI: 1.23–2.14), respectively. Similar estimates were observed in both sexes, and there was a clear dose–response trend across the three exposure categories (≤ 5, 5–10, > 10 mg/L). Sensitivity analyses excluding Italian participants did not materially change the findings.

- **Fathmawati et al. (Indonesia) [44]** the association between nitrate in drinking water and colorectal cancer in 75 cases recruited (2014–2016) from a hospital in Yogyakarta and 75 hospital controls who had undergone colon biopsy confirming non-neoplastic findings. All participants had lived in the city for at least three years. Nitrate concentrations measured in participants' wells (first quarter of 2016) were used as the exposure metric. Comparing lower (< 50 mg/L) versus higher (> 50 mg/L) nitrate concentrations in drinking water, the OR for colorectal cancer was 2.8 (95 % CI: 1.1–7.4).

Across all case–control studies, the estimated effect sizes generally suggested an increased risk of colorectal cancer with higher nitrate exposure, although not always statistically significant (specifically in [43, 45]). According to the DTU assessment, the studies reporting no or some suggestive association were rated as having low or moderate reliability [43, 45], whereas the two studies reporting significant associations [41, 44] were rated high and low reliability, respectively. Thus, the reliability ratings do not fully explain the divergent findings. However, these two studies reporting significant associations [41, 44] were conducted in populations with greater exposure contrast compared with those reporting unclear or no association [43, 45].

In summary, in the 2024 DTU report rated one cohort [1] and one case-control [41] study as having high reliability. Both studies reported relatively clear associations between nitrate concentrations in drinking water and colorectal cancer. The other studies varied in their findings, with some supportive evidence reported in three studies [43-45], whereas one study did not find an association [40]. Assuming that a causal dose-response relationship of weak to moderate strength exists between nitrate exposure in drinking water and colorectal cancer, studies conducted in populations with a small exposure contrast would be less likely to detect such an association. The partially divergent findings among the six included studies (see Table 1) are broadly consistent with this interpretation.

New literature on colorectal cancer since 2024

With colorectal cancer being identified in the 2024 DTU report as the outcome with the strongest evidence linking nitrate in drinking water with cancer a new literature search was performed by the working group to identify new studies on colorectal cancer published after the report. Two new studies were identified, and their findings are summarized below.

In an ecological study from the *US Cisneros et al* [46] examined the association between nitrate in drinking water, as provided by the *California State Water Resources Control Board*, with incidence of colorectal cancer between 2010-2015, extracted from the *Surveillance, Epidemiology, and End Results Program in California*. A total of 56,631 colorectal cancers were identified during the study period. Associations were examined for water nitrate concentrations from 1 and up to 20 years prior to diagnoses with different lag-times. The mean concentrations (SD) over this 20 year period was 14.8 mg/L (7.2). Significant associations between nitrate concentrations in drinking water and colorectal cancer were observed with relative risk estimates ranging from 1.02 to 1.07 per 1-mg/L increase in nitrate exposure depending on the exposure lag-time (1 to 20 years).

In a study based on the *Danish Diet, Cancer, and Health Cohort* (N = 54,610) *Erichsen et al.* examined the association between nitrate exposure from diet and drinking water and colorectal cancer risk [47]. Over 27 years of follow-up, a total of 2,235 colorectal cancer cases were identified, including 1,508 colon cancer cases. Dietary nitrate intake was assessed at baseline (1993–1997; participant age 50–65 years) using a validated food frequency questionnaire (FFQ). Nitrate exposure from drinking water was estimated by linking participants' residential addresses to nitrate concentrations in local water supplies (as provided to households) and by quantifying tap water intake based on FFQ data.

In the study Erichsen [47] neither total dietary nitrate intake nor nitrate from specific dietary sources was associated with colorectal cancer or its subtypes, with HRs tightly centered around the null. Similarly, quantified nitrate intake from tap water, based on self-reported consumption, was not associated with colorectal, colon, or rectal cancer. However, when nitrate exposure was classified according to concentrations in drinking water supplied to the household, the HRs comparing the highest (≥9.3 mg/L) with the lowest (< 1.3 mg/L) exposure categories were 1.29 (95% CI: 0.99–1.69) for colorectal cancer, 1.52 (95% CI: 1.11–2.07) for colon cancer. Below the highest exposure category there was no clear evidence of dose–response and no association was observed for rectal cancer [0.86 (95% CI: 0.49–1.50)]. For colorectal and colon cancer (but not rectal cancer), associations were more pronounced among individuals with established co-risk factors, including current or former smoking, high red meat intake, and low intake of antioxidants[8].

This new evidence, particularly the study from the Danish Diet, Cancer, and Health Cohort [47], strengthens the evidence base suggesting that nitrate in drinking water may be associated with an increased risk of colorectal cancer. Although the two large Danish studies [1, 47] complement each other, each has distinct strengths and limitations that warrant consideration. The Danish Diet, Cancer, and Health Cohort [47] allows for a more detailed assessment of individual-level factors compared with the larger registry-based cohort by Schullehner et al. [1], including more comprehensive control for confounding and explicit evaluation of dietary factors as both confounders and potential effect modifiers. However, despite its relatively large sample size, the statistical power of this cohort is lower than that of the registry-based study. This is not only related to sample size but also to the fact that smaller proportion of participants with higher nitrate exposure. Specifically, only about 2 % of participants in the Danish Diet, Cancer, and Health Cohort, whose participants were recruited within the greater areas of Copenhagen and Aarhus, were supplied with drinking water containing ≥ 9.3 mg/L nitrate, compared with approximately 20 % in the nationwide registry-based study by Schullehner et al. The lower exposure contrast in the Danish Diet, Cancer, and Health Cohort may contribute to the absence of a clear dose–response relationship observed in the new cohort study. Finally, it is worth noting that the recruitment periods, and therefore parts of the study populations, in the Erichsen (1993–1997) [47] and Schullehner (1978–2011) [1] studies overlap, although the age at exposure (20–35 years vs. 50–65 years) and the geographic recruitment areas differ.

***In conclusion*,** despite certain limitations and minor inconsistencies, the two large studies now available from Denmark [1, 47] are concordant in indicating a risk of colorectal cancer at higher nitrate exposures below the current parametric value of 50 mg/L. The newer study provides additional support for this association, particularly as its findings, when accounting for diet and other risk factors, are biologically plausible, consistent with the proposed mechanism of endogenous formation of N-nitroso compounds (NOCs) through nitrate ingestion[7]. As such the study by Erichsen et al. [47] provides added support to the findings previously reported by Schullehner et al [1].

[8] i.e., N-nitroso compound–inhibiting factors, see section om "Toxicokinetic and mechanistic considerations"

Meta-analyses

Several meta-analyses have examined the association between nitrate exposure from drinking water and colorectal cancer [31, 48-51][9]. Of these, two included dose–response analyses in addition to providing simple summary estimates [48, 49]. Among the five meta-analyses, two reported statistically significant summary estimates above one [48, 49], suggesting an increased risk of colorectal cancer, whereas the others reported non-significant estimates above one.

However, all of these meta-analyses were assessed as having *critically low* methodological quality in the 2024 DTU report, based on the AMSTAR 2 appraisal tool. AMSTAR 2 evaluates compliance with key components of systematic review methodology (but not risk bias per se) across seven critical domains. These include the use of a predefined protocol, an adequate literature search strategy, transparent justification for study exclusion, and other essential elements related to methodological rigor and transparency.

Perhaps more important, several other limitations were noted with some of the meta-analyses including studies on cancer mortality rather than incidence [31, 48], making errors in data extraction [49, 51, 52], or incorporated duplicate studies [31, 49]. Across all meta-analyses, heterogeneity among the included studies was substantial, complicating interpretation of the pooled estimates. Moreover, the merging of cohort and case–control studies, as done in all the meta-analyses, is far from ideal due to fundamental differences in design and risk of bias [53].

Despite these limitations, it is relevant to note that after correcting data-extraction errors and removing duplicate studies [52] in the meta-analysis by Hosseini et al. [49], which was based on the same studies reported in Table 1 above, the summary estimates for colorectal and colon cancer indicated significantly increased risks of 1.39 (95% CI: 1.09–1.78) and 1.27 (95% CI: 1.03–1.57), respectively.

In conclusion, given the small number of available studies for each design and their methodological variability, the working group concluded that limited information could be extracted from available meta-analyses. For the purpose of this assessment, it was also concluded that conducting a new dose–response meta-analysis would not be a feasible approach for evaluating the parametric value for nitrate in drinking water.

