Protect Our Water Jackson Hole

CERTIFIED MAIL, RETURN RECEIPT REQUESTED

September 23, 2025

Todd Parfitt, Director
Department of Environmental Quality
200 West 17th St.
Cheyenne, WY 82002

RE: COMPLAINT AND REQUEST FOR INVESTIGATION OF GROUNDWATER CONTAMINATION IN THE PUB PLACE AREA OF TETON COUNTY, WYOMING

Dear Director Parfitt:

We are writing pursuant to W.S. §35-11-701(a)[1] to request immediate action by the Department of Environmental Quality (DEQ) to determine the cause of groundwater contamination in the vicinity of Pub Place in Teton County and to develop and implement a remediation plan to eliminate that contamination. Specifically, we are requesting that the DEQ conduct an investigation to determine the cause or causes of elevated nitrate levels in groundwater that supplies public water systems and presumably private wells in this area.

Groundwater in the Pub Place area[2] is designated Class I under Wyoming DEQ Water Quality Rules and Regulations, meaning it is suitable for domestic use and must not exceed 10 mg/L for nitrate. Information available from the U.S. Environmental Protection Agency (EPA) reveals that nitrate concentrations in the Pub Place public water system (PWS)[3] have exceeded the EPA's maximum contaminant level (MCL), with concentrations as high as 14.8 mg/L reported.[4] According to Chapter 8 of the DEQ's water quality rules and regulations, concentrations of nitrate in Class 1 groundwater that exceed 10 mg/L are unsuitable for domestic use, and

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[1] W.S. §35-11-701(a) provides that, " If the director or the administrators have cause to believe that any persons are violating any provision of this act or any rule, regulation, standard, permit, license, or variance issued pursuant hereto, or in case any written complaint is filed with the department alleging a violation, the director, through the appropriate administrator, shall cause a prompt investigation to be made."

[2] Exhibit A, map depicting Pub Place area of concern.

[3] PWS No. WY5601258

[4] Exhibit B, Chem/Rad Sample Results for Pub Place PWS, collection date 3/20/2024. Drinking water quality data is available online through EPA's Region 8 Drinking Water Watch website at https://www.epa.gov/region8-waterops/drinking-water-watch-epa-region-8.

constitute a violation of the groundwater quality standards set forth in Chapter 8, Section 4, Table 1 (p.9).

The high nitrate level experienced by the Pub Place PWS in March of 2024 is not a one-off. Water quality data reported in consumer confidence reports (CCRs) for Pub Place over the past ten years show that nitrate concentrations in drinking water -and presumably in the groundwater upon which this system relies- have been trending upwards, reaching nearly 15 mg/L in 2024.[5] Nitrate concentrations exceeding 10 mg/L present a threat to the public health and safety of the residents of this area, and constitute a violation of Wyoming's quality standards for groundwater. Although the Pub Place PWS has taken corrective action to reduce concentrations of nitrates in the drinking water it distributes,[6] we are concerned that nitrate concentrations in the groundwater used by this PWS likely remain above the 10 mg/L limit set by Chapter 8, which may put nearby drinking water wells at risk.

Adding to the concern, two Public Water Systems in close proximity to Pub Place have also exhibited elevated nitrate levels. O Bar B (PWS No. WY5601693) showed levels at 6.1 mg/L in 2024[7,8] while Valley View Mutual Water Company (PWS No. 5601499) showed levels at 6.4 mg/L the same year.[9,10] Concentrations of nitrates well above natural background levels demonstrate that there is a persistent pollutant source in the area that has not been addressed. With respect to background levels, Table 1 of Teton Conservation District's *Teton County, Wyoming Drinking Water Quality Mapping Project* (January 20, 2021) shows median (0.13 mg/L) and average (0.58 mg/L) nitrate concentrations based on samples from 360 locations. The TCD report notes that:

> Nitrate provided the most spatially complete dataset available for this analysis, with 360 locations having available data (including Yellowstone National Park) and many of these sites containing duplicate records. ***The median value for the 4,827 sampling events for nitrate was 0.13 mg/L and is a testament to the low background concentrations typically found in the region.***

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[5] Exhibit C, graph of nitrate levels at Pub Place PWS, created using nitrate values reported in CCRs from the past ten years.

[6] Exhibit D, EPA Chem/Rad Sample Results for Pub Place PWS showing nitrate levels of 3.3 mg/L, collection date 8/19/2025.

[7] Exhibit E, EPA Chem/Rad Sample Results for O Bar B PWS showing nitrate level of 6.1 mg/L, collection date 9/4/2024.

[8] Exhibit F, graph of nitrate levels at O Bar B PWS, created using nitrate values reported in CCRs from 2016 to 2024.

[9] Exhibit G, EPA Chem/Rad Sample Results for Valley View PWS showing nitrate levels of 6.4 mg/L, collection date 8/12/2024.

[10] Exhibit H, graph of nitrate levels at Valley View PWS, created using nitrate values reported in CCRs from 2009 to 2024.

Id. at 6 (emphasis added).

In a letter to the Board of County Commissioners, the Teton District Board of Health wrote: "While naturally occurring, nitrate does not typically occur at concentrations above 2 mg/L in undisturbed surface or groundwater. Concentrations in excess of this are often indicative of human caused contamination."[11] Moreover, nitrates are considered an indicator species for other contaminants in human wastewater such as pharmaceuticals, viruses, and bacteria.[12]
Based on what is known about the presence of nitrate in Teton County's groundwater, the nitrate levels found in the two aforementioned PWSs clearly indicate human contamination.

Commercial and residential septic systems in the Pub Place area, a sewerage system (W.S. 35-11-103(c)(iii)), a nearby landfill, and heavy industrial uses in the area are amongst some of the potential causes of and/or contributions to the nitrate exceedances, but a conclusive determination as to the precise source and cause of high nitrate levels has not been made. Teton County GIS records show that approximately 55 buried leach fields, dozens constructed in the 1970s and eighties, exist within a 0.60 mile radius of the Pub Place PWS.[13] The advanced age of the septic systems coupled with the high density of the systems in this area –many on lots smaller than one acre– suggests they may have some role in the groundwater contamination.[14]

Although concerns about potential contamination of drinking water wells from residential septic systems in other areas south of town are well documented and are being addressed,[15] we are not aware of any effort by any regulatory agency to address rising nitrate levels in the Pub Place area.

Health Concerns Associated with Nitrates

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[11] The Teton Health District's letter to the Teton County BCC is attached as Exhibit J.

[12] *See* Schaider, Laurel A.; Ackerman, Janet M.; Rudel, Ruthann A., "Septic Systems as Sources of Organic Wastewater Compounds in Domestic Drinking Water Wells in a Shallow Sand and Gravel Aquifer," *Science of The Total Environment*547 (2016): 470–481, DOI:10.1016/j.scitotenv.2015.12.081.

[13] Exhibit K, map depicting Teton County wastewater overlay in Pub Place area of concern.

[14] *See, e.g.,* J.E. Bremer & T. Harter, Domestic Wells Have High Probability of Pumping Septic Tank Leachate, 16 Hydrol. Earth Syst. Sci. 2453 (2012), https://doi.org/10.5194/hess-16-2453-2012 (finding that domestic wells in areas with high septic system density face a significant probability of pumping septic leachate, thereby elevating risks to groundwater quality and public health).

[15] *See* Wyoming Dep't of Envtl. Quality, Investigation of Elevated Nitrates in the Hoback Junction Area, Teton County, Wyoming (Dec. 2024), https://deq.wyoming.gov (prepared in cooperation with Teton County and the Teton Conservation District) (finding that the major known source of elevated nitrates in Hoback Junction groundwater is the density of domestic septic systems, with some wells already exceeding EPA's 10 mg/L maximum contaminant level).

The U.S. EPA established the 10 mg/L MCL for nitrate in the early-1990s in response to blue baby syndrome.[16] Since then more recent studies suggest that adverse health effects can be associated with nitrate levels at 5 mg/L.[17]

Health concerns associated with the ingestion of nitrates are well known. A DEQ fact sheet on nitrates explains that:

> Ingestion of water containing high levels of nitrate or nitrite can be fatal for infants, especially bottle-fed infants under 6 months of age. Bacteria in the saliva and digestive tract convert nitrate to nitrite, this can interfere with the ability of blood to carry oxygen. In serious cases, this can lead to a disorder called methemoglobinemia, or 'blue baby syndrome.' Symptoms include shortness of breath or a blue coloring to the skin. Water containing nitrate or nitrite should not be used in food or formula preparation for children under 6 months of age. Nitrate and nitrite are not usually a problem for people over 6 months of age, although people with certain health conditions may be more susceptible to problems from nitrate or nitrite ingestion, such as:
>
> - Pregnant women
> - People with low stomach acid
> - People with gastrointestinal infections
> - People lacking the methemoglobin reductase enzyme
>
> People who consume unusually high levels of nitrates can experience decreases in blood pressure, increased heart rate, headaches, abdominal cramps, and vomiting.

The DEQ's nitrate fact sheet is available online at: https://www.tetoncountywy.gov/DocumentCenter/View/8836/Nitrate-Nitrite-Fact Sheet?bidId

Conclusion

In March of 2024, the Pub Place public water system reported nitrate levels of 14.8 mg/L, significantly exceeding the EPA's maximum contaminant level of 10 mg/L. Although the PWS has since instituted measures to reduce nitrate levels in the drinking water, the nitrate-contaminated groundwater used by this system has not been addressed, and is believed to contain levels that exceed the DEQ's groundwater quality standards for domestic use.

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[16] See https://wqa.org/wp-content/uploads/2022/09/2014_NitrateNitrite.pdf

[17] Allison R. Sherris et al., Nitrate in Drinking Water During Pregnancy and Spontaneous Preterm Birth: A Retrospective Within-Mother Analysis in California, 129 **Envtl. Health Persp.** 057001 (2021), https://doi.org/10.1289/EHP8205. Attached as Exhibit L.

