Health Effects of Nitrate in Drinking Water

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Overview

• Biologic effects of ingestion of nitrate in drinking water
  ✷ N-nitroso compound (NOC) formation

• Health effects
  ✷ Methemoglobinemia
  ✷ Adverse reproductive outcomes
  ✷ Cancers
  ✷ Thyroid disease
Nitrate in drinking water: Sources and exposures

• Nitrogen fertilizers, animal and human waste

• Regulatory limit (Maximum Contaminant Level [MCL]):
  • 10 mg/L as NO$_3$-N (USA)
  • 50 mg/L as NO$_3$ (EU)

• Highest exposures:
  • Residents of agricultural areas
  • Private wells
    – Not regulated
    – Spare measurements

Nolan et al, EST 2002
Nitrate in the diet: Sources and exposure

- High nitrate vegetables (e.g., green leafy vegetables, celery, beets)
- Vegetables also contain vitamin C, polyphenols, & other antioxidants inhibits formation of N-nitroso compounds (NOC)
- Beneficial effects on the cardiovascular system through nitric oxide (NO) formation
- Supplements with high nitrate (e.g. beet juice) can cause NOC formation (Berends et al. Random Contr Trial; 2019)
- Nitrate & nitrite added to processed/cured meats linked to colorectal and other cancers
N-nitroso compound (NOC) formation from ingested nitrate (drinking water & diet)

Oral bacteria: Nitrate -> nitrite

\[
\text{Nitrite} + \text{stomach acid} \rightarrow N_2O_3 + \text{amines/amides}
\]

\[
\downarrow \quad \downarrow
\]

NOC

Heme iron (red meat)
Thiocyanate (smoking)
Antioxidants (vitamin C)
Human studies of nitrate ingestion

- Direct relationship between nitrate conversion to nitrite and NOC concentrations in urine

- 5-8% of ingested nitrate reduced to nitrite by the oral bacteria

- Drinking water nitrate increases endogenous formation of NOC:
  - >10 mg/L NO$_3$-N (Mirvish 1992; Moller et al. 1989)
  - But not at levels <5 mg/L (Levallois, 2000)
N-nitroso compounds (nitrosamines, nitrosamides): Cancer and birth defects

• ~300 NOCs tested in animals: 90% were carcinogenic
  (International Agency Research on Cancer [IARC], 1995)

• Carcinogens in 39 animal species including nonhuman primates

• Cause tumors in multiple organs: e.g., lung, digestive tract, bladder, kidney, ovary, thyroid, brain tumors

• *In utero* exposure causes congenital malformations especially of the central nervous system
  ✷ First trimester most important for organ development
Methemoglobinemia
Nitrate and infant methemoglobinemia

September 8, 1945

CYANOSIS IN INFANTS CAUSED BY NITRATES IN WELL WATER

HUNTER H. COMLY, M.D.

- Nitrite binds to hemoglobin forming methemoglobin (MetHb) and interferes with oxygen transport when >20% MetHb
  - Infants <6 months most susceptible

- Investigation of 279 cases in 14 U.S. states found no cases below 20 mg/L NO₃-N (Walton, Am J Public Health 1951)

- Case in WI at 27 mg/L NO₃-N (Knobeloch et al. Env Health Perspect 2000)

- Ongoing public health problem in Eastern Europe, The Gaza Strip, Morocco
Adverse reproductive outcomes

- Spontaneous abortions
- Congenital malformations (birth defects)
- Few studies of other outcomes (low birth weight, preterm birth)
Adverse reproductive outcomes – spontaneous abortions

• High maternal MetHb can cause abortions in lab animals and livestock

• CDC MMWR report (1996) of a cluster of spontaneous abortions (miscarriage) in rural Indiana:
  • Women on private wells >20 mg/L NO₃-N
  • Switched to low nitrate water and had healthy live births

• Study in Massachusetts: no association with levels that were below 5.5 mg/L NO₃-N (Aschengrau, 1989)
Adverse reproductive outcomes: Central Nervous System (CNS) malformations

- **Dorsch, 1984 (Australia):** 3.5 increase in CNS defects for groundwater nitrate (<10 ppm nitrate-N)

- **Arbuckle, 1988 (Canada):** two-fold increase in CNS malformations with nitrate >5.5 ppm in private wells