Toxicokinetic and mechanistic considerations

The IARC Monograph "*Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins*" (Volume 94, 2010) evaluated separately the potential carcinogenic of ingested nitrate and nitrite from, i) food, ii) drinking water and iii) under conditions that promote endogenous nitrosation [2]. After assessing the evidence IARC concluded that

- the evidence for carcinogenicity of nitrate in food or drinking water was considered "*inadequate*" in both humans and animals
- The corresponding evidence for nitrite was considered "*limited*" in both humans and animals
- The evidence in experimental animals on intake of nitrate in combination with amines and other amides for carcinogenicity was considered as "*sufficient*".

On the basis of *"sufficient"* evidence that nitrate in combination with amines and other amides is carcinogenic in experimental animals and other mechanistic evidence, IARC concluded that "*Ingested*

[9] Reference 52 provides corrected analyses for reference 49 (Hosseini F et al 2021). All results cited here are based on those corrected numbers (see reference 52).

nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)". IARCs basis for this conclusion and its relevance to colorectal cancer is briefly summarized below.

Endogenous nitrosation and carcinogenicity

As reviewed in the IARC monograph [2], ingested nitrate is absorbed from the gastrointestinal tract, reaching peak plasma levels within an hour and clearing with a half-life of about five hours. Nitrate is widely distributed in the stomach, intestines, urinary system and bloodstream. It undergoes further metabolism in circulation, with nitrite interacting with hemoglobin and other molecules to participate in nitric oxide metabolism. The majority of nitrate is excreted through urine within 48 hours, though high urinary nitrate levels are associated with inflammatory conditions. Relative to dietary intake, endogenous nitrate synthesis, primarily through the arginine–nitric oxide pathway, contributes much less to the total circulating nitrate pool.

Once swallowed, nitrite enters the acidic stomach environment, where it can generate nitrosating agents capable of reacting with amines and amides to form N-nitroso compounds (NOCs), many of which are known carcinogens, Their endogenous formation follows a sequence of steps occurring in the gastrointestinal tract. That is, after ingestion from diet[10] or drinking water, nitrate is absorbed into the bloodstream but approximately 25% of it is recirculated in saliva, where oral bacteria reduce ~5% of it to nitrite. Once nitrite enters the acidic environment of the stomach it can generate nitrosating agents that react with dietary or endogenous amines and amides, leading to the formation of NOCs.

The presence of NOCs in the gastrointestinal tract is a major concern due to their role in colorectal carcinogenesis. A significant subgroup of NOCs, nitrosamines, are metabolized by cytochrome P450 enzymes into α-hydroxynitrosamines, which decompose into highly reactive molecules that cause premutagenic DNA lesions. One primary mechanism involves DNA alkylation, where NOCs transfer alkyl groups to DNA bases, leading to mutations such as O6-methylguanine lesions, which mispair during replication, causing G:C→ A:T transitions commonly found in colorectal tumors. Additionally, NOCs can inhibit DNA repair enzymes, such as O6-methylguanine-DNA methyltransferase (MGMT), impairing the cell's ability to correct mutations. Beyond direct DNA damage, NOCs contribute to oxidative stress and inflammation by generating reactive oxygen species (ROS), which damage cellular components and create a pro-inflammatory environment that fosters tumor development. Experimental studies confirm that NOCs induce tumor formation in animal models, particularly in the colon, by causing mutations in oncogenes and tumor suppressor genes, such as TP53.

Factors influencing NOC formation

The possible risk of colorectal cancer from endogenous NOCs is influenced by several factors

- **Diet**, particularly red and processed meat contain nitrite and heme from myoglobin, both of which promote NOC formation in the colon. High red meat intake increases the availability of amines and amides that can react with ingested nitrate-derived nitrite, further enhancing NOC production. Studies show that individuals with high red meat consumption exhibit a stronger association between nitrate exposure and colorectal cancer risk, indicating that dietary factors may influence the carcinogenic potential of ingested nitrate [41, 43, 47]. In contrast, vegetables are also a source of nitrate, but antioxidants such as ascorbic acid (vitamin C) and α-tocopherol (vitamin E), folate, and flavonoids inhibit nitrosation and therefore NOC formation [41, 43, 47]. Vegetable consumption can therefore mitigate the harmful effects of nitrate both from

[10] With caratin vegetables and processed meat being major sources

vegetables themselves and from other sources, and the adverse effects of drinking-water nitrate appear less pronounced in individuals with high antioxidant intake.

- The human **gut microbiota** also plays a crucial role in nitrate metabolism and NOC formation. Certain bacterial species with nitrate reductase activity enhance the conversion of nitrate to nitrite, increasing NOC synthesis in the lower gastrointestinal tract. Individual differences in gut microbiota composition, shaped by genetics, diet, and antibiotic use, may therefore influence the extent of endogenous nitrosation and, consequently, colorectal cancer risk [54].
- **Lifestyle factors** such as smoking, alcohol consumption and physical activity influence the formation of NOCs. For example, tobacco smoke contains preformed NOCs, which may independently contribute to colorectal cancer risk. Similarly, alcohol intake can increase endogenous nitrosation and oxidative stress, further promoting carcinogenesis. Conversely, physical activity has been shown to reduce colorectal cancer risk by modulating inflammation, insulin sensitivity, and gut motility, potentially mitigating the impact of nitrate exposure [55].

Both diet and lifestyle factors may influence (or modify) the association between ingested nitrate and colorectal cancer through their role in NOC formation. In addition, these factors might act as confounders if they are associated with colorectal cancer through other mechanisms (than NOC formation). Separating the role of these factors, as confounders or effect modifiers, through covariate adjustment can be challenging in epidemiological studies (some studies do not collect sufficiently detailed information to address this).

Other uncertainties

Several other uncertainties associated with assessing the association between nitrate in drinking water health outcomes, including colorectal cancer, are worth noting.

- Co-exposure to other contaminants, such as trihalomethanes, may potentially confound the relationship between nitrate and colorectal cancer. However, in the case-control study by Espejo-Herrera et al [41], adjustment for trihalomethanes produced only minimal changes in the risk estimates
- A key methodological challenge in studies of drinking-water contaminants is accurately quantifying exposure, as the proportion of tap and bottled water in total fluid intake varies considerably between populations [56, 57]. Furthermore, exposure may fluctuate over time due to residential changes, which can introduce misclassification of the exposure estimate. These two limitations may partly explain inconsistencies across studies. Still, it is unclear how much accuracy is gained in terms of assessing exposure by relying on simple questions on use of tap versus bottled water at one point in time at least in populations where the variation in use of bottled water is modest. Studies conducted in populations such as Denmark, where tap water is the dominant source of drinking water and nitrate exposure from tap water can be traced through registry linkage, are therefore likely to have higher certainty in exposure assessment.

Evidence for colorectal cancer - conclusion

As reviewed by IARC [2] and in the 2024 DTU report (which also included newer studies), the relationship between nitrate/nitrite ingestion and endogenous nitrosation reactions under experimental conditions is well documented in both humans and animals. Evidence of formation of NOCs in the gastrointestinal tract therefore provides a direct mechanistic link with colorectal carcinogenesis. However, as reviewed by IARC and in the DTU report, the few studies directly assessing carcinogenicity of nitrate in combination with nitrosable amines and amides have been suggestive but not fully consistent. Variation in study design with respect to inclusion of substrates may partly explain such findings.

In line with IARC, other agencies such as JECFA in 2003 [58] and EFSA in 2017 [4] have concluded that nitrate per se is not carcinogenic. The primary focus of these agencies has been to assess the direct link between ingestion of nitrate or nitrite and cancer in both humans and animals, where in line with IARC the evidence from human observational studies and studies in experimental animals was considered inconclusive. In the absence of such evidence, mechanistic evidence for cancer was given less weight by EFSA and JECFA. It is, however, worth noting that none of the human studies on colorectal cancer reviewed in this report had been published when JECFA did their assessment in 2003 and four of the studies reviewed here were not published when EFSA did their assessment [1, 40, 44, 47] of which all except one [40] reported significant associations. As such the evidence for an association between nitrate from drinking water and colorectal cancer has been substantially strengthened.

Furthermore, the two large studies from Denmark [1, 47] could individually and/or combined address some uncertainties listed through use of registry based (but not self-reported) exposure assessment, confounder control and assessing dietary and lifestyle factors associated with NOC formation. With support from other studies and combined with biologically plausible explanation for the observed association between nitrate in drinking water and colorectal cancer, the working group agrees with the conclusion from the 2024 DTU report that *"nitrate in drinking water poses a potential health risk."*

At the same time the working group acknowledges that several uncertainties exists, and better characterization of the association observed between nitrate in drinking water and colorectal cancer observed in some but not all studies assessed in this report is warranted. Despite these uncertainties the overall evidence is considered sufficiently robust to justify a derivation of a new parametric value for nitrate in drinking water as a precautionary approach. The working group considers that the study from Schullehner et al [1] is the most suitable for dose response modelling given its large sample size and exposure contrast.

