Health Effects of Nitrate in Drinking Water

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Chemical Toxicants in Water: A GeoHealth Workshop in the Context of Climate Change
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Overview

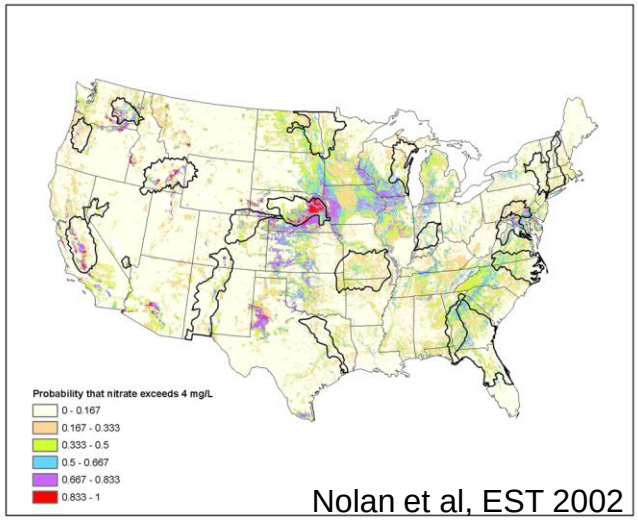
- **Biologic effects of ingestion of nitrate in drinking water**
  - **N-nitroso compound (NOC) formation**

- **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)**

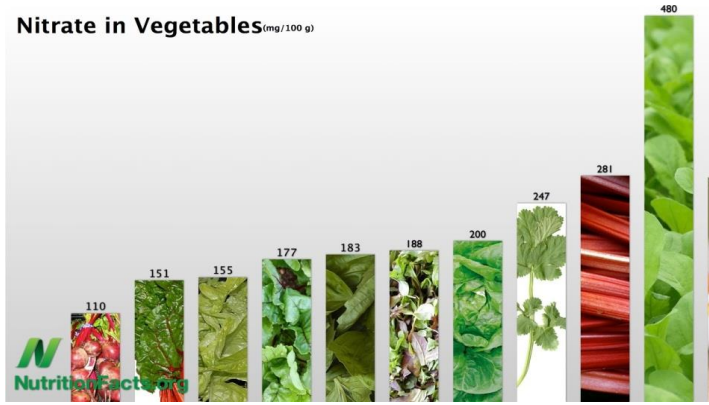


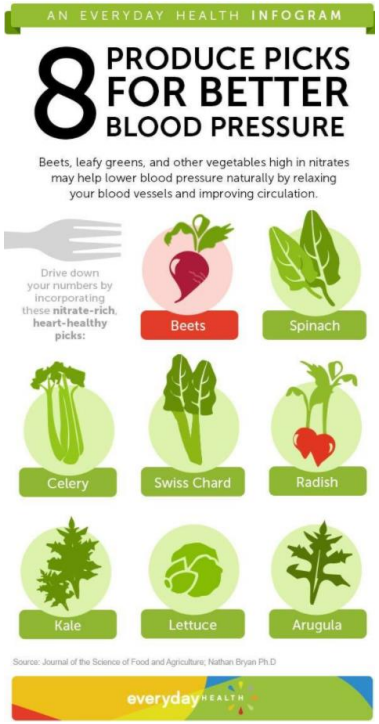
Nolan et al, EST 2002

- **Highest exposures:**
  - **Residents of agricultural areas**
  - **Private wells**
    - **Not regulated**
    - **Spare measurements**