At the same time, nitrate concentrations at two nearby public water systems, O Bar B and Valley View Mutual Water Company, have reported nitrate levels of 6.1 mg/L and 6.4 mg/L, respectively, levels that clearly indicate human-caused contamination in the groundwater.

Chapter 8 Section 3(c) of the DEQ's water quality rules establishes that: "Protection shall be afforded all underground water bodies (including water in the vadose zone). Water being used for a purpose identified in W.S. 35-11-102 and 103(c)(i) shall be protected for its intended use and uses for which it is suitable." Groundwater used for drinking water and other domestic purposes is an identified use and therefore "shall" be protected. Chapter 8 further provides: "A discharge or activity that impacts an underground source of water ... shall not make the affected water unsuitable for its intended use or uses, at any place or places of withdrawal or natural flow to the surface." Ch. 8 Sec. 4(c).

In accordance with W.S. §35-11-701(a), POWJH alleges, based on the facts and evidence presented herein, that "discharges or activities" have rendered groundwater in the Pub Place area unsuitable for domestic use. We therefore request a prompt investigation into the sources and causes of exceedances of DEQ's groundwater quality standards for nitrates in the Pub Place area. We appreciate your attention to this matter and look forward to your reply.

Sincerely,

Jennifer Evans

Jennifer Evans
Advocacy Director
Protect Our Water Jackson Hole

Dan Heilig
Board Member
Protect Our Water Jackson Hole

CC (via email):

Lily Barkau, Groundwater Section Manager, WDEQ/WQD

Jennifer Zygmunt, Administrator, WDEQ/WQD

Wendy Cheung, UIC Permitting Section Supervisor, Environmental Protection Agency

Teton County Board of County Commissioners

Dr. Travis Riddell, Teton County Health Department

Teton County District Board of Health

Chris Peltz, Water Resources Coordinator, Teton County

Heather Overholser, Director of Public Works, Teton County

Carlin Girard, Executive Director, Teton Conservation District

Amy Ramage, Public Works County Engineer, Teton County

Johnny Ziem, Acting Public Works Director, Town of Jackson

Teton County Water Quality Advisory Board

Enclosures:

Ex. A: Map depicting Pub Place area of concern.

Ex. B: Chem/Rad Sample Results for Pub Place PWS, collection date 3/20/2024.

Ex. C: Graph of nitrate levels at Pub Place PWS, created using nitrate values reported in CCRs from the past ten years.

Ex. D: EPA Chem/Rad Sample Results for Pub Place PWS showing nitrate levels of 3.3 mg/L, collection date 8/19/2025.

Ex. E: EPA Chem/Rad Sample Results for O Bar B PWS showing nitrate level of 6.1 mg/L, collection date 9/4/2024.

Ex. F: Graph of nitrate levels at O Bar B PWS, created using nitrate values reported in CCRs from 2016 to 2024.

Ex. G: EPA Chem/Rad Sample Results for Valley View PWS showing nitrate levels of 6.4 mg/L, collection date 8/12/2024.

Ex. H: Graph of nitrate levels at Valley View PWS, created using nitrate values reported in CCRs from 2009 to 2024.

Ex. J: The Teton Health District's letter to the Teton County BCC.

Ex. K: Map depicting Teton County wastewater overlay in Pub Place area of concern.

Ex. L: Allison R. Sherris et al., Nitrate in Drinking Water During Pregnancy and Spontaneous Preterm Birth: A Retrospective Within-Mother Analysis in California, 129 **Envtl. Health Persp.** 057001 (2021), https://doi.org/10.1289/EHP8205.

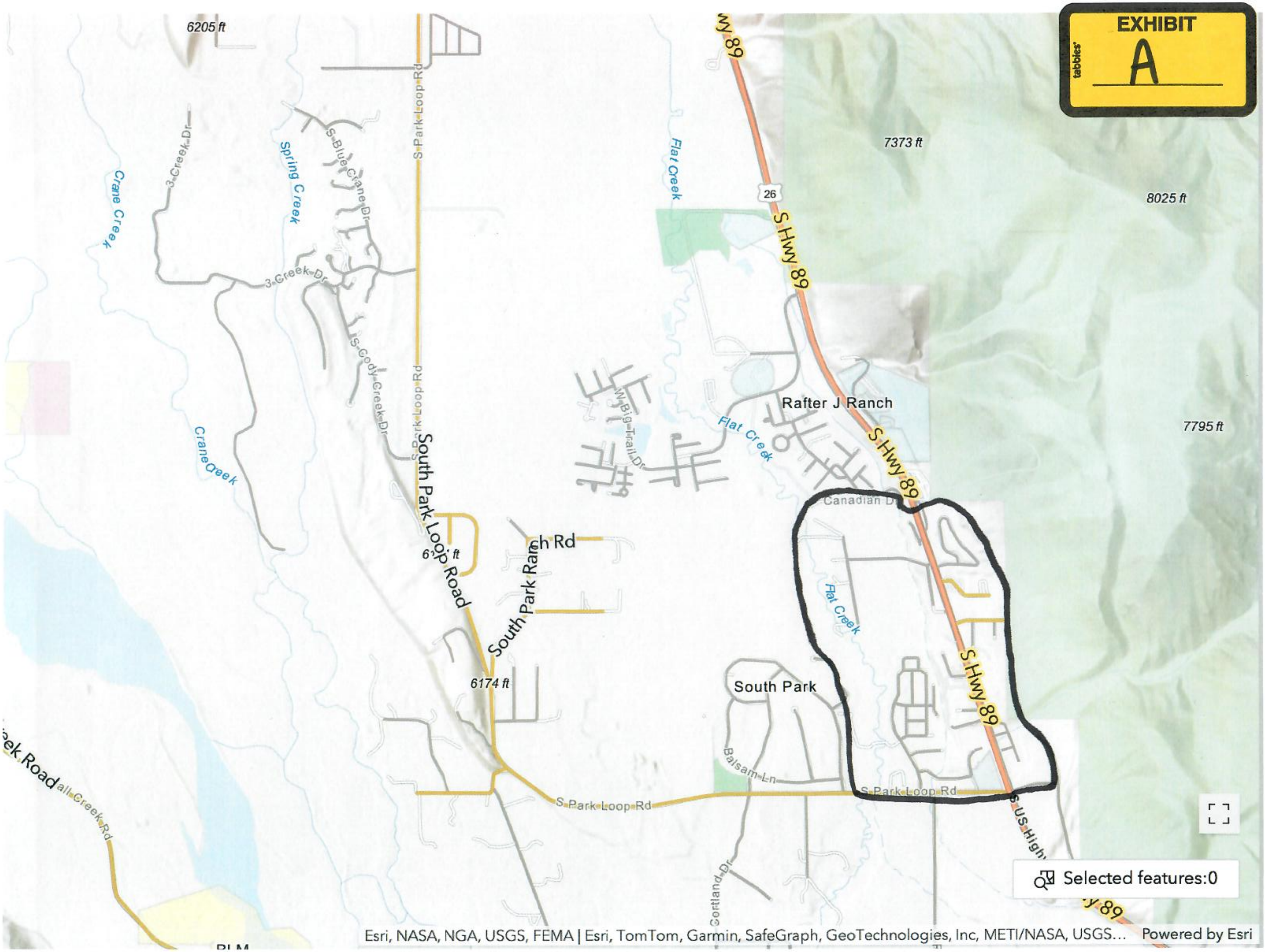


EXHIBIT
A
tabbies
6205 ft
7373 ft
8025 ft
7795 ft
S Hwy 89
26
Rafter J Ranch
Flat Creek
Canadian Dr
W Big Trail Dr
South Park Loop Road
S Park Loop Rd
South Park Ranch Rd
6174 ft
South Park
Balsam Ln
Cortland Dr
S US Highway 89
S Blue Crane Dr
S Cody Creek Dr
3 Creek Dr
Spring Creek
Crane Creek
Fall Creek Rd
Selected features:0
Esri, NASA, NGA, USGS, FEMA | Esri, TomTom, Garmin, SafeGraph, GeoTechnologies, Inc, METI/NASA, USGS... Powered by Esri

EXHIBIT B

Drinking Water Branch

Chem/Rad Sample Results

Water System No. :	WY5601258	**Federal Type :**	NTNC
Water System Name :	PUB PLACE	**State Type :**	NTNC
Principal County Served :	TETON	**Primary Source :**	GW
Status :	A	**Activity Date :**	06-01-1996
Lab Sample No. :	C24030567-001B	**Collection Date :**	03-20-2024

This list displays sample/results of all non-microbial analytes (TSAANLYT.TYPE_CODE <> MOR) associated to the selected sample. Results for Microbial Analytes are not included. If there are no sample results in the Concentration Level column, then the results were lower than the lab Reporting Level.