Evaluating thresholds and non-linearity in nitrate exposure and colorectal cancer risk

The epidemiological literature on nitrate in drinking water and colorectal cancer apparently points to a non-linear, context-dependent association rather than a simple linear dose–response extending from zero exposure.

Existing studies and meta-analyses illustrate why defining a single "safe" level is scientifically problematic. Based on pairwise comparison relative to the lowest quintile (1.3 mg/L) the study by Schullehner et al [1] found statistically significant increases in colorectal cancer risk beginning at mean drinking-water nitrate levels above 3.9 mg/L, well below the conventional 50 mg/L guideline (equivalent to 11.3 mg/L nitrate–N[11]). The next highest (3.8-9.3mg/L) and highest-exposure group (≥9.3 mg/L nitrate ion) showed a HR of 1.11 (95%CI: 1.02, 1.20) and 1.15 (95% CI 1.07–1.24), respectively, compared with the lowest group (<1.3mg/L), with stronger associations for colon than rectal cancer. These findings point to an exposure range at which colorectal cancer risk begins to rise, but they do not identify a distinct threshold below which no risk occurs. On the other hand the more recent study from Danish Diet, Cancer, and Health Cohort [47] suggests a significant increase only above 9.3 mg/L when using the same exposure categories as used by Scullehner et al [1].

Studies that have examined factors influencing endogenous NOC formation—such as diet and smoking—demonstrate that these factors may have substantial effects, supporting a role for dietary

[11] In the United States, nitrate concentrations in drinking water are typically reported as mg/L NO_3-N, whereas in Europe they are more often reported as mg/L NO_3. In the latter case, values can be converted to mg/L NO_3-N using a factor of 0.226 (National Research Council (US) Subcommittee on Nitrate and Nitrite in Drinking Water. Nitrate and Nitrite in Drinking Water. Washington (DC): National Academies Press (US); 1995. PMID: 25101396). https://nap.nationalacademies.org/read/9038/chapter/4#10

nitrosation modifiers in shaping the underlying dose–response relationship between drinking-water nitrate exposure and colorectal cancer. This distinction between nitrate sources, addressed in some studie, helps explain why dietary nitrate intake (primarily from vegetables) shows no association with colorectal cancer [41, 47], while stronger associations are observed for nitrate in drinking water among individuals with low antioxidant intake [41, 43, 47] or higher consumption of red or processed meat [41, 43, 47]. This source distinction explain how waterborne nitrate may confer risk even at levels where vegetable-derived nitrate appears protective

In summary, the current evidence does not support a single, universal biological threshold for nitrate exposure with respect to colorectal cancer. Use of different exposure categories, limited sample sizes at high exposures, exposure misclassification, and residual confounding may explain varying effect estimates and dose-response observed in existing studies.

Regulatory frameworks that treat nitrate solely as a threshold compound for acute toxicity, such as infant methemoglobinemia, may underestimate long-term cancer-related risks. A more protective approach, incorporating lower monitoring or action levels and accounting for dietary context, source-specific exposure, and nitrosation potential, is more consistent with current evidence.

Derivation of a parametric value through benchmark dose modelling

Benchmark dose (BMD) modelling was performed based on results from the paper by Schullehner et al. [1] using Bayesian Model Averaging. The modelling was performed following the principles outlined in the *JECFA*[12] *Guidance Document* [59] and the EFSA Scientific Committee Guidance on the *'Use of the Benchmark Dose Approach in Risk Assessment'* [60]. Because these guidance documents primarily address experimental toxicological data, adaptations and sensitivity analyses were implemented to account for specific challenges associated with modelling human observational data which have been described specifically elsewhere [61].

The primary analyses were performed using the EFSA 'Bayesian BMD' webtool, which applies the R package *BMABMDR* for data preparation, model fitting, model averaging, and plotting[13].

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Modelling considerations

Modelling was performed using adjusted numbers of cases. The adjusted number of cases were calculated from the adjusted hazard ratio (HR) and case distribution in the Schullehner et al 2018 study [1]. This approach was applied by JECFA in its 2011 assessment of inorganic arsenic [62] and later adopted by EFSA in its 2024 assessment of inorganic arsenic [63]. The data used for these analyses and modelling results are presented in **Appendix II.** Modelling results are presented in **Appendix III**.

Selection of a benchmark response (BMR)

In EFSA's 2022 guidance on benchmark dose (BMD) modelling, the benchmark response (BMR) is the pre-specified change in the endpoint used to derive the BMD [60]. This change (the BMR) should be "*measurable, considered relevant to humans or to the model species"* and be "*biologically relevant"* (i.e. adverse). For quantal data the EFSA guidance defines the BMR as "*an increase in the incidence of the lesion/response scored, compared with the background incidence*", with the background incidence, in toxicological experiments referring to the control animals (or "zero dose"). In human observational studies an "unexposed control group" does not exists but a BMR relative to no exposure

[12] JECFA: The Joint FAO/WHO Expert Committee on Food Additives

[13] https://r4eu.efsa.europa.eu/

is obtained by extrapolating the fitted curve to zero [60, 61] or relative to a lowest exposure group [64][14].

In Bayesian analyses the lower credibility interval around the BMD (or the BMDL) is then defined as the "*dose below which the change in response is likely to be smaller than the* BMR". If the BMR is chosen as the smallest measurable change in response that is considered biologically relevant (or adverse) the corresponding BMDL would reflect a dose at which no biologically relevant health risk is to be expected. By convention the credibility interval around the BMD is usually estimated at 90% level meaning that the BMDL reflects the 5th percentile in the BMD probability distribution. Other credibility intervals can also be derived but this must be justified on a case-by-case basis.

For animal cancer studies, a 10% extra risk ($BMR_{10\%}$) is commonly used as the default benchmark response, as it represents the smallest effect size that standard OECD carcinogenicity studies, typically involving approximately 50 animals per sex per dose group, are adequately powered to detect

In contrast, human epidemiological studies generally involve much larger sample sizes, and the definition of a small but measurable increase in risk depends strongly on study design, background incidence, and sample size. Only a few prior examples of BMD modelling in human observational data for quantal outcomes exist. In its 2011 assessment of inorganic arsenic, JECFA modelled an extra risk of 0.5% for cancer [58], while in its more recent evaluation, a smaller 0.1% extra risk was used [65], reflecting a shift to a more conservative approach. In those assessments, the cumulative cancer incidence in the underlying studies ranged from approximately 1.5% to 5%. In its more recent arsenic assessment, EFSA applied an added risk corresponding to a relatively 5% increase in incidence above the lowest exposure category[15]. This approach was used consistently across quantal-based outcomes, including cancer, birth defects, and cardiovascular disease [59].

For disease outcomes with low background incidence, such as colorectal cancer [66], an extra risk corresponding to a 5% relative increase above the referent may, in some cases, be close to or below the limits of what is reasonable to model as reflected in some existing analyses where such modelling has been performed on human cancer data [63]. This supports the conclusion that a 5% relative increase (or a RR of 1.05) is at the lower end of what is reasonable to model in terms of measurable effect size above background variation.

The Schullehner study used for this assessment [1] is a large nationwide cohort with sufficient statistical power to detect small effect sizes, such as a hazard ratio (HR) of 1.05. Although modest, this level of association represents an adverse and meaningful effect. Accordingly, an extra risk corresponding to a 5% increase in HR was considered a conservative benchmark response (BMR), reflecting the smallest measurable change that would be reasonable to model. An extra risk corresponding to a 10% increase in HR was also modelled to represent a clearer adverse effect size that should be more accurately quantified and of high public health relevance.

Based on these considerations, the working group modelled the BMR of 5% and 10% increase in cancer incidence using data relative to the background incidence defined as the incidence in the lowest exposure category, and approximated the BMR relative to a nitrate concentration of 0 mg/L through

[14] This latter approach is less conventional and has not been used by EFSA or JECFA for modelling of epidemiological data.