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

Nitrite + stomach acid

↓

***N_2O_3* +**

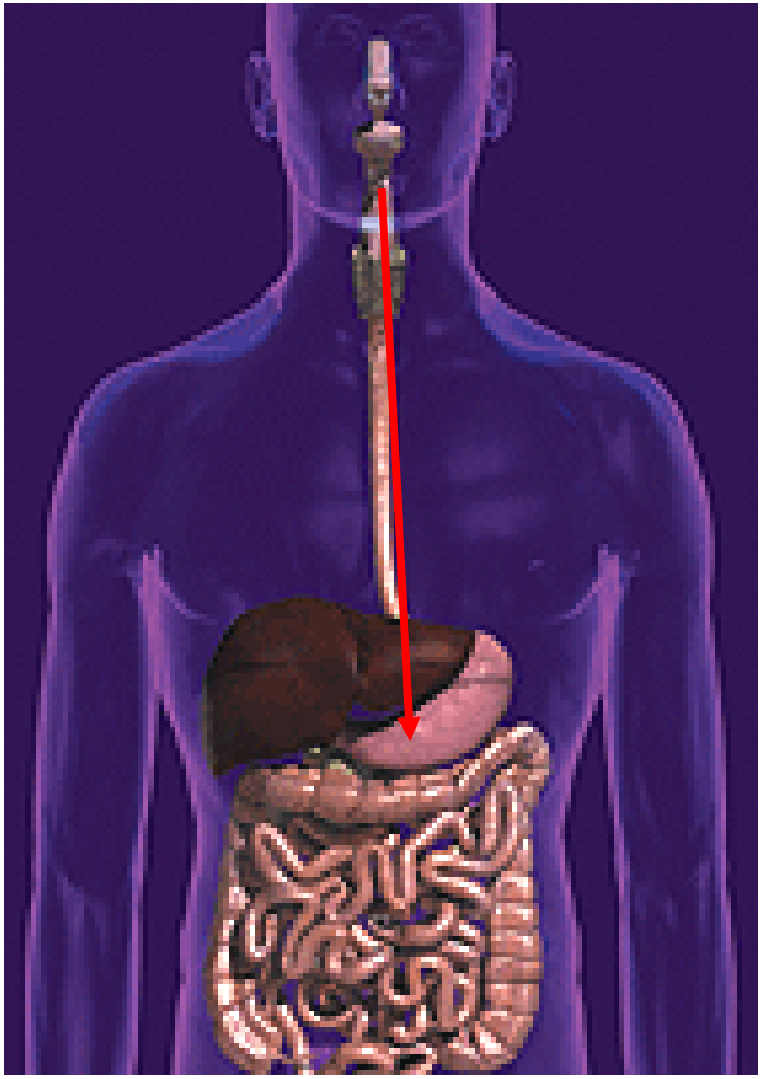
amines/amides

↓

NOC

Heme iron
(red meat)
Thiocyanate
(smoking)

Antioxidants
(vitamin C)

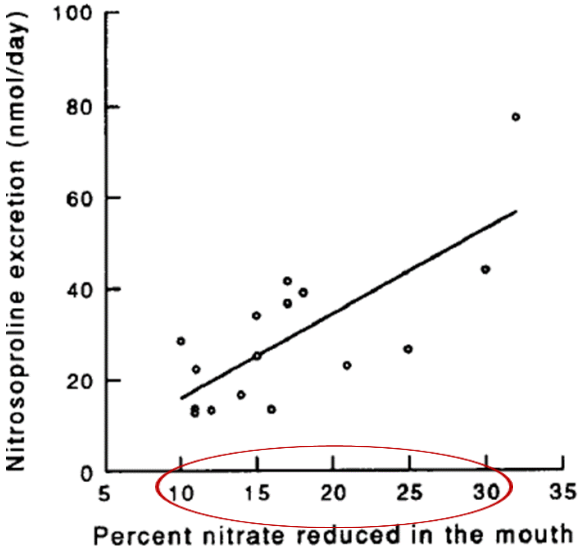


Human studies of nitrate ingestion

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

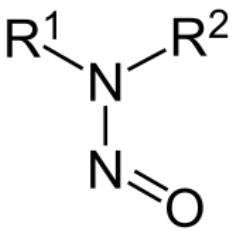
- **5-8% of ingested nitrate reduced to nitrite by the oral bacteria**

- **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)**



Shapiro et al, Fd Chem Tox 1991

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

Methemoglobinema

Nitrate and infant methemoglobinemia

September 8, 1945

CYANOSIS IN INFANTS CAUSED BY NITRATES IN WELL WATER

HUNTER H. COMLY, M.D.