- **Croen, 2001 (California):** increased risk anencephaly for nitrate >10 mg/L

- **Brender, 2004/2005 (Texas):** two-fold increase in neural tube defects (NTDs) for nitrate >3.5 mg/L

- **Brender et al, 2013 (Texas, Iowa):** two-fold increase in specific defects including spina bifida, limb deficiencies, cleft lip/palate

- **Holtby, 2014 (Canada):** no association nitrate <MCL and all defects combined
Adverse reproductive outcomes: CNS malformations

- **Brender, 2004/2005 (Texas):** two-fold increase in neural tube defects (NTDs)
  - Highest risk for women with high nitrate in their drinking water and who took prescription or over-the-counter drugs that can form NOC (nitrosatable drugs)

- **Brender, 2013 (Texas, Iowa):** 2-fold increase in various defects: spina bifida, limb deficiencies, cleft lip/palate
  - Highest risk for women with high total nitrate intake (diet and drinking water) and nitrosatable drug use during pregnancy

**Summary:** 5 of 6 studies found positive associations nitrate concentrations were <10 mg/L in 4 studies
Cancer
Ingested Nitrate and Nitrite (IARC vol. 94):
2A - probably carcinogenic to humans when ingested under conditions favorable for endogenous nitrosation

- Animal studies
  - 90% of NOC are carcinogens
  - Carcinogenic in all species tested
  - Many cancer sites

- Human mechanistic studies
  - NOC exposure increases with nitrate ingestion

- Epidemiology studies
  - Dietary nitrate – no studies with increased risk
  - Drinking water nitrate- inadequate evidence:
    - Few studies of specific cancer types
    - Low exposures - population using public water supplies
    - Historical exposures not always evaluated
    - Factors affecting endogenous nitrosation not always considered
Cancer: # studies (# positive in at least one subgroup):

- **Colorectal**: 5 studies (4 positive)
- **Brain** (children, young adults): 4 studies (4 positive)
- **Bladder**: 4 studies (2 positive)
- **Kidney**: 2 studies (2 positive)
- **Non-Hodgkin lymphoma**: 3 studies (1 positive)
- **Pancreas**: 2 studies (0 positive)
- **Brain (adults)**: 2 studies (0 positive)
Iowa Women Health Study:
- 42,000 postmenopausal women across Iowa
- 73% use public water supplies, 25% private wells


NO$_3$-N

Total Trihalomethanes (THMs)

City

N = 473

Median duration (years)

- 4
- 8
- 16
- 34

Exposure Metrics

Average NO$_3$-N and THMs

Years >1/2 Maximum Contaminant Level

82% used supply 16+ years
Drinking water nitrate and cancer: early results

- Weyer et. al. (2001)

**Ovarian cancer** (n = 109)
- Public water: $HR_{Q4vs.Q1} = 2.0 \ (1.01 - 4.1)$
- Private well users (vs Q1): $HR = 1.6 \ (0.8 - 3.1)$

**Bladder cancer** (n=57)
- Public water: $HR_{Q4vs.Q1} = 2.8 \ (1.1 - 7.2)$
- Private well users (vs Q1): $HR = 1.3 \ (0.5 - 3.5)$

- No association for colon, rectum, lung, breast, kidney, pancreas, NHL, melanoma
Updated results: Ovary and Bladder Cancers

• >20 years of follow-up:
  – 315 ovary, 263 bladder cases
  – Similar increased risk (Inoue-Choi, 2015; Jones, 2016)
  – Updated analyses of colon, rectum, pancreas cancers (Jones, 2019; Quist, 2018)
  – Increased risk of kidney cancer in highest exposure group (Jones, 2017)

• Exposure assessment:
  – Trihalomethanes ($\rho=0.24$ with nitrate)
  – Dietary nitrite

• Factors affecting NOC formation:
  – Vitamin C and red meat
  – Smoking (bladder)
Average nitrate (mg/L) and ovarian cancer

P-trend=0.003

adjusted for age, BMI, hormone use, reproductive factors, THMs

Inoue-Choi..Ward Int J Cancer 2015
Average nitrate and ovarian cancer, by vitamin C intake