[15] To clarify the difference between modelling extra risk versus relative risk for the benchmark response (BMR). Let's say that the incidence in the lowest exposure category is x. Modelling 0.1% extra risk results in a BMR = x +0.001. Modelling a relative increase of 5% above background means that the BMR=x*1.05. Regardless of what the incidence (x) in the lowest exposure category absolute increase would always reflect the same additional risk while the added risk related to relative increase is highly dependent on the incidence in the study population under consideration.

extrapolation. The resulting posterior distributions of the benchmark dose (BMD) estimates for these two BMRs were then compared to evaluating the level of protection for both BMRs.

Selection of a reference point

The results of the modelling based on the study by Schullehner et al. (2018) [1] are presented in Table 2. The corresponding benchmark doses (BMDs) for 5% and 10% relative increases in incidence were 11.9 mg/L and 17.8 mg/L, respectively. These values represent the expected nitrate concentrations in drinking water associated with the specified effect sizes. The credible intervals were relatively wide, indicating uncertainty across an exposure ranging from ~3 to ~40 mg/L (for the 5th to 95th percentiles of the BMD). The benchmark dose lower bounds (BMDLs) based on a 90% credible interval when modelling BMR corresponding to of 5% and 10% relative increase over background, were 3.2 mg/L and 6.1 mg/L, respectively.

Table 2. Benchmark dose results[1] for modelling a BMR of 5% and 10% relative increase in cancer incidence above background in the Schullehner et al 2018 study. The table shows the percentile distributions for the estimated BMD when modelling 5% and 10% relative increase.

Percentiles[2]	$BMD_{5\%}$[3]	$BMD_{10\%}$[3]
5th	**3.2**	**6.1**
10th	4.9	8.8
15th	**6.1**	10.6
20th	7.1	12.0
50th (BMD)	11.9	17.8
80th	18.5	25.6
75th	21.4	28.7
90th	27.1	33.3
95th	37.7	40.6

[1] Modelling was performed using the EFSA Bayesian BMD platform: https://r4eu.efsa.europa.eu/app/bmdbayesian
[2] The BMD is the 50th percentile (median) in the BMD distribution while the 5th 10th and 15th percentile reflect the benchmark dose lower bound (or BMDL) for the 90%, 80% and 70% credible intervals, respectively
[3] the subscript refers to modelling of a BMR corresponding to 5% and 10% increase in cancer incidence above background, respectively.

To achieve a protective level for an effect size corresponding to a 5% relative increase over background, the nitrate concentration in drinking water would need to be <3.2 mg/L, based on the conventional 90% credible interval. However, using a 70% credible interval, the corresponding BMDL is 6.1 mg/L. At this concentration, the probability of maintaining a protective level against a 5% relative increase in risk is approximately 85%. Thus, while 6.1 mg/L would not be considered protective under the conventional 90% credibility interval, the overall risk to human health would still be relatively low. Moreover, this same concentration of 6.1 mg/L would provide full protection under the 90% credible interval for a 10% relative increase in risk.

Given the limited number of studies available on nitrate in drinking water and colorectal cancer, and the fact that different modelling approaches produced BMDL estimates varying by only a few milligrams per Liter, the expert group considered that the most conservative estimate (3.2 mg/L) may reflect greater uncertainty in the data rather than a true health risk threshold. In contrast, a BMDL of

6.1 mg/L could be justified as providing protection against a 10% relative increase in cancer incidence while still affording a reasonable level of protection for the more conservative 5% relative increase.

Based on this evaluation, the expert group decided to recommend a parametric value of 6 mg/L for nitrate in drinking water. This recommendation is grounded in evidence from two Danish population-based studies indicating adverse effects on cancer risk at or slightly below 10 mg/L [1, 48], while also considering the uncertainties inherent in the modelling and the limited number of available studies. Deviations from this recommended value can be justified and quantitatively assessed based on the distributions around the BMD presented in Table 2.

Sensitivity analyses

The primary analyses performed using the EFSA 'Bayesian BMD' webtool by modelling the BMR of 5% and 10% increase in cancer incidence using data relative to the background incidence defined as the incidence in the lowest exposure category, and approximated the BMR relative to a nitrate concentration of 0 mg/L through extrapolation. As reported in table 2, this resulted in a $BMDL_{5\%}$ and $BMDL_{10\%}$ of 3.2 and 6.1mg/L, respectively when using a 90% credible interval around the BMD. The other, less conventional, alternative is to estimate the BMR relative to the nitrate exposure observed in the lowest exposure category, where the median concentration was 0.69 mg/L. That approach resulted in a slightly higher $BMDL_{5\%}$ and $BMDL10_{\%}$ of 3.4 and 6.7mg/L, respectively. Given the difference between these two definitions of a BMR is less than 10%, the same conclusions are supported.

To further evaluate the modelling approach the expert group also used two alternative benchmark-dose modelling approaches: *A Web-Based System for Bayesian Benchmark Dose Estimation* developed by Shao et al. [67] was used to model the same BMRs corresponding to relative increases of 5% and 10% above the background incidence (as done using the EFSA software) as well modelling the hazard ratios directly (which is not possible for the EFSA software). Model structure specific BMDL estimates were similar for different modelling approaches. These results are not included in the appendix as they were only used for sensitivity analyses and to make judgement on the consistency across different modelling platforms.

Possible health benefits of a revised parametric value

To estimate the possible benefits in terms of cases avoided if the exposure pattern was reduced, the working group used the exact exposure categories and hazard ratios as reported in the Schullehner paper (see table 3). More recently the average exposure to nitrate in drinking water appears to have been reduced in Denmark and to account more recent estimates as reported by Jacobsen et al were used [5][16]. For the assessment it is also assumed that annual number of colorectal cases are 4543 as reported in the Nordcan database for Denmark between 2019 to 2023[17]. Table 3 also shows the estimated number of annual cases according to the exposure categories in the Schullehner paper.

[16] That paper by Jacobsen et al provided an update assessment on the exposure distribution across the same exposure cutoffs as used in the Schullehner et al paper

[17] https://nordcan.iarc.fr/en

Table 3. Distribution of population, relative risks and estimated number of annual colorectal cancer (CRC) cases as categorized in the Schullehner et al 2018 paper.

Exposure category (mg/L)	Population percentage[1]	CRC[2] HR	CRC Cases per year
<1.27	26.9	1.00	1165
1.27-2.3	26.9	1.06	1234
>2.3-3.9	26.9	1.03	1200
>3.9-9.3	9.9	1.11	476
>9.3	9.4	1.15	468
Total	100%		4543

[1] as reported by Jacobsen et al [5]. In that paper the distribution was 80.7% in the first three exposure groups and here it is assumed the three first groups are the same size (80.7/3=26.9%.).
Effect estimates as reported by Schullehner et al [1]

Based on the estimated number in table 3 the likely benefit in terms of cases which could be prevented under various scenarios can be estimated. Those estimates assume that i) the HR from the Schullehner study represents a causal association in each group, ii) the exposure distribution as reported by Jacobsen et al (2024) reflects current exposure distribution. To assess possible case reduction **five different scenarios** defined by the categories cut-points used in the Schullehner study are assessed (see Table 4).

Table 4: Expected reduction in cases by different intervention scenarios (see text below table for explanation)

		Case distribution for different intervention scenarios				
Exposure category (mg/L)	Case distribution without intervention[1]	1	2	3	4	5
<1.3	1165	4330	1572	1165	2001	1165
1.3-2.3	1234		1234	1234	1234	1234
2.3-3.9	1200		1200	1200	1200	2061
>3.9-9.3	476		476	928		
>9.3	468					
Total	4543	4330	4482	4527	4435	4460
Case reduction		**213**	**61**	**16**	**108**	**83**

[1]number of cases per exposure group prior to reduction in exposure

Scenario 1: Exposure is reduced to a minimum and everybody is exposed to less than 1.3 mg/L. This intervention would result in 213 fewer cases per year

Scenario 2: Exposure is controlled to 9.3 mg/L and those >9.3 mg/L are reduced to a minimum, (<1.3 mg/L). This intervention would result in 61 fewer cases per year

Scenario 3: Exposure is controlled to 9.3 mg/L and those >9.3 are reduced to the group below (3.9-9.3mg/L). This intervention would result in 16 fewer cases per year

Scenario 4: Exposure is controlled to 3.9 mg/L and those >3.9mg/L are reduced to a minimum. This intervention would result in 108 fewer cases per year

Scenario 5: Exposure is controlled to 3.9 mg/L and people currently >3.9 are reduced to the group below (2.3-3.9 mg/L). This intervention would result in 83 fewer cases per year

The results in table 4 clearly show that how exposure is modified (in terms of intervention) has a relatively large impact. For example, for the 9.4% of the population exposed to over 9.3 mg/L, if they are reduced to the minimum (<1.3mg/L) then 61 cases might be prevented, but if they are only reduced to just below 9.3, then 16 cases would be prevented. Thus, there is a range of possible benefits from 16 to 61cases and the average of these two scenarios would be 39 cases.