» Author Affiliations

JAMA. 1945;129(2):112-116. doi:10.1001/jama.1945.02860360014004

- **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 NO_3-N (Walton, Am J Public Health 1951)**
- **Case in WI at 27 mg/L NO_3-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 NO_3-N**
  - **Switched to low nitrate water and had healthy live births**

MMWR Weekly
July 05, 1996 / 45(26);569-572

- **Study in Massachusetts: no association with levels that were below 5.5 mg/L NO_3-N (Aschengrau, 1989)**

Adverse reproductive outcomes: Central Nervous System (CNS) malformations

Birth Defect Research for Children
Because Every Birth Defect has a Cause

- **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)

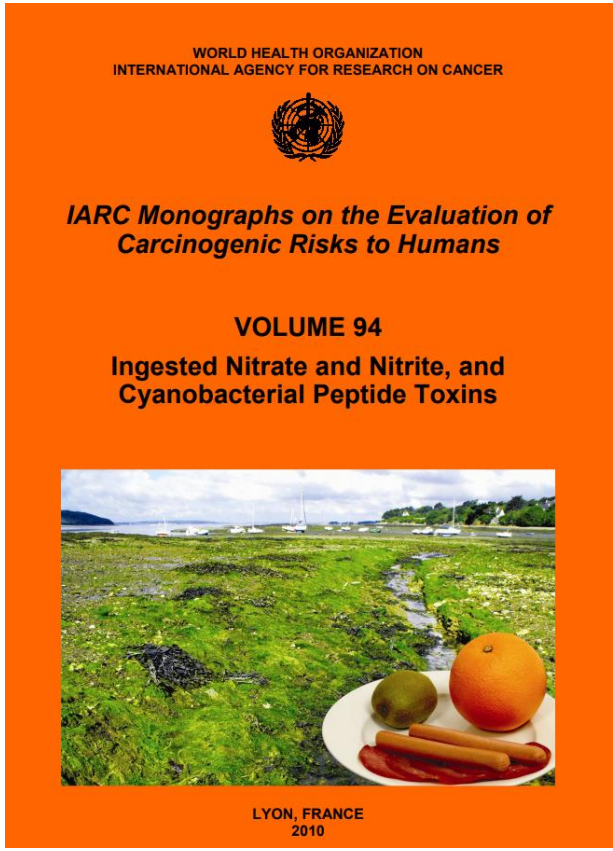
- **Brender, 2013 (Texas, Iowa):** 2-fold increase in various defects: spina bifida, limb deficiencies, cleft lip/palate
  - Highest risk for women with high total nitrate intake (diet and drinking water) and nitrosatable drug use during pregnancy

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



IARC , 2010

- 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

Case-control or cohort studies of drinking water nitrate and cancer: cancer sites with 2+ studies

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

Public Water Supply Monitoring Data, 1955-1988

NO_3-N

Total Trihalomethanes (THMs)

Median duration (years)