Inoue-Choi..Ward, *Int J Cancer* 2015

**Vitamin C > 190 mg/d**

*P*-trend = 0.12

**Vitamin C ≤ 190 mg/d**

*P*-trend = 0.005

*p*-interaction = 0.01
Summary and limitations of studies of drinking water nitrate and cancer

- Most consistent evidence for colorectal cancer and childhood brain tumors
- Still few studies of specific cancer sites
- Limitations of most studies:
  - Private well users (highest exposures) usually not considered due to lack of measurement data
  - Factors affecting NOC formation not always evaluated

Thyroid disease
Nitrate inhibition of iodide uptake by the thyroid

Inhibition of iodide uptake in thyroid

Plasma $T_3$, $T_4$, ↓ plasma TSH ↑

Thyroid hypertrophy/hyperplasia

Hypothyroidism

Metabolic effects

Impaired development and growth (fetus, child)

=> Cancer?
Nitrate in drinking water and hypothyroidism

1. Subclinical hypothyroidism (measured by ultrasound) was increased in children in Slovakia with high nitrate drinking water (Tajtakova et al, Chemosphere 2006)

2. Postmenopausal women in Iowa with higher nitrate ingestion (diet and drinking water) had higher prevalence of hypothyroidism (Ward et al, Epidemiology 2010)

3. Amish women (but not men) in Pennsylvania with drinking water nitrate >6 mg/L NO₃-N had higher serum TSH & subclinical hypothyroidism (Aschebrook-Kilfoy et al, Env Health 2012)
Modeled nitrate levels in well water supplies and prevalence of abnormal thyroid conditions among the Old Order Amish in Pennsylvania (Lancaster, Chester, Lebanon counties) with measurements of thyroid hormones (N=2543)

- Nitrate measurements for 3,613 private wells
Nitrate in drinking water and clinical and subclinical hypothyroidism

Table 5 Odds ratios (ORs) and 95% confidence intervals (CIs) for the prevalence of hypothyroidism associated with nitrate levels in residential wells

<table>
<thead>
<tr>
<th>Overall</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/L nitrate-nitrogen</td>
<td>Cases</td>
<td>OR 95%CI</td>
</tr>
<tr>
<td><strong>Clinical Hypothyroidism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low nitrate (&lt; 6.5)</td>
<td>29</td>
<td>1.0</td>
</tr>
<tr>
<td>High nitrate (≥ 6.5)</td>
<td>27</td>
<td>0.89 (0.52-1.52)</td>
</tr>
<tr>
<td><strong>Subclinical Hypothyroidism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low nitrate (&lt; 6.5)</td>
<td>96</td>
<td>1.0</td>
</tr>
<tr>
<td>High nitrate (≥ 6.5)</td>
<td>132</td>
<td>1.32 (1.0-1.75)</td>
</tr>
</tbody>
</table>

Models adjusted for age and BMI; model with men and women combined is also adjusted for gender; nitrate concentrations in mg/L

Limitations: no information on water intake, dietary intakes
Farmers and their spouses in Iowa and North Carolina (N=~90,000)

70% used private wells at enrollment in 1993-1997

Estimated nitrate in private wells (random forest models)

Public water supply measurements: nitrate, disinfection by-products

Dietary nitrate/nitrite from food frequency questionnaire

Manley.. Ward, in prep
Random forest model of nitrate in private wells

- ~34,000 nitrate measurements (1980-2000s)
- Evaluated >150 variables (e.g., land use, animal feeding operations, geology, soils)

Wheeler et al. STOTEN; 2015
Research needs

• Clarify relationship between nitrate ingestion & NOC formation below MCL in controlled studies

• Evaluate additional factors that influence endogenous nitrosation
  – E.g. oral microbiome can be measured

• More studies in populations with high exposures
  – Private well users, small public supplies
  – Inequality in exposures
  – Exposures increasing and will be exacerbated by climate change
Collaborators

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Reviews of nitrate in drinking water and health

Workgroup Report: Drinking-Water Nitrate and Health—Recent Findings and Research Needs

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Review

Drinking Water Nitrate and Human Health: An Updated Review


Mary H. Ward 1,* , Rena R. Jones 1 ℹ, Jean D. Brenner 2, Theo M. de Kok 3, Peter J. Weyer 4, Bernard T. Nolan 5, Cristina M. Villanueva 6,7,8,9 ℹ and Simone G. van Breda 3