To estimate the benefit from regulating below 6 mg/L we used linear interpolation based on the figures int table 4. For reduction from 6 mg/L down to the minimum (<1.3mg/L) the benefit would be estimated as 90 cases per year (linear interpolation of benefits in scenario 2 and 4) , and the benefit of dropping to just below 6 would be 57 cases per year (linear interpolation of benefits in scenario 3 and 5), and the **average** of these two scenarios would be **73 cases**.

The estimates presented here in terms of case reduction should be interpreted with some caution. Firstly, the HRs carry uncertainty, as reflected in their 95% confidence intervals. It is also important to note that exposure has already decreased due to reductions in nitrate levels implemented by water suppliers [5] , relative to the exposure estimated by Schullehner et al. for the period 1978–2011 [1]. The estimates in Table 4 therefore represent the possible additional benefits of further reducing nitrate concentrations in drinking water. Some caution is also needed, as current risks reflect past exposures because of the latency periods required for cancer development. A further source of uncertainty relates to differences between current and past dietary patterns, which may influence the extent to which endogenous nitrosation of ingested nitrate occurs."

In conclusion, the benefit from regulating below 6 mg/L is estimated by the expert group to range between 57 (1.3% of cases) and 90 (2.0% of cases) depending on the specific intervention strategy with an average of 73 cases (1.6% of cases).

Derivation of a parametric value - uncertainties

Several uncertainties are associated with deriving a parametric value through benchmark dose (BMD) modelling. Firstly, the available data included only summary data for five exposure categories, which is rather limited for characterizing the underlying dose–response relationship. Having divided the data into a larger number of exposure categories (ideally >10) would have reduced this uncertainty. In the case of the data used in the Schullehner et al. study, the underlying registry-based dataset was no longer accessible because such data must be deleted after a defined retention period, in accordance with current legislation. Modelling a higher number of exposure categories was therefore not possible.

Despite these limitations, modelling the dose–response relationship through BMD analysis provides a more robust basis for deriving a parametric value and for quantifying the associated uncertainties than relying on pairwise comparisons between a few (n = 5) exposure categories. The use of BMD modelling is now recommended by several international agencies [59], and alternative approaches are advised only when the available data are not suitable for modelling, which was not the case here.

Another source of uncertainty is that only one study was considered suitable for benchmark dose modelling. As discussed above, variation in diet and lifestyle, factors that influence endogenous N-nitroso compound formation, is likely to differ across populations and may therefore affect the observed dose–response relationship. Assessing the dose–response curve in multiple populations would be preferable. On the other hand, the study by Schullehner et al. encompasses the entire Danish population over a well-defined period, which is a major strength.

Finally, it is important to recognize that data limitations cannot be resolved by any modelling approach, and identifying a reference point that reflects no appreciable risk with very low uncertainty is rarely achievable. Nevertheless, dose–response modelling makes better use of the available data, and when its estimates and quantified uncertainties are interpreted in the context of the overall evidence, it provides an appropriate basis for establishing a parametric value. The proposed value of 6 mg/L is grounded in those considerations.

Conclusions

Based on the evidence synthesis, modelling and discussion above the working group concludes the following:

Assessment

- Since a previous assessment by other international agencies [2, 4, 58] the evidence for an association between nitrate in drinking water and colorectal cancer has been substantially strengthened.
- In line with the DTU 2024 report, the working group concludes that the totality of the available evidence indicates that nitrate in drinking water poses a potential health risk for colorectal cancer.
- The working group considers that, as a precautionary approach, derivation of a parametric value for nitrate in drinking water based on colorectal cancer is sufficiently supported by the current evidence.

Derivation of a parametric value:

- The nationwide registry-based cohort study by Schullehner et al. was considered the most appropriate basis for deriving a health-based parametric value.
- In accordance with existing guidance [59-61], benchmark-dose (BMD) modelling was used for deriving the reference point.
- Among several available software platforms, the EFSA Bayesian BMD webtool was selected because it applies model averaging, has undergone testing, and is used in regulatory contexts.
- Based on the modelling results, their associated uncertainties, and the totality of the evidence, the **working group proposes a parametric value of 6 mg/L nitrate in drinking water.**
- The uncertainties surrounding this estimate, including those reflected in the BMD credible intervals (Table 2) and in the estimated range of potentially preventable colorectal cancer cases (Table 4), were quantified to inform risk managers about the implications of deviations from the proposed value. As discussed above these numbers should be interpreted with some caution.

Uncertainties:

- Access to more granular exposure data (i.e., a larger number of exposure categories) would likely reduce uncertainty in the BMD estimates.
- Sensitivity analyses confirmed that the proposed value of 6 mg/L is not materially influenced by extrapolating to no exposure (below the lowest exposure category) or the choice of BMD platform, when modelling uncertainties are taken into account.

Future perspective

- To further assess the robustness of the proposed value, it is recommended that any revisions to the current drinking-water standard be accompanied by ongoing evaluation of their implications for disease prevalence in future studies.

Appendix I

Terms of reference as provided to the working group

Miljøministeriet
Departementet

Drinking water and chemicals
Ref. KARKR
23 September 2024

Terms of reference for international expert group - Evaluation of the parametric value for nitrate in drinking water

I. Background

In 2019, the Ministry of Environment and Food of Denmark initiated an evaluation of the parametric value for nitrate in drinking water in view of a Danish nationwide population-based cohort study from 2018 that showed a statistically significant correlation between nitrate in drinking water and colorectal cancer[18]. The Danish Environmental Protection Agency (DEPA) asked DTU National Food Institute to perform the evaluation.

The evaluation assessed acute and chronic effects of nitrate in drinking water and the main concern related to a potential carcinogenic effect, in particular colorectal cancer (Annex 1 and 2). Based on epidemiological studies, the DTU National Food Institute found some level of evidence that intake of nitrate from drinking water may lead to the development of colorectal cancer also at nitrate concentrations under the existing parametric value for nitrate in drinking water of 50 milligram per litre (mg/L). Still, a considerable uncertainty exists with respect to the results of the epidemiological studies and therefore a firm conclusion could not be drawn and DTU did not propose a revised parametric value for nitrate in drinking water. Based on DTU's evaluation, DEPA concluded in 2020 that there was not sufficient data to lower the existing EU parametric value for nitrate in drinking water of 50 mg/L – a value also in line with the recommendations of WHO.

In 2021-2022, three additional epidemiological studies were published on nitrate in drinking water and various health effects in children (reduced fetal growth[19], childhood cancer[20], and birth defects[21]) based on Danish data. DTU National Food Institute also assessed these and concluded after each assessment that the most substantiated potential health risk relating to nitrate in drinking water was the association to colorectal cancer. Therefore, DEPA maintained its conclusion from 2020.

In 2023, a health-economic valuation of lowering nitrate standards in drinking water related to colorectal cancer in Denmark was published by Danish researchers[22]. The valuation, which was based on the findings of the Danish cohort study from 2018, showed an annual net gain for society of $302 million with a new parametric value of 4 mg/L by which it was assumed that 127 annual colorectal

[18] https://doi.org/10.1002/ijc.31306
[19] https://doi.org/10.1289/EHP7331
[20] https://doi.org/10.1016/j.envint.2021.106613
[21] https://doi.org/10.1016/j.lanepe.2021.100286
[22] https://doi.org/10.1016/j.scitotenv.2023.167368

cancer cases could be avoided. By a lowering of the parametric value to 9 mg/L and an assumption of 72 fewer annual colorectal cancer cases, the researchers estimated an annual net gain of $170 million.

In light of the health-economic evaluation, the Ministry of Environment of Denmark initiated an update of the evaluation of the parametric value for nitrate in drinking water in 2023 with a particular focus on new data correlating nitrate in drinking water and cancer, especially colorectal cancer.

In the updated evaluation from 2024 (Annex 3, 4 and 5), DTU National Food Institute finds that drinking water is positively associated with risk of developing colorectal cancer based on the overall knowledge. DTU therefore finds that nitrate in drinking water constitutes a health risk. This assessment also applies to exposure to nitrate in drinking water below the existing parametric value of 50 mg/L. DTU notes, however, that the assessment of the parametric value in relation to the risk of developing colorectal cancer is subject to some uncertainty.

In addition, DTU finds that it cannot be excluded that nitrate in drinking water is a risk factor for certain other types of cancer. DTU also notes that beneficial effects of nitrate were not included in the assessment.