City

N = 473

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**

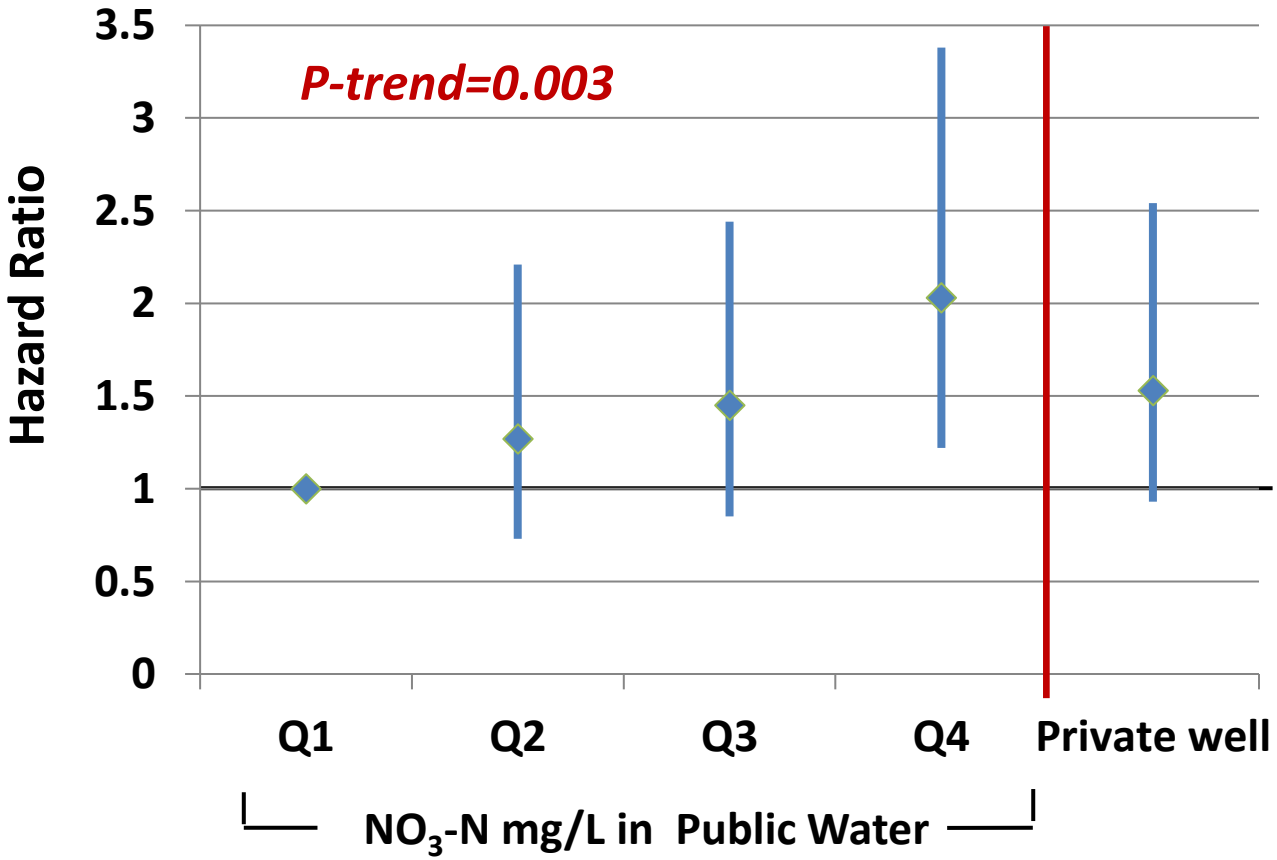
Updated results: Ovary and Bladder Cancers

- **>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 (ρ=0.24 with nitrate)
  - Dietary nitrite

- **Factors affecting NOC formation:**
  - Vitamin C and red meat
  - Smoking (bladder)

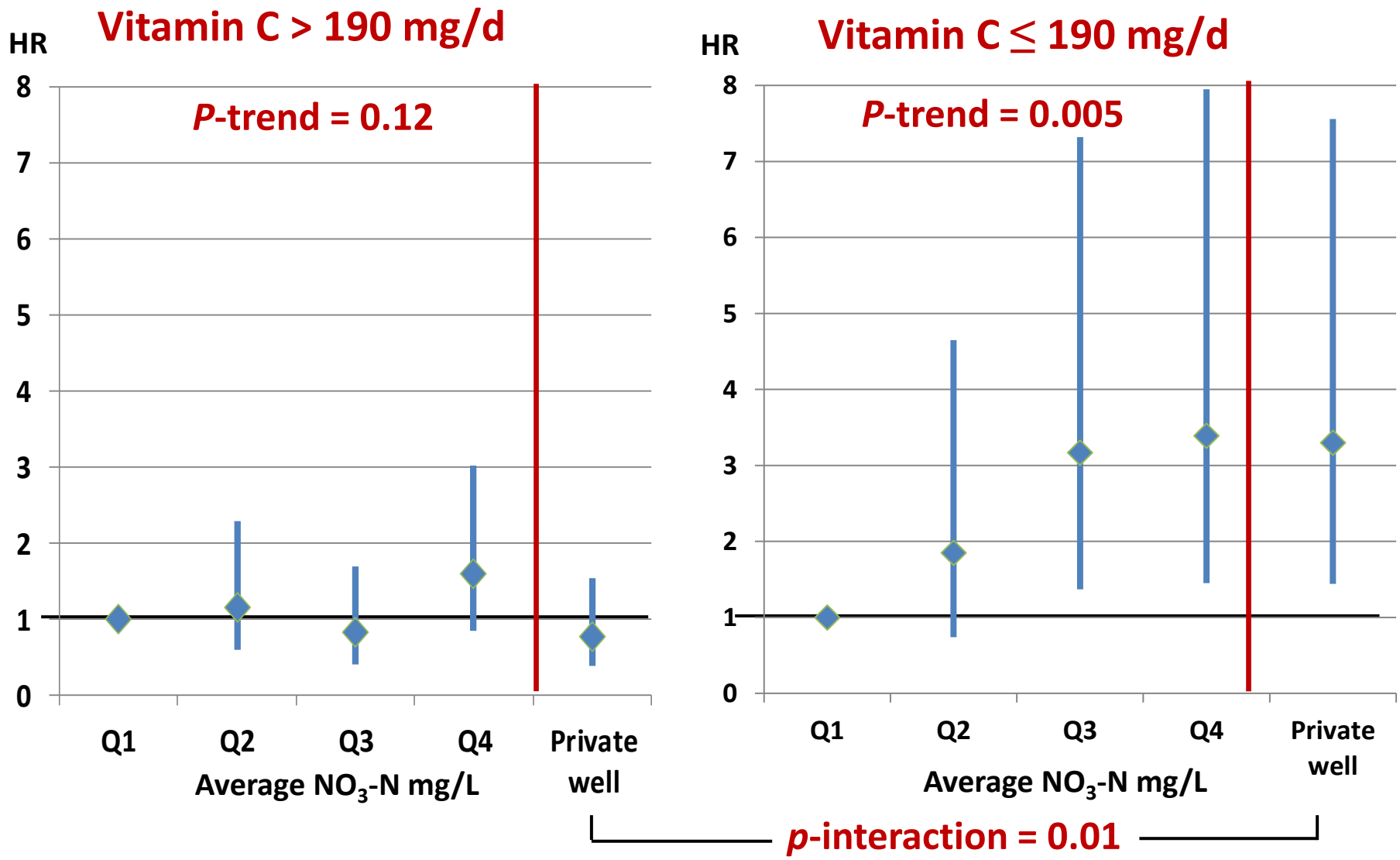
Average nitrate (mg/L) and ovarian cancer