Analyte Code	Analyte Name	Method Code	Less than Indicator	Level Type	Reporting Level	Concentration level	Monitoring Period Begin Date	Monitoring Period End Date
1038	NITRATE-NITRITE	353.2		MRL	0.1 MG/L	14.8 MG/L	01-01-2024	03-31-2024
1040	NITRATE	353.2		MRL	0.05 MG/L	14.8 MG/L		
1041	NITRITE	null		MRL	0	0 MG/L		

Total Number of Records Fetched = 3

EXHIBIT C

2014-2024 Nitrate Levels (mg/L) at Pub Place, Teton County

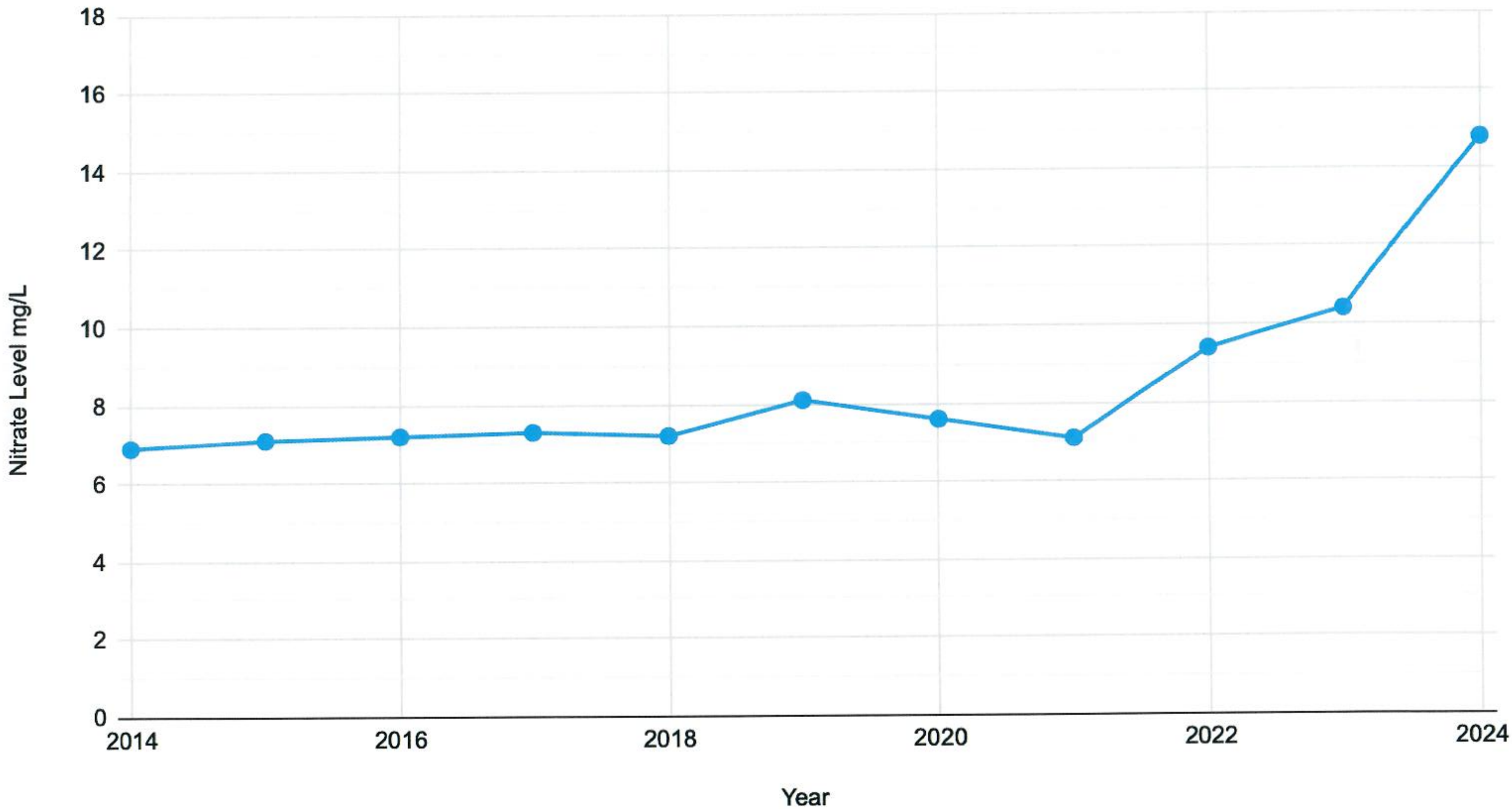


EXHIBIT D

Drinking Water Branch

Chem/Rad Sample Results

Water System No. :	WY5601258	**Federal Type :**	NTNC
Water System Name :	PUB PLACE	**State Type :**	NTNC
Principal County Served :	TETON	**Primary Source :**	GW
Status :	A	**Activity Date :**	06-01-1996
Lab Sample No. :	C25080793-002C	**Collection Date :**	08-19-2025

This list displays sample/results of all non-microbial analytes (TSAANLYT.TYPE_CODE <> MOR) associated to the selected sample. Results for Microbial Analytes are not included. If there are no sample results in the Concentration Level column, then the results were lower than the lab Reporting Level.

Analyte Code	Analyte Name	Method Code	Less than Indicator	Level Type	Reporting Level	Concentration level	Monitoring Period Begin Date	Monitoring Period End Date
1038	NITRATE-NITRITE	353.2		MRL	0.1 MG/L	3.3 MG/L		

Total Number of Records Fetched = 1

EXHIBIT E

Drinking Water Branch

Chem/Rad Sample Results

Water System No. :	WY5601693	**Federal Type :**	NTNC
Water System Name :	O BAR B IMPROVEMENT & SERVICE DISTRICT	**State Type :**	
Principal County Served :	TETON	**Primary Source :**	GW
Status :	A	**Activity Date :**	05-21-2016
Lab Sample No. :	C24090228-001A	**Collection Date :**	09-04-2024

This list displays sample/results of all non-microbial analytes (TSAANLYT.TYPE_CODE <> MOR) associated to the selected sample. Results for Microbial Analytes are not included. If there are no sample results in the Concentration Level column, then the results were lower than the lab Reporting Level.

Analyte Code	Analyte Name	Method Code	Less than Indicator	Level Type	Reporting Level	Concentration level	Monitoring Period Begin Date	Monitoring Period End Date
1038	NITRATE-NITRITE	353.2		MRL	0.1 MG/L	6.1 MG/L	07-01-2024	09-30-2024