DTU considers that it is not possible for them at present to recommend a specific health-based quality criterion for a nitrate content in drinking water where the risk is assessed to be negligible.

Based on the updated evaluation, it was decided to appoint an international expert group to evaluate and potentially devise a lower health based parametric value for nitrate in drinking water based on epidemiologic studies and the overall evidence.

II. Role of the expert group

Scope

The scope of the expert group is to provide scientific expertise in areas of relevance for evaluating and determining health-based reference values, in particular when the available data are from epidemiological studies.

Using a multidisciplinary approach, the expert group will contribute to the protection of human health by evaluating the existing parametric value for nitrate in drinking water and potentially devise a proposal for a revised parametric value based on the best available scientific knowledge and evidence. The evaluation by the expert group could be based on the two evaluations from the DTU National Food Institute and should be supplied with other relevant information including any new scientific literature of relevance for the scope until two months before the termination of this mandate.

Tasks

The tasks of the expert group are:

- to evaluate the parametric value for nitrate in drinking water in order to determine if it is sufficiently protective for the public with respect to the risk of developing cancer, in particular colorectal cancer.

- if required, to devise a health-based proposal for a revised parametric value for nitrate in drinking water based on the overall evidence.
    - This should consider also the overall exposure to nitrate and its transformation product nitrite from all sources.
    - If relevant, it may include a meta-analysis and modelling of exposure/response relationships based on data from high-quality epidemiologic studies.
    - If possible, the feasibility in terms of technologic/economic possibilities for obtaining a revised parametric value for nitrate in drinking water should be considered.
    - If possible, to estimate the decrease of risk to the Danish public in terms of the development of cancer, in particular colorectal cancer, if the parametric value for nitrate in drinking water is changed to a certain lower level.
- to propose additional studies to be performed if a lack of data is identified in order to evaluate if the existing parametric value of nitrate in drinking water is sufficiently protective of human health.

Members

Danish and international experts within relevant areas of expertise constitute the 5-8 members of the expert group to be found e.g. from the bodies below:

- A chairperson and two or three additional representatives associated with The European Food Safety Authority, EFSA, e.g. members of EFSA's Panel on Contaminants in the Food Chain
- One or two representatives associated with the World Health Organization's, WHO's, work on setting indicative health-based values for substances in drinking water
- One or two representatives from research institutions
- One or two representatives from universities

Observers

Observers are invited to attend the meetings of the expert group and they are permitted to participate in discussions at the meetings and in the collective effort to obtain the scope of the terms of reference. However, observers cannot object to decisions of the expert group or to the content of the final scientific report to be delivered by the expert group. Experts within relevant areas of expertise constitute the observers with the below constitution:

- One representative of the Danish Health Authority
- One representative of the Danish Patient Safety Authority

Secretariat and meeting frequency

The secretariat function will be provided by DEPA. This includes meeting logistics and providing minutes of the meetings. Minutes are sent for commenting to the expert group members and observers within two weeks after a meeting for commenting and written approval within a specified time.

DEPA will as soon as possible invite the members of the expert group and observers to a first meeting in order to decide on a work plan and time schedule, including meeting frequency. Meetings are expected to be held primarily online. Travel and daily allowance expenses for one or two meetings in person in Copenhagen incurred under this mandate will be reimbursed for both members and observers.

Deliverables

A scientific report with an outline of the outcome of the tasks prescribed in the terms of reference shall be delivered to the Danish Ministry of Environment and Gender Equality within [6-12 months] from the date of issue of this mandate [before 1 Xxx 2025].

Appendix II

Data from the Schullehner et al 2018 [1] paper as used for modelling:

A	B	C	D	E	F	G	H
Dose	**Dose_scaled**	**N**	**HR**	**CasesRaw**	**CasesAdj**	**rawIncidence**	**adjIncidence**
0.69	0	348419	1	1266	1123	0.00363356	0.0032231
1.79	1.1	348419	1.06	820	1190	0.00235449	0.00341649
2.95	2.26	348419	1.03	809	1157	0.00232192	0.0033198
5.72	5.03	348419	1.11	1268	1247	0.0036393	0.00357765
18.59	17.9	348419	1.15	1845	1291	0.00529535	0.00370657
				(Ctrl)			

Dose: Refers to the *median* nitrate concentration in drinking water for each exposure group in the Schullehner et al. paper. This variable was used in the main BMD analyses, and the resulting BMR is estimated relative to **zero dose** (i.e., extrapolating to a water concentration of 0 mg/L).

Dose_scaled: Same as *Dose*, except that 0.69 mg/L (the lowest observed exposure category) has been subtracted. As a result, the modelling estimates the BMR relative to the lowest exposure category (0.69 mg/L). These analyses were performed as sensitivity analyses.

N: Total number of subjects in each exposure group.

HR: The central estimate of the hazard ratio (HR) reported for each exposure group in the Schullehner et al. study.

CasesRaw: The unadjusted number of colorectal cancer cases in each exposure group.

CasesAdj The adjusted number of colorectal cancer cases per exposure group (estimated from the HR).

rawIncidence and **adjustedIncidence:** The unadjusted and adjusted incidence rates in each exposure category, respectively (CasesRaw/N and CasesAdj/N).

The modeling approach used here (shown in Appendix I and II) is the same approach as recently used by EFSA in their opinion on inorganic arsenic [63].

Appendix III

Modelling results

The working group modelled the BMR of 5% and 10% increase in cancer incidence using data relative to the background incidence defined as the incidence in the lowest exposure category, and approximated the BMR relative to a nitrate concentration of 0 mg/L through extrapolation. Only the full modelling output using a BMR of 5% relative increase using a 90% credible interval are shown below. The main results using a BMR corresponding to 10% relative increase are also presented with the same credible interval. Estimates for the alternative credible intervals reported in Table 2 are not included, as these represent the same analyses repeated with different credibility levels.

Results from the sensitivity analyses where the BMR was estimated relative to the exposure in the lowest category (or 0.69mg/L) are also reported. A 90% credible interval was used for these models as well. Other results from the sensitivity analyses are not reported

Note: The adjusted incidence in the lowest exposure category (defined as background) is 0.00322 (~0.322%). A BMR corresponding to a 5% and 10% relative increase in cancer incidence corresponds to extra risks of 0.00016[23] and 0.00032, respectively.

1. **Modelling a BMR corresponding to a 5% relative increase in incidence above background (BMR approximated relative to a nitrate concentration of 0 mg/L). Full modelling report.**

Benchmark Dose Modeling: Report

European Food Safety Authority (EFSA)

[23] In the EFSA modelling report, CES stands for 'critical effect size', and for quantal data the default definition of CES is extra risk. In our modelling, the BMR was defined as a 5% relative increase above background. With a background incidence of 0.00322, a 5% relative increase corresponds to 0.00322 × 1.05 = 0.00338. The corresponding CES (expressed as extra risk) is therefore 0.00338 – 0.00322 ≈ 0.00016

Table of Contents

A. Data Description

Brief general description of the data. This section should include a table summarizing the data. In case that raw data is available, resulting in a too large table, summary statistics may be given instead. For quantal endpoints both the number of responding animals and the total number of animals should be given for each dose level; for continuous endpoints either the individual responses or the mean responses and the associated SDs (or SEMs) and sample sizes should be given for each dose level.

The endpoint to be analyzed is: CasesAdj.

Data used for analysis:

Dose	CasesAdj	N
0.69	1123	348419
1.79	1190	348419
2.95	1157	348419
5.72	1247	348419
18.59	1291	348419

B. Software Used

Results are obtained using the EFSA web-tool for Bayesian BMD analysis, which uses the R-package [BMABMDR] version 0.1.17 for the underlying calculations.