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

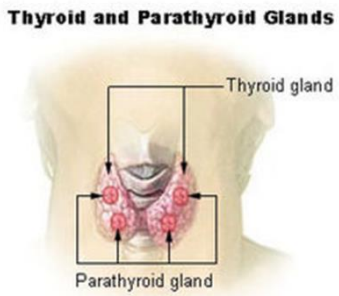
Summary and limitations of studies of drinking water nitrate and cancer

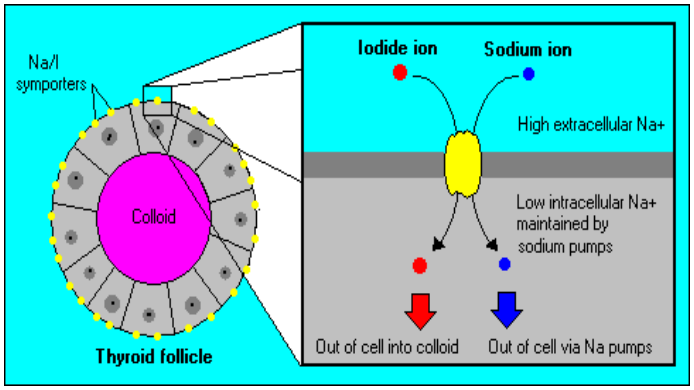
- **Most consistent evidence for colorectal cancer and childhood brain tumors**
- **Still few studies of specific cancer sites**
- **Limitations of most studies:**
  - Private well users (highest exposures) usually not considered due to lack of measurement data
  - Factors affecting NOC formation not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

Ward et al. Int J Environ Res Pub Health, 2018

Thyroid disease

Nitrate inhibition of iodide uptake by the thyroid





NO_3^- → Inhibition of iodide uptake in thyroid

↓

Plasma T_3, T_4, ↓ plasma TSH ↑

↓

Thyroid hypertrophy/hyperplasia => Cancer?