Total Number of Records Fetched = 1

EXHIBIT F

2016-2024 Nitrate Levels (mg/L) at O Bar B Improvement Service District

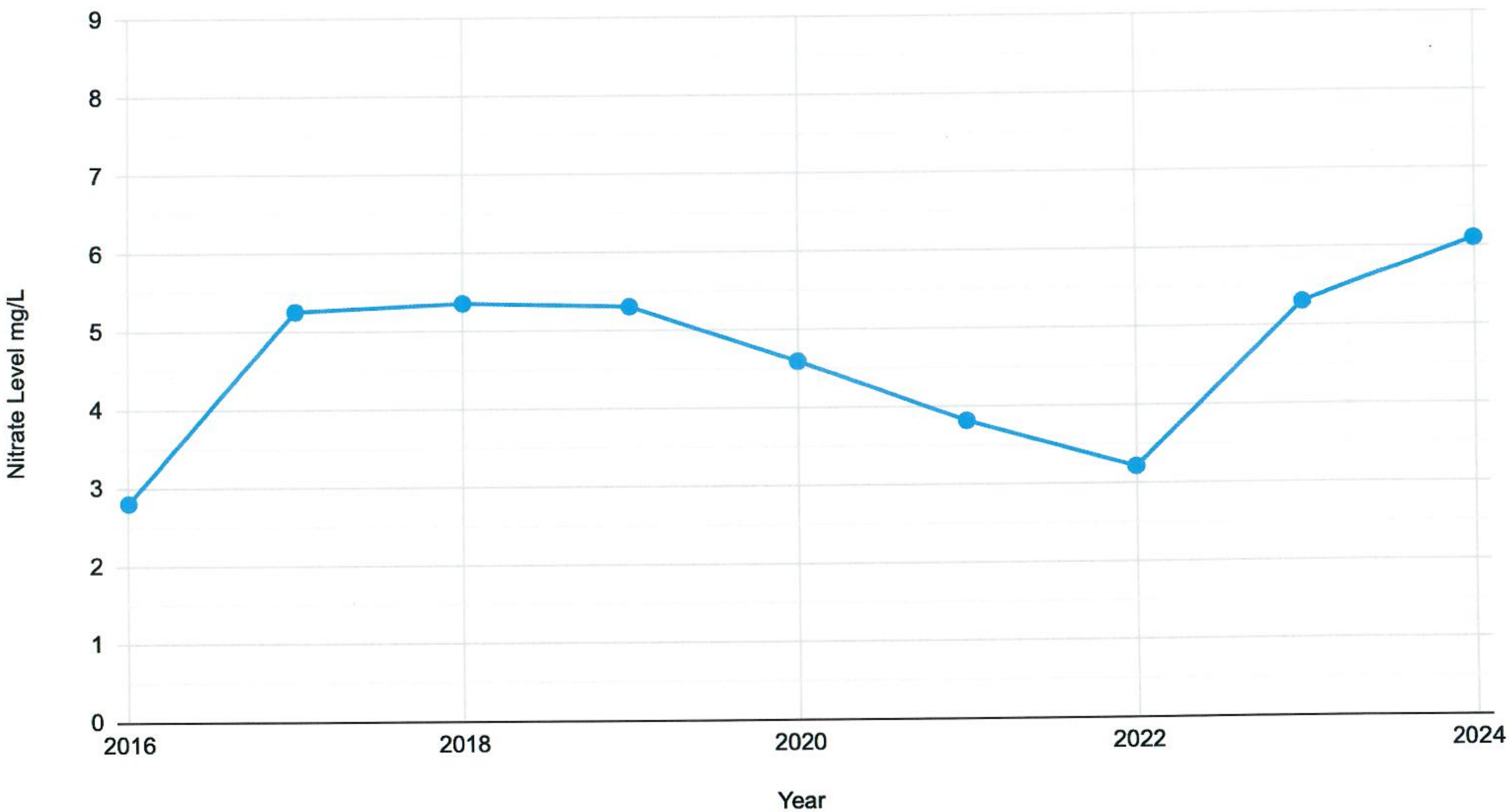


EXHIBIT G

Drinking Water Branch

Chem/Rad Sample Results

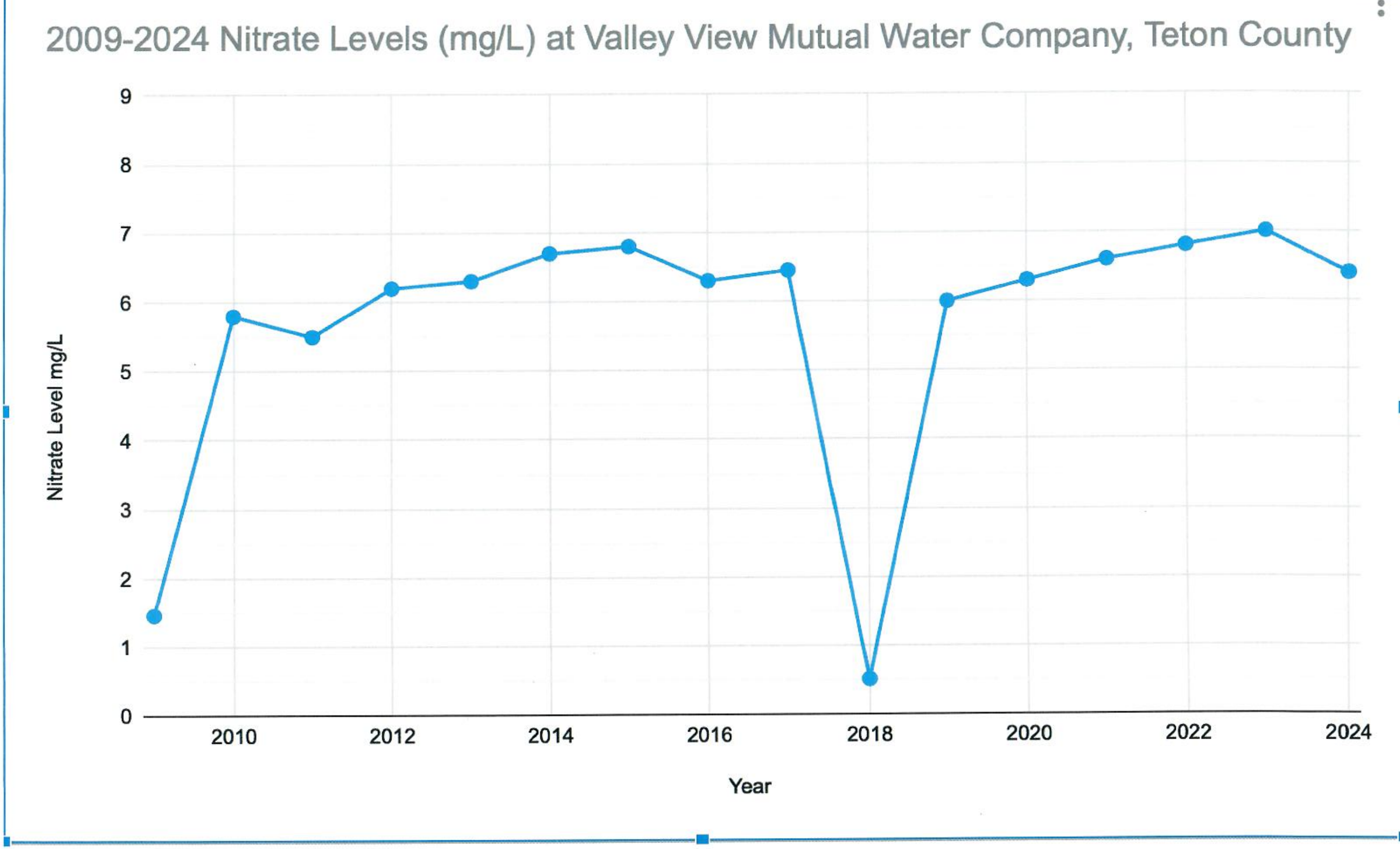
Water System No. :	WY5601499	**Federal Type :**	C
Water System Name :	VALLEY VIEW MUTUAL WATER COMPANY	**State Type :**	C
Principal County Served :	TETON	**Primary Source :**	GW
Status :	A	**Activity Date :**	12-21-2001
Lab Sample No. :	C24080639-001A	**Collection Date :**	08-12-2024

This list displays sample/results of all non-microbial analytes (TSAANLYT.TYPE_CODE <> MOR) associated to the selected sample. Results for Microbial Analytes are not included. If there are no sample results in the Concentration Level column, then the results were lower than the lab Reporting Level.

Analyte Code	Analyte Name	Method Code	Less than Indicator	Level Type	Reporting Level	Concentration level	Monitoring Period Begin Date	Monitoring Period End Date
1038	NITRATE-NITRITE	353.2		MRL	0.1 MG/L	6.4 MG/L	07-01-2024	09-30-2024

Total Number of Records Fetched = 1

EXHIBIT H



2009-2024 Nitrate Levels (mg/L) at Valley View Mutual Water Company, Teton County
Nitrate Level mg/L
0
1
2
3
4
5
6
7
8
9
2010
2012
2014
2016
2018
2020
2022
2024
Year

EXHIBIT J

Dear Teton County Board of County Commissioners,

The following letter was prepared by a partnership between the Teton Conservation District (TCD), the Teton County Health Department (TCHD), and the Teton County District Board of Health. The purpose of this letter is to provide an update to the Board of County Commissioners on the drinking water concerns in the Hoback Junction area.

Introduction

In Wyoming, the United States Environmental Protection Agency (EPA) and the Wyoming Department of Environmental Quality (WYDEQ) regulate public water supplies by requiring regular testing and public reporting. While privately-owned wells are not subject to any government-mandated testing, the results of voluntary water tests offered to property owners by the TCD and TCHD offer insight into the water quality of such drinking water sources throughout Teton County. By observing both of these sources of data, it has come to the attention of TCD, TCHD, and the Teton District Board of Health that significant water quality issues exist within the Hoback Junction area. Nitrate concentrations have at times exceeded the EPA's drinking water standards for public systems. The TCHD has observed frequent positive bacteriological tests from private and public systems in the area. Other problems affecting drinking water in the Hoback area include the presence of sulfur in many private systems and poor or inconsistent well productivity. These water quality issues have created concern for the public's health and warrant continued exploration for suitable water sources and governmental support.

Concerns of Nitrate in Drinking Water

The presence of nitrate in drinking water is of concern for several reasons:

1) Nitrate can be an indicator of human-caused contamination. In the Hoback area, the observed nitrate is suspected to originate from wastewater sources such as septic systems. This could indicate a cross-connection between wastewater and drinking water systems, suggesting that other potentially harmful contaminants such as cleaning products and pharmaceutical drugs could enter the drinking water. Routine testing of public and private water systems does not include screening for many such chemicals.

2) High concentrations of nitrate in drinking water have been associated with a condition known as methemoglobinemia, or 'blue baby syndrome', wherein hemoglobin in the blood is modified and its ability to transport oxygen is reduced, resulting in hypoxia. Infants and young children are particularly susceptible to this condition.

3) Other human health effects of nitrate are not currently well-understood. While some epidemiological research has observed an association between nitrate in drinking water and certain cancers and birth defects, other research has demonstrated no such correlation. The degree of risk posed by nitrate in this regard remains uncertain. Further research is needed on these subjects. TCHD will continue to monitor and

review the scientific literature as it is published and update any recommendations as necessary to best protect public health.

Presence of Nitrate in the Hoback Area

While naturally occurring, nitrate does not typically occur at concentrations above 2 mg/L in undisturbed surface or groundwater. Concentrations in excess of this are often indicative of human-caused contamination. Testing from the Hoback Junction area has often revealed concentrations significantly above the expected naturally occurring level. Concentrations at or exceeding 10 mg/L (the EPA's maximum allowable level for public systems) have also been observed.

Records from public water systems provide the most robust data source on this issue and indicate that nitrate contamination is a growing problem in the area. Routine monitoring from public systems such as the J-W Subdivision demonstrate a steady increase in nitrate concentrations in some areas over several years. *Figure 1* shows nitrate concentrations in that system from 1984 to 2016.

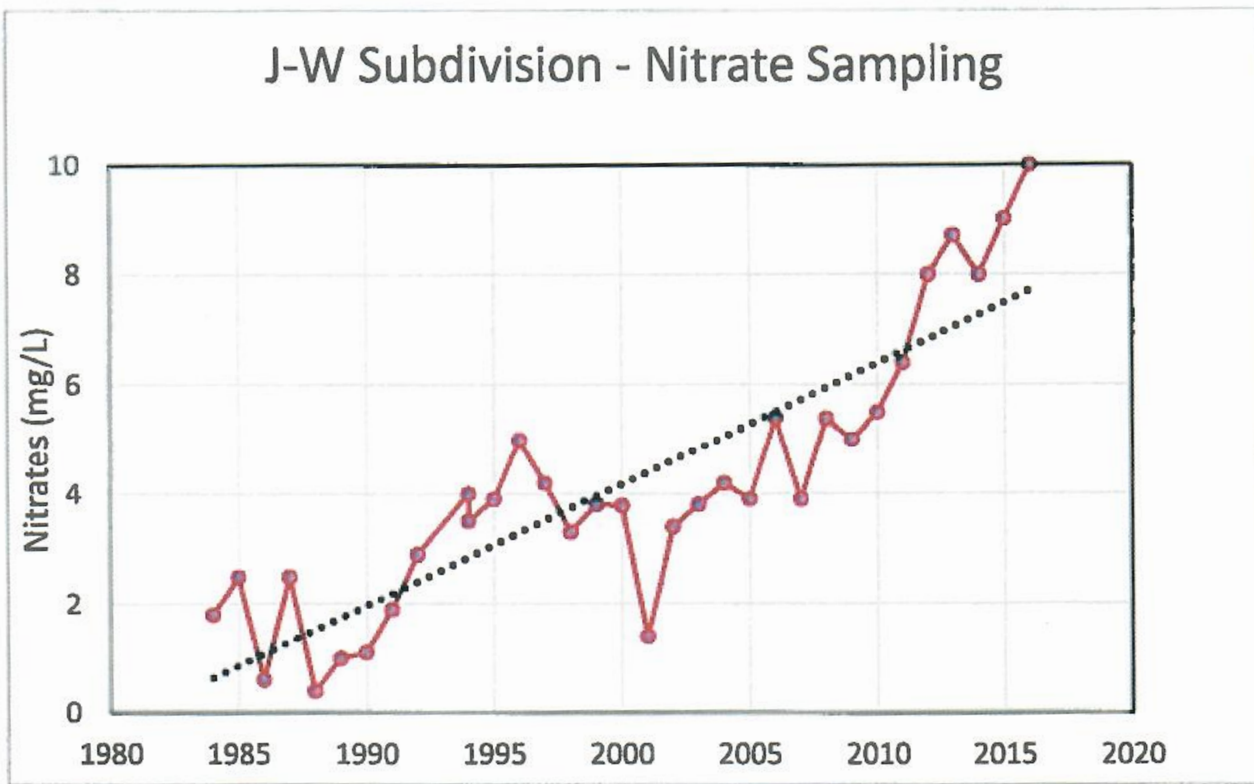


Figure 1. Nitrate concentrations from J-W Subdivision water supply from 1984-2016, James Brough.