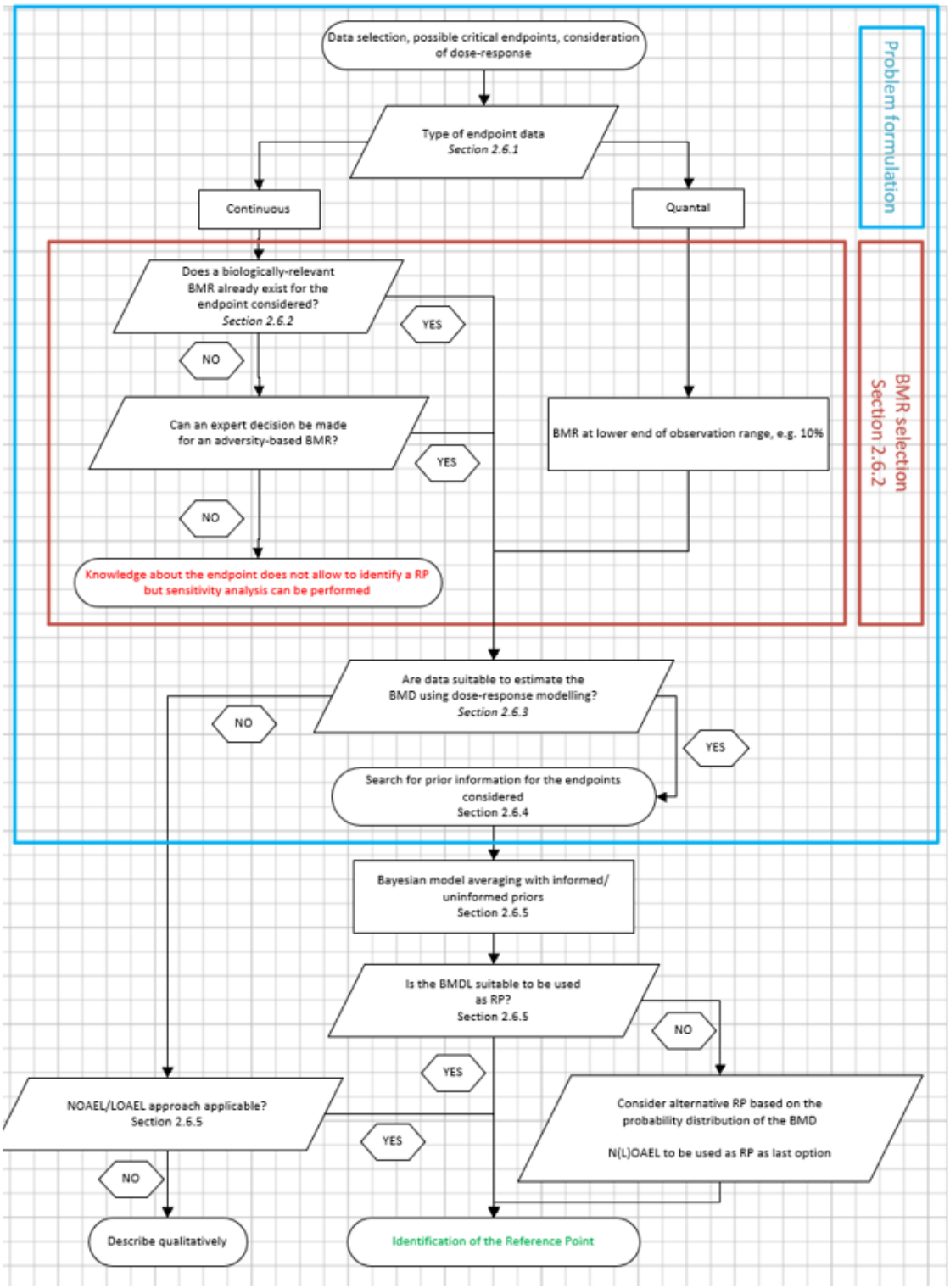
C. Justification of any deviation from the procedure and assumptions

- *In case another approach than Bayesian model averaging was used, the rationale and details for deviating from the recommended approach should be provided.*
- *Assumptions made when deviating from the recommended defaults in this guidance document (e.g. gamma distributional assumption instead of normal and*

log-normal, heteroscedasticity instead of homoscedasticity).

- *Other models than the recommended ones listed in Tables 2 and 3 of this guidance document that were fitted should be listed, with the reasons to include them.*
- *Description of any deviation from the procedure described in the flow chart to obtain the final BMD credible interval.*

The 'Value for CES' is set to 0.0001611 which differs from the default value (0.1). Please justify this deviation.


Problem formulation
Data selection, possible critical endpoints, consideration of dose-response
Type of endpoint data
Section 2.6.1
Continuous
Quantal
BMR selection
Section 2.6.2
Does a biologically-relevant BMR already exist for the endpoint considered?
Section 2.6.2
YES
NO
Can an expert decision be made for an adversity-based BMR?
YES
NO
Knowledge about the endpoint does not allow to identify a RP but sensitivity analysis can be performed
BMR at lower end of observation range, e.g. 10%
Are data suitable to estimate the BMD using dose-response modelling?
Section 2.6.3
NO
YES
Search for prior information for the endpoints considered
Section 2.6.4
Bayesian model averaging with informed/ uninformed priors
Section 2.6.5
Is the BMDL suitable to be used as RP?
Section 2.6.5
NO
YES
NOAEL/LOAEL approach applicable?
Section 2.6.5
YES
NO
Consider alternative RP based on the probability distribution of the BMD
N(L)OAEL to be used as RP as last option
Describe qualitatively
Identification of the Reference Point

Flowchart to derive a Reference Point (RP) from a dose-response dataset of a specified endpoint, using BMD analysis

D. Results

Information pertaining to this endpoint.

TRUE

Goodness of Fit

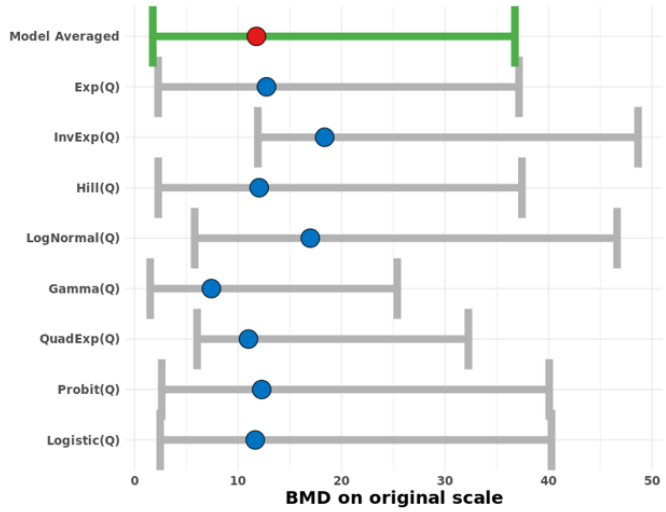
Best fitting model fits sufficiently well (Bayes factor in favor of saturated model is 7.39e-15).

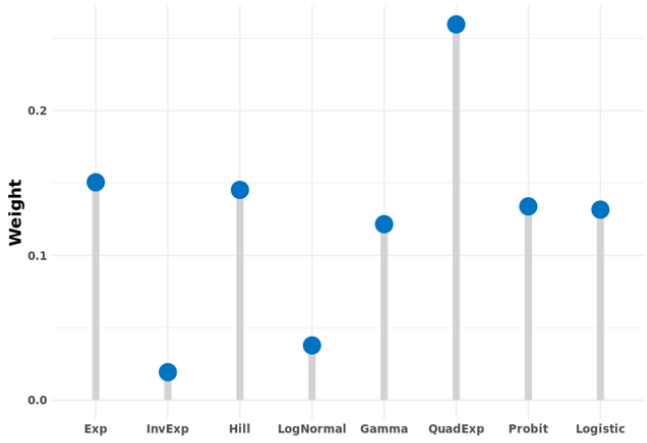
Model Averaged BMD

Model	Type	BMDL	BMD	BMDU
Model Averaged	BS	3.167	11.875	37.748

Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model Weights	Converged
E4_Q	2.279	12.756	37.144	0.151	1
IE4_Q	11.920	18.368	48.642	0.019	1
H4_Q	2.295	12.047	37.424	0.145	1
LN4_Q	5.813	16.985	46.615	0.038	0
G4_Q	1.516	7.426	25.381	0.122	0
QE4_Q	6.049	11.013	32.255	0.260	1
P4_Q	2.630	12.288	40.064	0.134	1
L4_Q	2.482	11.662	40.282	0.132	1