↓

Hypothyroidism

↓

Metabolic effects

Impaired development and growth (fetus, child)

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 NO_3-N had higher serum TSH & subclinical hypothyroidism (*Aschebrook-Kilfoy et al, Env Health 2012)*

Aschebrook-Kilfoy *et al. Environmental Health* 2012, **11**:6

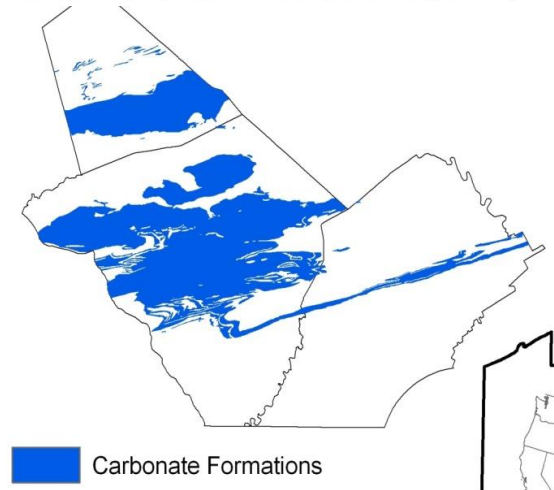
RESEARCH Open Access

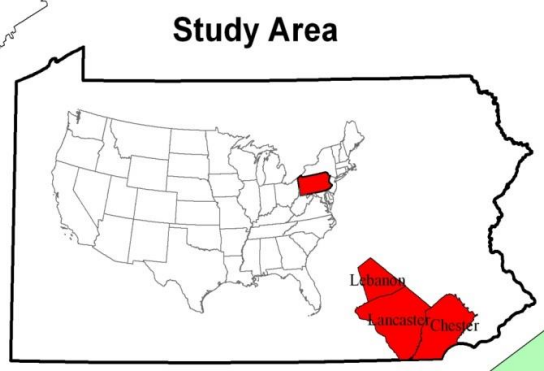
Modeled nitrate levels in well water supplies and prevalence of abnormal thyroid conditions among the Old Order Amish in Pennsylvania

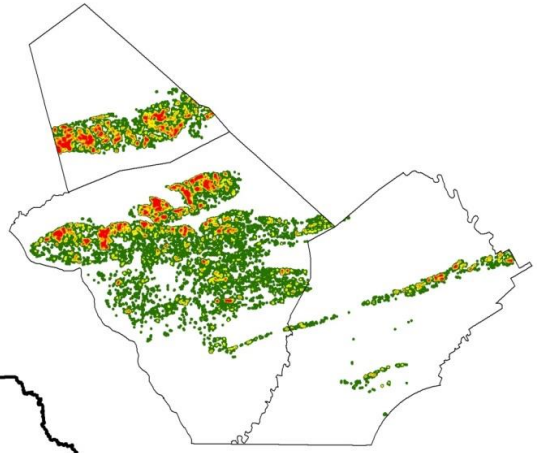
Briseis Aschebrook-Kilfoy[1,2,9], Sonya L Heltshe[3], John R Nuckols[4], Mona M Sabra[5], Alan R Shuldiner[6,7], Braxton D Mitchell[6], Matt Airola[8], Theodore R Holford[2], Yawei Zhang[2] and Mary H Ward[1*]

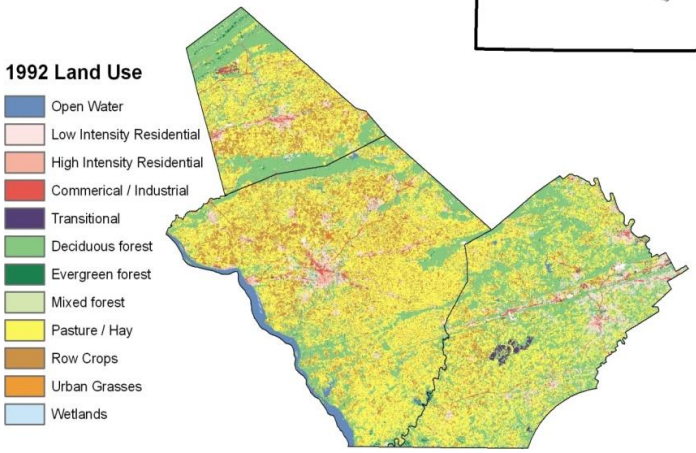
- Cohort of Old Order Amish in SE Pennsylvania (Lancaster, Chester, Lebanon counties) with measurements of thyroid hormones (N=2543)