Other public water supplies in the area have already exceeded the drinking water standard for nitrate. In 2004, testing of the water from the Hoback Market system measured nitrate at 57.4

mg/L and 59.4 mg/L. Hoback RV Park began approaching the regulatory limit as early as 1995, testing at 9.8 mg/L, and exceeded it for the first time in 1997. Such systems that would otherwise consistently exceed 10 mg/L are now required to treat the water prior to its use.

Data from these systems and from the J-W system clearly demonstrate that the area immediately north of the Hoback River/Snake River confluence has a persistent and, in places, growing problem with nitrate in drinking water. Private well data from the vicinity also demonstrate a similar trend to that seen in J-W and other public systems, with several homes increasing over time or already exceeding 10 mg/L.

Based on the available information, concerning nitrate concentrations have been observed in the area North of the Snake River/Hoback River confluence to roughly the HWY 89 Snake River Bridge at Henry's Road. The primary focus, however, is on the immediate Hoback Junction area as seen in *Figure 2.* This problem has persisted for over twenty years and while there have been attempts at mitigation, progress has been limited and halting. However, there has been and remains interest in more comprehensive solutions. In 2006, the Wyoming Water Development Commission funded a Level One Water Supply Study. More recently, TCD and TCHD hosted an agency/stakeholder meeting on September 13th, 2018. This was followed by a public meeting to build common knowledge about this issue. A survey was conducted at the meeting to assess public interest in receiving governmental assistance in addressing this issue. A strong majority of respondents supported such assistance.

While public systems that are approaching or exceeding the nitrate maximum contaminant level should also be addressed, particular attention should be paid to private systems. Such wells and springs are primarily owned and managed by homeowners and are not subject to any required testing or mitigation. Efforts should be made to ensure those on private wells are educated as to the need for periodic water testing. Alternative water sources for owners and other forms of assistance should be provided.

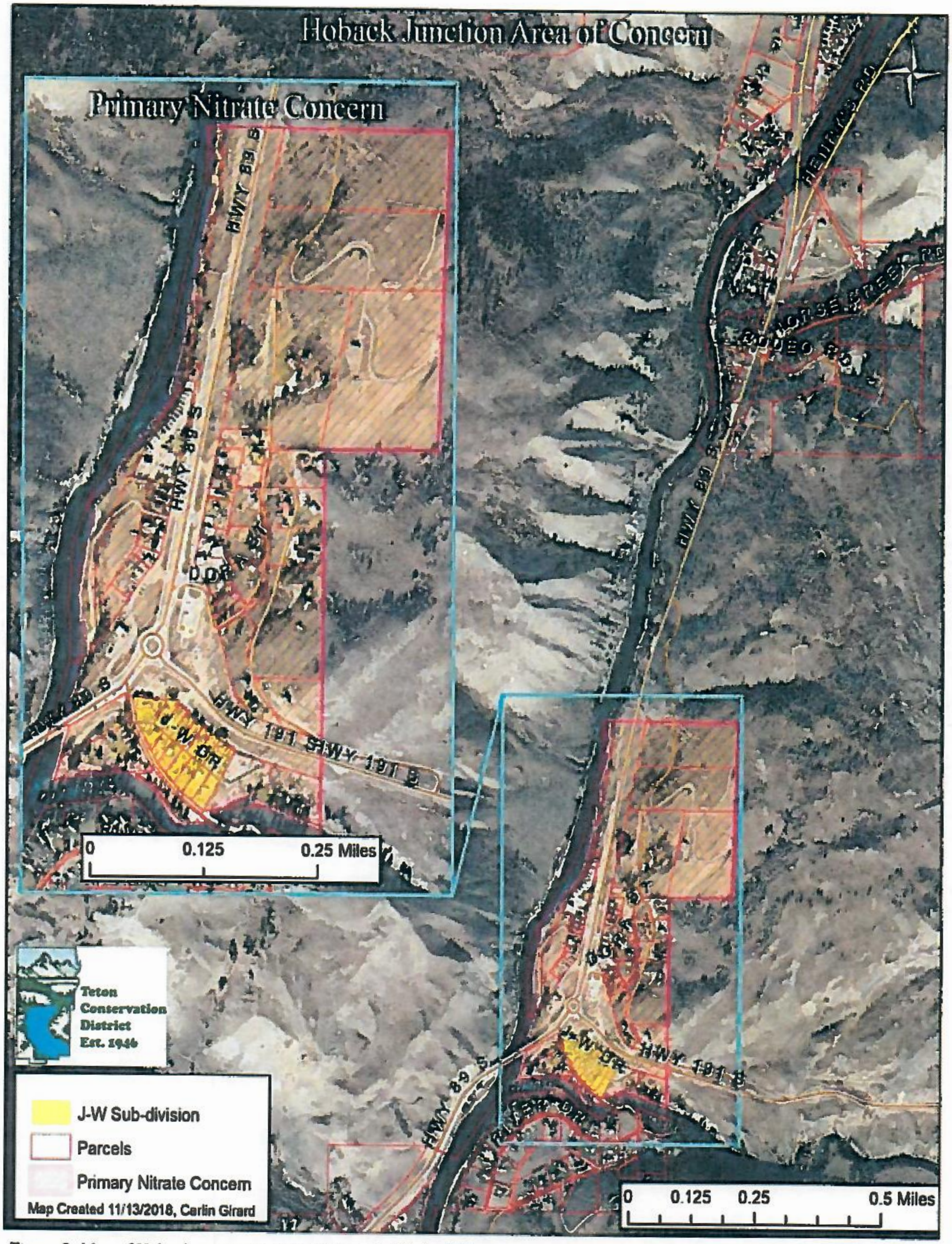


Figure 2. Map of Hoback Junction Area with insert of Hoback Junction.

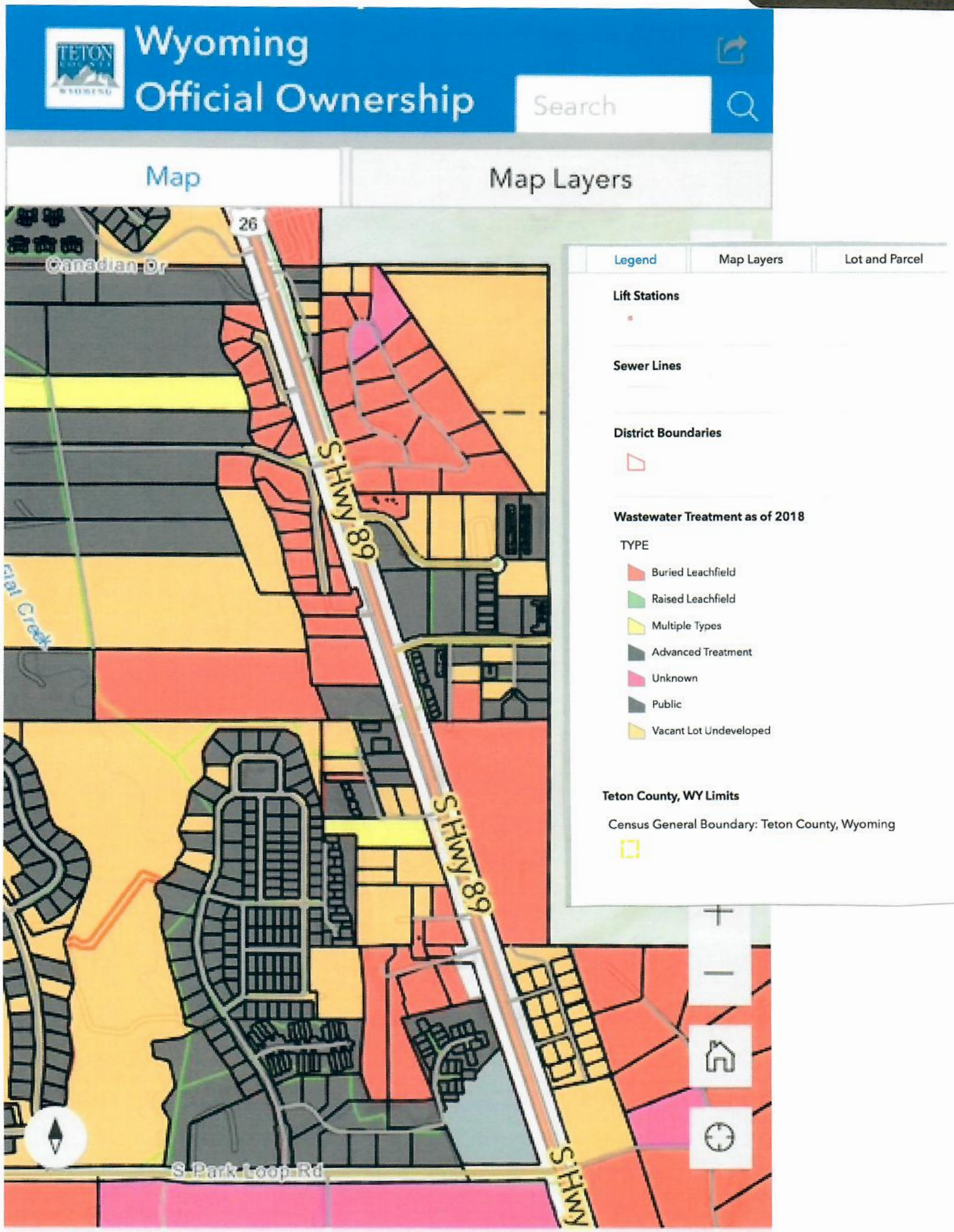
Questions regarding nitrate concentrations and distribution within the Hoback area can be directed to the Teton Conservation District at (307) 733-2110. Further information, including the basis for much of this memorandum, can also be found at https://www.tetonconservation.org/news/2018/8/27/hoback-junction-drinking-water-meeting.