Model Averaged
Exp(Q)
InvExp(Q)
Hill(Q)
LogNormal(Q)
Gamma(Q)
QuadExp(Q)
Probit(Q)
Logistic(Q)
0
10
20
30
40
50
BMD on original scale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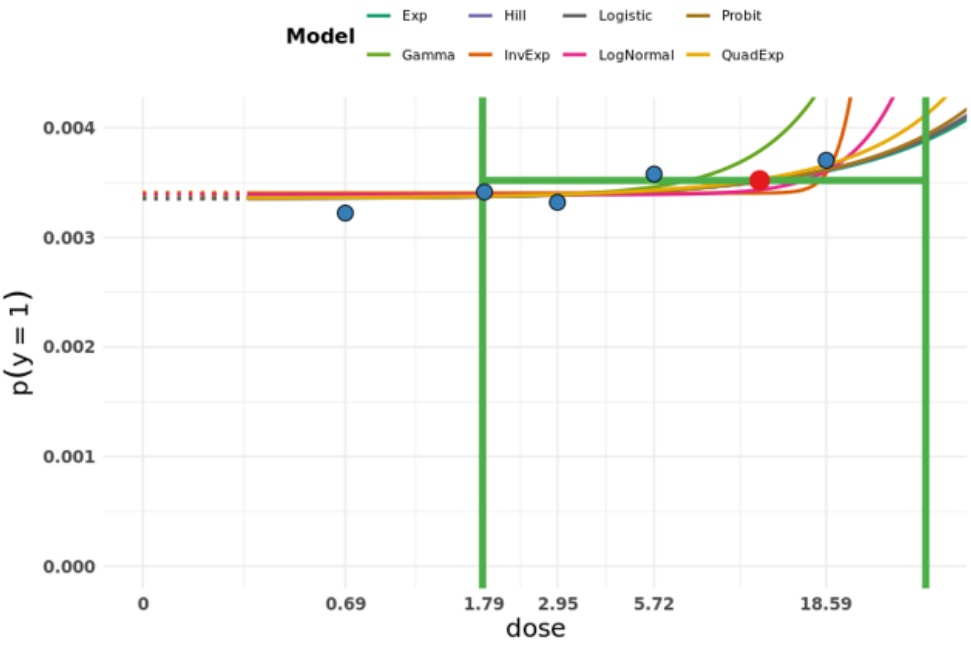
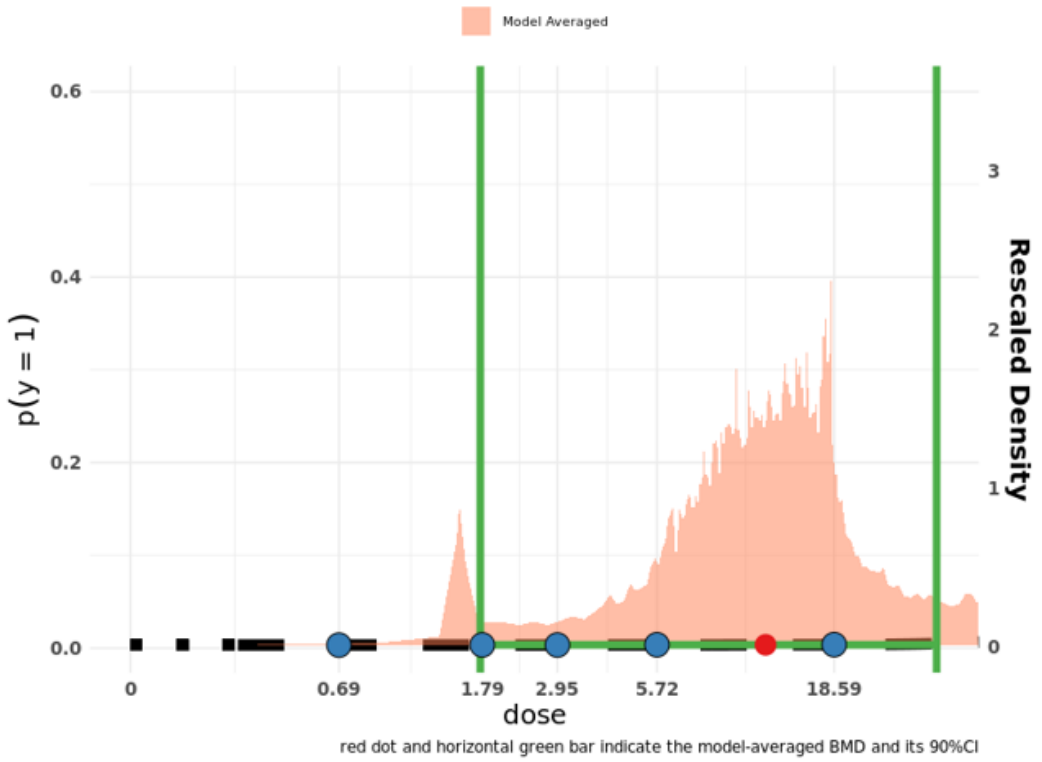
0.0
Exp
InvExp
Hill
LogNormal
Gamma
QuadExp
Probit
Logistic



red dot and horizontal green bar indicate the model-averaged BMD and its 90%CI



E. Conclusions

This section should summarize the results for each endpoint (dataset) that was analysed and provide a discussion of the rationale behind selecting the critical endpoint. The BMD confidence interval of the critical endpoint (and the BMDL selected as RP) should be reported and discussed.

2. **Modelling a BMR corresponding to a 10% relative increase in incidence above background (BMR approximated relative to a nitrate concentration of 0 mg/L).**

The 'Value for CES' is set to 0.0003223

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor in favor of saturated model is 4.71e-15).

Model Averaged BMD

Model	Type	BMDL	BMD	BMDU
Model Averaged	BS	6.098	17.794	40.618

Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model Weights	Converged
E4_Q	5.305	17.638	42.972	0.152	1
IE4_Q	15.241	19.615	49.409	0.011	0
H4_Q	5.064	17.781	41.467	0.159	1
LN4_Q	11.019	19.327	45.836	0.030	0
G4_Q	5.070	16.587	30.862	0.134	1
QE4_Q	11.523	18.459	39.660	0.228	1
P4_Q	5.134	17.694	43.552	0.144	1
L4_Q	4.944	17.776	43.265	0.142	1

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor in favor of saturated mc 4.71e-15).

Model Averaged BMD

Model	Type	BMDL	BMD	BMDU
Model Averaged	BS	6.098	17.794	40.618

Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model Weights	Converged
E4_Q	5.305	17.638	42.972	0.152	1
IE4_Q	15.241	19.615	49.409	0.011	0
H4_Q	5.064	17.781	41.467	0.159	1
LN4_Q	11.019	19.327	45.836	0.030	0
G4_Q	5.070	16.587	30.862	0.134	1
QE4_Q	11.523	18.459	39.660	0.228	1
P4_Q	5.134	17.694	43.552	0.144	1
L4_Q	4.944	17.776	43.265	0.142	1

3. **Sensitivity analyses modelling a BMR corresponding to a 5% relative increase in incidence above background. Here the BMR is estimated relative to a nitrate concentration of 0.69 mg/L.**

Note: 0.69 needs to be added to all the BMD estimates to get the correct BMD values

Dose_scaled	CasesAdj	N
0.00	1123	348419
1.10	1190	348419
2.26	1157	348419
5.03	1247	348419
17.90	1291	348419

The 'Value for CES' is set to 0.0001611

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor in favor of saturated mode
7.35e-15).

Model Averaged BMD

Model	Type	BMDL	BMD	BMDU
Model Averaged	BS	2.663	11.339	36.661

Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model Weights	Converged
E4_Q	2.093	10.942	36.417	0.141	1
IE4_Q	12.032	17.409	46.279	0.016	0
H4_Q	2.207	11.563	38.000	0.153	1
LN4_Q	4.948	16.288	44.832	0.035	1
G4_Q	2.276	10.827	27.152	0.139	1
QE4_Q	6.145	10.985	35.449	0.236	1
P4_Q	2.289	11.586	38.304	0.139	1
L4_Q	2.341	11.288	38.709	0.141	1

4. **Sensitivity analyses modelling a BMR corresponding to a 10% relative increase in incidence above background. Here the BMR is estimated relative to a nitrate concentration of 0.69 mg/L.**

Note: 0.69 needs to be added to all the BMD estimates to get the correct BMD values

Dose_scaled	CasesAdj	N
0.00	1123	348419
1.10	1190	348419
2.26	1157	348419
5.03	1247	348419
17.90	1291	348419

The 'Value for CES' is set to 0.0003223

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor in favor of saturated moc
4.73e-15).

Model Averaged BMD

Model	Type	BMDL	BMD	BMDU
Model Averaged	BS	5.974	17.318	41.546

Estimated BMDs per model

Model	BMDL	BMD	BMDU	Model Weights	Converged
E4_Q	5.548	17.761	44.495	0.163	1
IE4_Q	14.711	18.666	45.671	0.010	0
H4_Q	5.367	17.549	44.023	0.171	1
LN4_Q	9.986	18.538	43.428	0.027	0
G4_Q	5.135	15.474	29.517	0.126	1
QE4_Q	11.376	18.052	36.934	0.190	1
P4_Q	5.823	17.568	43.442	0.158	1
L4_Q	5.574	17.542	43.546	0.156	1

Reference

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