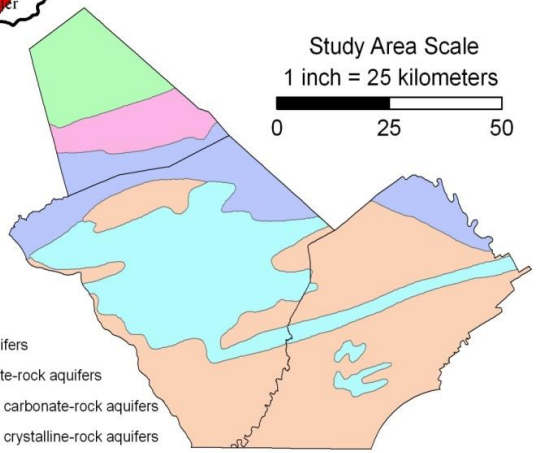
- Nitrate measurements for 3,613 private wells











Nitrate in drinking water and clinical and subclinical hypothyroidism

Aschebrook-Kilfoy *et al. Environmental Health* 2012, **11**:6

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

Overall			*Men*		*Women*	
mg/L nitrate-nitrogen	Cases	OR 95%CI	Cases	OR 95%CI	Cases	OR 95%CI
Clinical Hypothyroidism						
Low nitrate (< 6.5)	29	1.0	11	1.0	18	1.0
High nitrate (= > 6.5)	27	0.89 (0.52-1.52)	12	0.98 (0.43-2.25)	15	0.82 (0.41-1.66)
Subclinical Hypothyroidism						
Low nitrate (< 6.5)	96	1.0	44	1.0	52	1.0
High nitrate (= > 6.5)	132	1.32 (1.0-1.75)	49	0.98 (0.63-1.52)	83	**1.60 (1.11-2.32)**
Subclinical Hypothyroidism						
Q1 [0.34-4.46]	48	1.0	20	1.0	28	1.0
Q2 [4.47-6.53]	49	0.99 (0.65-1.51)	24	1.06 (0.56-2.00)	25	0.93 (0.52-1.64)
Q3 [6.54-8.55]	69	1.45 (0.97-2.15)	23	0.97 (0.51-1.83)	46	**1.84 (1.11-3.06)**
Q4 [8.56-16.4]	62	1.23 (0.82-1.84)	26	1.12 (0.60-2.09)	36	1.28 (0.75-2.16)
p-trend		0.57		0.91		0.45

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

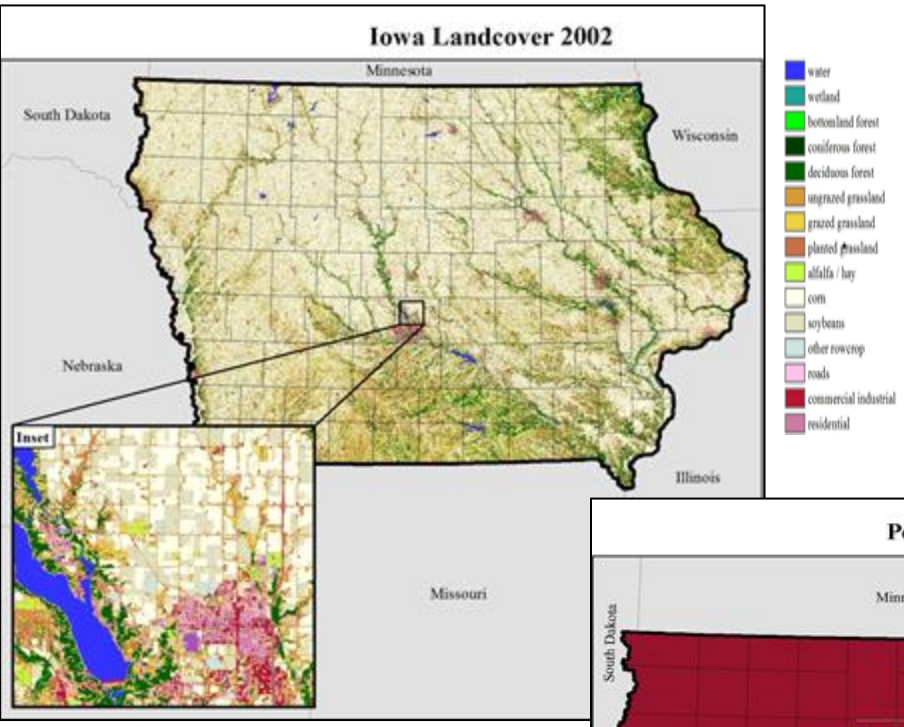
Ongoing research on drinking water contaminants - the Agricultural Health Study cohort