Questions regarding the health effects of nitrate can be directed to the Environmental Health Division of the Teton County Health Department at (307) 732-8490.

Sincerely,

Dan Forman, DVM
Teton District Board of Health
Chairman

EXHIBIT K


Wyoming
Official Ownership
Search
Map
Map Layers
26
Canadian Dr
S Hwy 89
S Hwy 89
S Park Loop Rd
S Hwy
Legend
Map Layers
Lot and Parcel
Lift Stations
Sewer Lines
District Boundaries
Wastewater Treatment as of 2018
TYPE
Buried Leachfield
Raised Leachfield
Multiple Types
Advanced Treatment
Unknown
Public
Vacant Lot Undeveloped
Teton County, WY Limits
Census General Boundary: Teton County, Wyoming

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP8205.

Nitrate in Drinking Water during Pregnancy and Spontaneous Preterm Birth: A Retrospective Within-Mother Analysis in California

Allison R. Sherris,[1] Michael Baiocchi,[2] Scott Fendorf,[3] Stephen P. Luby,[4] Wei Yang,[5] and Gary M. Shaw[5]

[1]Emmett Interdisciplinary Program in Environment and Resources, Stanford University, Stanford, California, USA
[2]Department of Epidemiology and Population Health, Stanford University, Stanford, California, USA
[3]Department of Earth System Science, Stanford University, Stanford, California, USA
[4]Department of Medicine, Stanford University, Stanford, California, USA
[5]Department of Pediatrics, Stanford University, Stanford, California, USA

Background: Nitrate is a widespread groundwater contaminant and a leading cause of drinking water quality violations in California. Associations between nitrate exposure and select adverse birth outcomes have been suggested, but few studies have examined gestational exposures to nitrate and risk of preterm birth (before 37 wk gestation).

Objective: We investigated the association between elevated nitrate in drinking water and spontaneous preterm birth through a within-mother retrospective cohort study of births in California.

Methods: We acquired over 6 million birth certificate records linked with Office of Statewide Health Planning and Development hospital discharge data for California births from 2000–2011. We used public water system monitoring records to estimate nitrate concentrations in drinking water for each woman's residence during gestation. After exclusions, we constructed a sample of 1,443,318 consecutive sibling births in order to conduct a within-mother analysis. We used separate conditional logistic regression models to estimate the odds of preterm birth at 20–31 and 32–36 wk, respectively, among women whose nitrate exposure changed between consecutive pregnancies.

Results: Spontaneous preterm birth at 20–31 wk was increased in association with tap water nitrate concentrations during pregnancy of 5 to <10 mg/L [odds ratio (OR) = 1.47; 95% confidence interval (CI): 1.29, 1.67] and ≥ 10 mg/L (OR = 2.52; 95% CI: 1.49, 4.26) compared with <5 mg/L (as nitrogen). Corresponding estimates for spontaneous preterm birth at 32–36 wk were positive but close to the null for 5 to <10 mg/L nitrate (OR = 1.08; 95% CI: 1.02, 1.15) and for ≥ 10 mg/L nitrate (OR = 1.05; 95% CI: 0.85, 1.31) vs. <5 mg/L nitrate. Our findings were similar in several secondary and sensitivity analyses, including in a conventional individual-level design.

Discussion: The results suggest that nitrate in drinking water is associated with increased odds of spontaneous preterm birth. Notably, we estimated modestly increased odds associated with tap water nitrate concentrations of 5 to <10 mg/L (below the federal drinking water standard of 10 mg/L) relative to <5 mg/L. https://doi.org/10.1289/EHP8205

Introduction

Preterm birth (delivery before 37 wk gestation) is the primary contributor to perinatal morbidity and mortality in the United States, affecting nearly 12% of births (Goldenberg et al. 2008). Preterm infants are at greater risk of adverse health outcomes even in childhood and later life (Saigal and Doyle 2008). The etiologies of specifically spontaneously occurring preterm birth are largely unknown, though several environmental contaminants have been suggested as risk factors (Wigle et al. 2008). The potential association between drinking water contaminants and preterm birth remains unclear—particularly at concentrations at or below regulatory limits (Ferguson et al. 2013; Bove et al. 2002).

Nitrate (NO^3) is among the most common groundwater contaminants globally, largely because of widespread use of synthetic fertilizers and manure application in agricultural regions (Spalding and Exner 1993). Prior studies suggest associations between gestational exposure to nitrate and adverse birth outcomes, including birth defects (Croen et al. 2001; Brender et al. 2013; Blaisdell et al. 2019) and intrauterine growth restriction (Migeot et al. 2013; Stayner et al. 2017; Coffman et al. 2021). Nitrate exposure can also cause hypoxia and cyanosis in infants (blue baby syndrome) because of oxidation of hemoglobin to methemoglobin. Fetal hemoglobin is particularly susceptible to oxidation, and elevated cord blood methemoglobin levels have been found in women exposed to nitrate during pregnancy (Tabacova et al. 1998; NRC 1981).

Oxidative stress in pregnancy is one of the posited mechanisms for spontaneous preterm birth (Tarquini et al. 2018; Aung et al. 2019). Despite its prooxidant properties, very few studies have investigated links between gestational nitrate exposure and preterm birth. Those that have been conducted have yielded inconclusive results and been limited by unmeasured confounding, possible misclassification bias, and small sample sizes (Ward et al. 2018; Manassaram et al. 2006).

We aimed to investigate the potential association between elevated nitrate in drinking water and spontaneous preterm birth through a within-mother analysis of over 1 million singleton live births in California from 2000–2011. By comparing outcomes between siblings with different gestational exposure to nitrate, we control for maternal structural genetic factors and many behavioral and socioeconomic sources of confounding that have arisen in prior studies.

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Methods

Study Population

We used data on all California singleton live births delivered at nonmilitary hospitals from 1 January 2000 through 31 December 2011, representing 6,267,905 births. This data set includes around 98% of births delivered in the state during the study period. Maternal residential addresses at the time of birth were extracted from birth certificates and geocoded using the California Environmental Health Tracking Program Geocoding Service, as described in Shaw et al. (2018a). Geocoding was successful in 95% of births to the street level and an additional 4% to the zip code level. From this population, we included singleton births with birth weights of 500 – 5,000 g and gestational ages 20–41 wk; births below these cutoffs have low rates of survival and potentially distinct etiologies (Salihu et al. 2013). We then linked birth certificate data with maternal and infant hospital discharge data from the Office of Statewide Health and Planning and Development (OSHPD) (>99% successful linkages). Maternal covariates included age, parity, education, race/ethnicity, highest educational attainment, payer type for delivery, and whether prenatal care was initiated by the fifth month of pregnancy. Unique maternal IDs were identified from maternal social security numbers in the linked OSHPD database.

We aimed to investigate the effect of nitrate on spontaneous preterm birth, rather than indicated birth or preterm birth mediated by comorbidities that may have different etiologies. Pregnancies resulting in spontaneous preterm birth were defined as those with preterm premature rupture of membranes, premature labor, or those in which tocolytic medications were administered, based on hospital discharge records or birth certificate codes. We excluded pregnancies resulting in medically indicated birth, defined as those with a code for medical induction or artificial rupture of membranes or for which there was cesarean delivery. We further excluded births accompanied by pregestational diabetes, gestational diabetes, gestational hypertension, chronic hypertension, and preeclampsia/eclampsia (except for births occurring at 20 to 23 wk of gestation). Spontaneous preterm birth was subclassified as 20–31 or 32–36 wk of gestation because of the suspected differences in etiology between early and near-term preterm births (Menon 2008).

The above criteria resulted in 4,698,830 eligible births in the study population. The study population was further refined to perform a within-mother analysis as described below and in Figure 1.

This study was approved by the Stanford University Institutional Review Board (IRB) and the California State Committee for the Protection of Human Subjects. The data employed in this study were made available by California OSHPD and Vital Records. The use of such data is possible to researchers who apply for their use and follow all procedures for their use as stipulated by IRB protocols, including stringent measures to ensure participant confidentiality and privacy. This use does not require further contact with human subjects.

Exposure Assessment

Our goal was to estimate the average concentration of nitrate in public tap water served to each woman during her pregnancy. The steps involved in this process included *a*) linking births to community water systems based on maternal residential addresses at time of birth; *b*) preparing community water system monitoring data; and *c*) averaging appropriate monitoring data to estimate tap water nitrate concentrations during pregnancy for each birth.