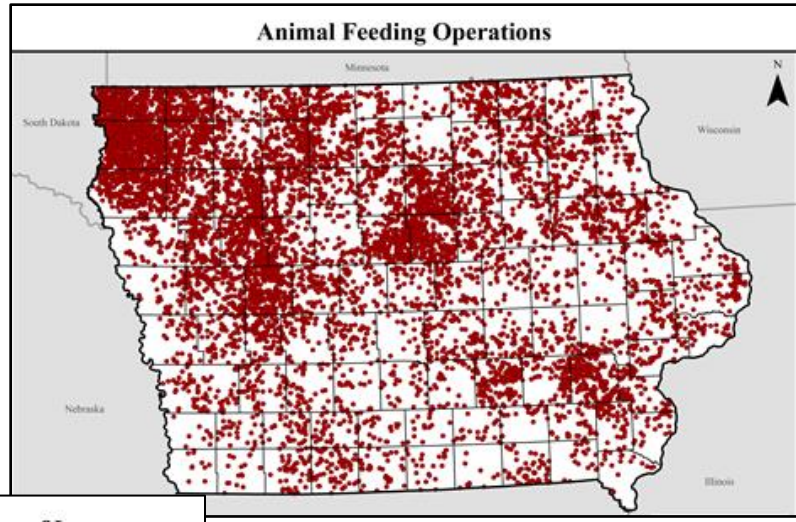
- Farmers and their spouses in Iowa and North Carolina (N=~90,000)
- 70% used private wells at enrollment in 1993-1997
- Estimated nitrate in private wells (random forest models)
- Public water supply measurements: nitrate, disinfection by-products
- Dietary nitrate/nitrite from food frequency questionnaire

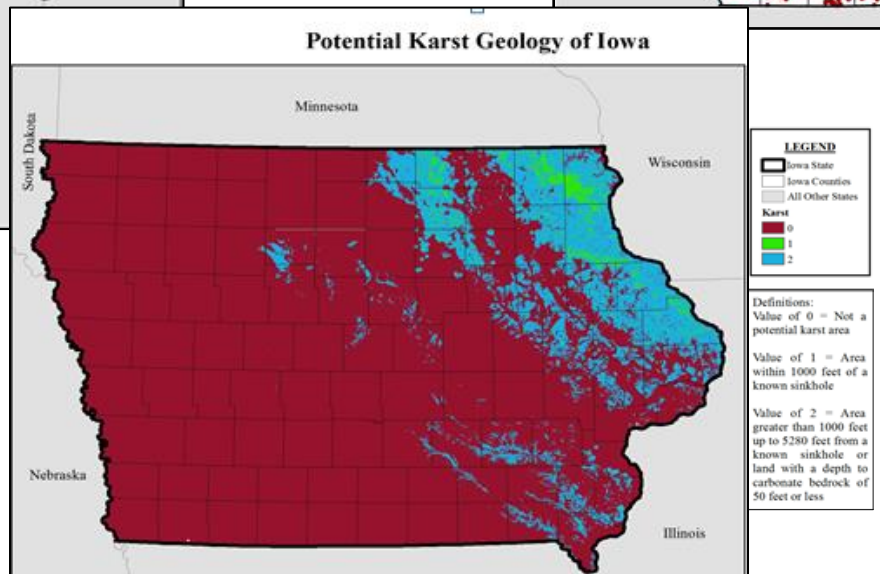
Manley.. Ward, in prep

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

National Cancer Institute

Brisa Aschebrook-Kilfoy
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Maki Inoue-Choi
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Virginia Commonweath University

David Wheeler

Colorado State University

John Nuckols

Reviews of nitrate in drinking water and health

ehp Environmental Health Perspectives

Research

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

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Mary H. Ward,[1] Theo M. deKok,[2] Patrick Levallois,[3] Jean Brender,[4] Gabriel Gulis,[5] Bernard T. Nolan,[6] and James VanDerslice[7]

International Journal of *Environmental Research and Public Health*

MDPI

Review

Drinking Water Nitrate and Human Health: An Updated Review

Int. J. Environ. Res. Public Health **2018**, 15, 1557

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