Linking births to community water systems. We first determined the water utility serving each woman's geocoded residence at time of birth using the Water Boundary Tool (Tracking

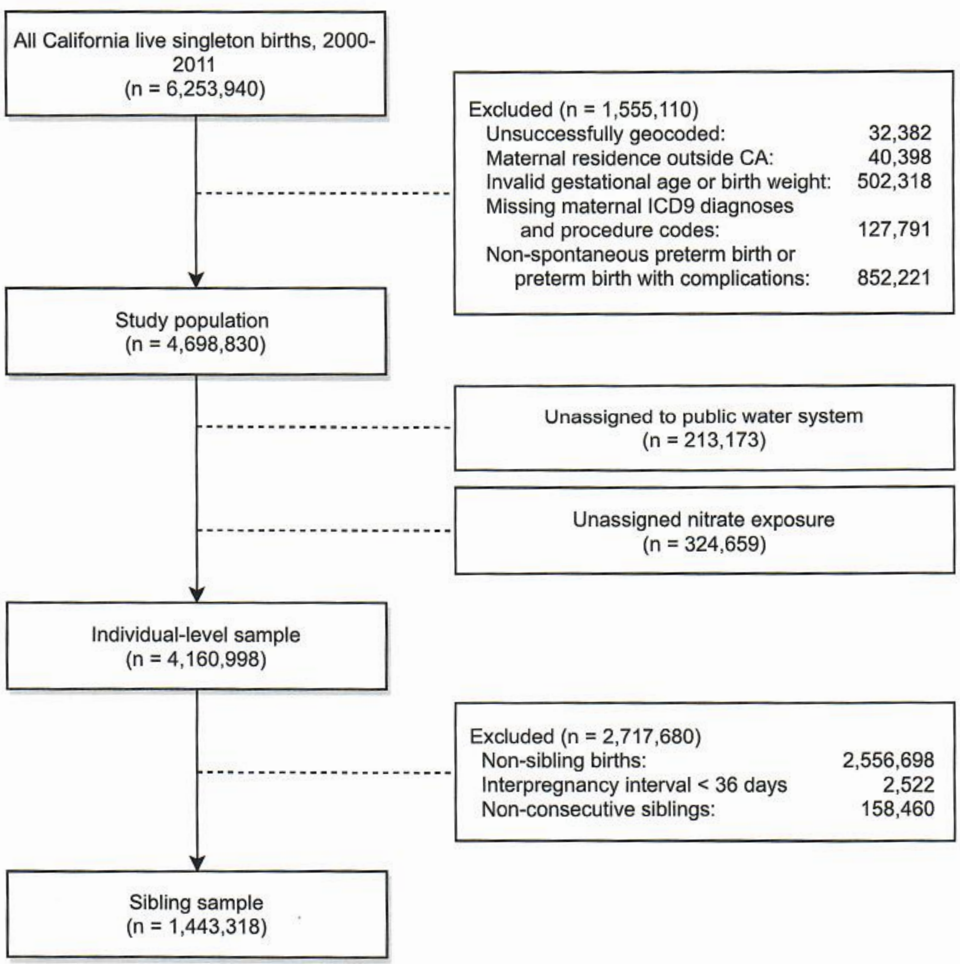

Figure 1. Inclusion and exclusion criteria for the study population, sample for conventional individual-level analysis, and sibling sample for within-mother analysis.

California Public Health Institute 2019), which maps the service areas of 2,644 community water systems serving around 90% of Californians (Figure S1). Community water systems are defined as public water utilities serving ≥25 residents year-round or with ≥15 connections. We excluded wholesaler systems (those that sell to other systems but do not directly serve consumers) based on federal fee codes (SWRCB 2018).

Out of the 4,698,830 births in the study population, 93% had maternal residences within a community water system boundary. An additional 3% of births were linked to community water systems within 0.5 km of the maternal residence to account for potential imprecision in geocoded residences and system boundary mapping. In some cases, the boundaries of large water systems encompass smaller systems that may purchase water for delivery to consumers. We therefore linked births that were located within more than one community water system to the smaller water system, which was assumed to be the direct supplier; if the overlapping water systems had the same area, the birth was linked to the water system with the larger population served. We excluded 213,173 births that remained unassigned, likely representing regions reliant on private wells or community water systems with service areas that are unmapped or have changed over time. Excluded births were more likely to have non-Hispanic white maternal race/ethnicity, pay for delivery with private insurance, and reside in the San Francisco Bay Area and San Joaquin Valley (Table S1).

Preparing community water system monitoring data. We acquired historical public monitoring records (1998–2012) from statewide community water systems from the State Water Resources Control Board (2019). Community water systems are required to monitor for nitrate quarterly or annually, depending on the type of water source and compliance history (Monitoring and Compliance–Nitrate and Nitrite. 22 CCR §64432.1). Water systems typically collect monitoring samples from sources in the supply system (i.e., groundwater wells, surface water intakes, and treatment plants) rather than from tap water served to consumers. We excluded samples from monitoring wells and agricultural supply sources, nondetect samples with reporting limit (RL) greater than half the maximum contaminant level for nitrate, and outliers (≥444 mg/L as nitrogen). Replicate samples from the same source on a given day were averaged. Approximately 10% of samples used in this study were below detection limits; because of the low number of nondetect samples, these results were interpolated at half the provided RL (Lubin et al. 2004). If no RL was provided, the result was interpolated at half the most common RL (0.4 mg/L as nitrogen).

Averaging appropriate monitoring data. For each birth, we identified nitrate monitoring data collected from the public water system linked to the maternal residence during the pregnancy exposure window. This window was defined as between the dates of conception and birth if monitoring data were available. If no data were available during this exposure window, we identified monitoring data collected from 15 months before conception to 12 months after the date of birth.

To estimate tap water nitrate concentrations, we used sampling data from the most proximal source(s) to the distribution system (point-of-entry sources). Our goal was to exclude data from any sources that were later treated or blended, using data only from sources that flowed directly into the distribution system, such as treatment plants or raw sources that are not treated for nitrate prior to distribution. This approach, based on methodologies of Balazs et al. (2011) and Balazs et al. (2019), relies on flow path data accessed upon request from the Division of Drinking Water on 11 August 2020. These data describe the raw sources that flow into associated receiving sources, such as treatment facilities. After identifying all samples collected during the exposure window for each birth, we excluded any source that flowed into another source with available data. Data on the relative flow contributions of sources were not available; we therefore averaged nitrate concentrations from each point-of-entry source to estimate tap water concentrations in the distribution system during the exposure window.

Exposure assessment was successful for 93% of births linked to community water systems; 324,659 births without assigned nitrate exposure were excluded. These births were more likely to have higher maternal age, Asian and non-Hispanic white maternal race/ethnicity, and private insurance relative to the study population and were more likely to reside in the San Francisco Bay Area or Inland Empire (Table S1).

Births were assigned the following exposure categories based on estimated nitrate concentrations: low (<5 mg/L), medium (≥5 mg/L and <10 mg/L), or high (≥10 mg/L). Nitrate concentrations throughout the article are given in units of nitrate nitrogen. The high exposure cutoff was selected to correspond to the U.S. federal maximum contaminant level (MCL) for nitrate in drinking water (10 mg/L as nitrogen, or 45 mg/L as nitrate). The medium exposure category was selected to correspond to half the MCL (5 mg/L as nitrogen).

Analytical Approach

We leveraged the occurrence of sibling births within our longitudinal data set to conduct a within-mother analysis. We aimed to evaluate preterm birth risk in women who experienced different nitrate exposure between pregnancies. By isolating within-mother effects, this design controls for factors that remain constant between pregnancies (including maternal genetic predisposition) or are largely time invariant (including socioeconomic status and many health behaviors).

The sample for the within-mother analysis was consecutive siblings with successful nitrate exposure assessment. To achieve this sample, we first excluded all nonsibling births from the analysis ($n = 2{,}556{,}698$). Siblings of any parity were included; however, we included only consecutive siblings to reduce the average temporal separation between sibling births and allow estimation of interpregnancy intervals (IPIs), defined as the period between birth and subsequent conception. In order to retain consecutive sibling sets, we excluded 157,460 births without consecutive parity (that is, with one or more unobserved births between siblings). Births following an IPI of less than 36 d were considered biologically implausible and excluded (Shachar et al. 2016). The above criteria resulted in a sample of 1,443,318 sibling births. Women whose nitrate exposure category changed between pregnancies were discordant in exposure; those who remain in the same category were concordant.

Our statistical approach relied on conditional logistic regression with the outcome of log odds of preterm birth relative to term birth; separate models were conducted for early preterm birth (20–31 wk of gestation) and near-term birth (32–36 wk). Each model excluded siblings with spontaneous preterm birth outside the gestational range of interest. A small number of sibling sets (<0.2%) contained both early and near-term preterm births and were therefore unmatched. Conditional logistic regression estimates a likelihood function conditional on mother ID, effectively stratifying the analysis by sibling sets. Thus, only exposure-discordant sibling sets contribute to effect estimates for nitrate exposure. The exposure of interest was the category (i.e., low, medium, or high) of nitrate in tap water during pregnancy, with low exposure as the reference group. Models were additionally adjusted for the following characteristics of each birth: maternal age as a five-level categorical variable (<20, 20–24, 25–29, 30–34, or ≥35 y), maternal parity as a three-level categorical variable (1, 2, or ≥ 3), and an indicator variable for IPI less than 1 y. This indicator variable was modeled as 0 for IPI greater than 1 y, first-parity births, and births with unobserved prior parity (i.e., consecutive sibling sets where the first observed parity was greater than 1). A very small number of births ($n = 7$) with missing data on maternal age were excluded. We did not adjust for maternal education level, which functions instead as a proxy for socioeconomic status and is not expected to change between pregnancies except as a function of maternal age. Analyses were conducted in R (version 3.5.3; R Development Core Team) using the Survival package.

We performed various secondary analyses to account for limitations of the within-mother design and analysis and to enable comparison between our findings and prior studies using conventional statistical techniques.

First, the within-mother analysis relies on exposure-discordant sibling sets, which reduces sample size and generalizability. Categorization of the exposure variable also can introduce nondifferential misclassification of exposure (Brenner and Loomis 1994). To address this limitation, we performed a secondary analysis in which nitrate was modeled as a continuous variable among the full sibling sample.

Second, maternal mobility between pregnancies could introduce bias into the within-mother analysis. For example, if a woman moves to an area with poor water quality between pregnancies, her change in nitrate exposure may be accompanied by other changes in quality of life. Women may also move in response to poor water quality. In both cases, mobility is tied to socioeconomic and demographic characteristics. In an ideal target trial, mothers would remain in the same water system for all births. We therefore performed a subanalysis among 1,032,961 sibling births to 478,761 women who were assigned to the same water system for multiple births, suggesting that women did not move or moved only a short distance between pregnancies.

We also performed a secondary individual-level case–control analysis to allow comparison between the within-mother approach and prior studies. This design estimates the effect of nitrate on preterm birth based on between-mother, rather than within-mother, variation. Thus, it also has the advantage of mitigating potential bias from factors that have higher within- than between-mother variance, such as maternal age and parity. The sample for this analysis was the individual-level population described in Figure 1. We used mixed-effects logistic regression to evaluate the log odds of preterm birth as a function of nitrate exposure category, fitting models separate models for early preterm birth (20–31 wk of gestation, excluding later preterm births) and near-term birth (32–36 wk, excluding earlier preterm births). These models incorporated a random intercept for mother ID and water system ID to account for the nonindependence of siblings and births assigned to the same water system. Model covariates included parity and maternal age (as defined above), health care payer for delivery, maternal race/ethnicity, maternal educational attainment (as categorical variables defined in Table 1), and prenatal care initiation by the fifth month of gestation (as a binary indicator variable). Births with missing covariate data were excluded from the analysis. Analytic code is available at https://github.com/arsherris/nitrate-and-preterm-birth.

Sensitivity Analyses

To explore the internal validity of the exposure assessment, we created a metric of the uncertainty in our estimate of gestational nitrate concentrations. The estimates with higher expected uncertainty were those with samples unavailable during the period of gestation or where the water system had <4 sources (indicating a data-sparse supply network) or ≥ 50 sources (indicating a very complex supply network). We then stratified births by level of exposure uncertainty and repeated the primary analysis within each stratum.

To evaluate the sensitivity of the findings to model specifications and assumptions, we compared the results of the unadjusted and adjusted conditional logistic regression models, as well as models additionally adjusted for birth year, month of conception, and county of maternal residence. We also generated bootstrapped standard errors for the primary adjusted model by sampling sibling sets with replacement and evaluating the 95th percentile of the distribution of the resulting estimates. This nonparametric approach does not assume normality of the input variables or symmetrical confidence intervals (CIs).

To investigate potential effect modification, we stratified the analysis on the following factors: maternal race, maternal educational attainment, parity, infant sex, region, and time period (2000–2006 or 2007–2011). Regions were defined as per the State Water Resource Control Board regional district management areas (Figure S1) in order to investigate potential effect modification because of changes in water system characteristics and management. Time periods were selected to explore effect modification because of a change in the methodology used to estimate gestational age: prior to 2007, gestational age was estimated based on the last menstrual period; during later years, the approach switched to best obstetric estimate. Parity was stratified into three categories: 1, 2, and ≥ 3. The stratified analysis on the subset of first-parity births also allows us to investigate the potential influence of time-dependent confounding, which could be introduced if the outcome of a birth impacts exposure in subsequent pregnancies—for example, if a preterm birth causes a woman to later avoid drinking tap water.

To investigate effect modification by maternal race/ethnicity and education, we stratified on the sibling sample and repeated the primary analysis within each stratum. Because the remaining covariates were not constant between siblings, we stratified on the individual-level population and applied mixed-effects logistic regression models as described above. To statistically evaluate the presence of effect modification, we conducted a likelihood ratio test comparing models with and without interaction terms for the variable of interest.

Finally, it is possible that certain comorbidities may mediate the relationship between nitrate exposure and spontaneous preterm birth. We therefore repeated the within-mother analysis after including an additional 276,053 consecutive sibling births with comorbid conditions in the sample (for a total of 1,719,371 siblings) in order to investigate this alternative causal pathway.

Results

The study population mothers were predominantly Hispanic or non-Hispanic white and with more than high school education (Table 1). Most women initiated prenatal care by the fifth month of gestation and paid for delivery with private insurance. Demographic trends were similar among births with assigned exposure (the individual-level sample). Births in the sibling sample were more likely to have non-Hispanic white maternal race/ethnicity, higher maternal education, and private insurance. The sibling sample also overrepresented second-parity births.

Of the 1,443,318 births in the sibling sample, 4.0% met the definition of spontaneous preterm birth; 0.6% were delivered at 20–31 wk of gestation and 3.4% at 32–36 wk of gestation (Table 2). Approximately 11% of the target study population was in the medium nitrate exposure category (5 to <10 mg/L as nitrate), and 0.6% was in the high exposure category (at or above the regulatory limit of 10 mg/L as nitrate). The percentage of exposed

Table 1. Characteristics of the study population, individual-level samples, and sibling samples for within-mother design.

Characteristic	Study population ($n = 4{,}698{,}830$)	Individual-level sample ($n = 4{,}160{,}998$)	Sibling sample		
			All siblings ($n = 1{,}443{,}318$)	Siblings in exposure-concordant sets ($n = 1{,}254{,}754$)	Siblings in exposure-discordant sets ($n = 188{,}564$)
Number of mothers	3,641,407	3,274,933	652,926	571,880	81,046
Maternal age (y)					
<20	9.7	10	8.7	8.5	9.6
20–24	23	24	24	24	27
25–29	27	27	28	28	30
30–34	24	24	25	25	23
≥35	16	15	14	14	11
Missing	0.0043	0.0043	0.0026	0.0024	0.0037
Maternal race/ethnicity					
Hispanic	50	52	42	41	48
Non-Hispanic white	29	27	35	36	31
Asian	12	12	13	13	12
Non-Hispanic black	5.6	5.9	6.8	6.9	5.9
Other	2.2	2.1	2.0	2.0	1.8
Missing	1.3	1.1	1.2	1.2	0.96
Maternal education					
Less than high school	27	28	18	17	19
High school	27	27	28	28	30
More than high school	44	43	52	52	49
Missing	2.6	2.4	2.1	2.1	1.8
Payer type for delivery					
Medi-Cal	46	48	37	37	41
Private	50	48	59	59	56
Other	2.0	1.8	1.1	1.1	1.1
Uninsured	2.0	2.1	2.5	2.5	2.3
Missing	0.18	0.18	0.17	0.15	0.25
Prenatal care initiated by fifth month of gestation					
Yes	94	94	95	95	95
No	4.6	4.6	3.9	3.8	4.3
Missing	1.2	1.2	1.1	1.1	1.1
Parity					
1	39	39	31	31	29
2	32	32	40	40	38
3+	28	29	29	29	33
Interpregnancy interval					
<1 y	N/A	N/A	12	12	13
≥1 y	N/A	N/A	43	43	45
Unknown or first parity	N/A	N/A	45	45	42
Female infant sex	51	51	51	51	51
Birth year					
2000	7.8	7.7	5.9	5.8	6.3
2001	7.7	7.6	6.1	6.1	6.5
2002	7.7	7.6	7.2	7.1	7.7
2003	7.9	7.8	8.2	8.2	8.3
2004	7.9	7.8	8.8	8.7	9.0
2005	8.2	8.2	9.5	9.5	9.1
2006	8.5	8.5	9.9	9.9	9.8
2007	9.4	9.5	11	11	10
2008	9.3	9.3	10	10	9.9
2009	8.8	8.9	9.0	9.0	8.8
2010	8.5	8.6	7.6	7.6	7.4
2011	8.3	8.4	7.2	7.3	6.9
Month of conception					
January	9.0	9.0	8.9	9.0	8.6
February	7.6	7.6	7.5	7.5	7.1
March	8.6	8.6	8.4	8.4	8.4
April	7.9	7.9	7.7	7.7	7.9
May	8.3	8.3	8.2	8.2	8.2
June	8.0	7.9	8.0	8.0	7.9
July	8.2	8.2	8.3	8.4	8.3
August	8.2	8.1	8.3	8.3	8.6
September	8.1	8.1	8.3	8.2	8.6
October	8.6	8.6	8.7	8.7	8.9
November	8.6	8.6	8.6	8.6	8.7
December	9.1	9.1	9.1	9.1	8.7
Region					
Northern California	7.8	7.7	9.4	10	2.8
San Francisco Bay Area	20	18	17	18	13
San Joaquin Valley	12	12	14	14	17
South Coast	32	34	31	31	31
Inland Empire	28	27	28	26	36

Note: Values are given as percent unless otherwise noted. Percentages may not equal 100 because of rounding.

Table 2. Nitrate exposure and gestational age categories in the analytic samples.

Gestational age of spontaneous preterm birth	Nitrate exposure category: Low (<5 mg/L)	Medium (5 to <10 mg/L)	High (≥10 mg/L)	Total
Individual-level sample ($n = 4{,}160{,}998$)				
20–31 wk	27,008	3,886	196	31,090
32–36 wk	129,222	16,013	824	146,059
37–40 wk	3,540,140	420,674	23,035	3,983,849
Sibling sample ($n = 1{,}443{,}318$)				
20–31 wk	7,789	1,199	64	9,052
32–36 wk	43,303	5,515	272	49,090
37–40 wk	1,226,196	150,628	8,352	1,385,176
Concordant sibling sets ($n = 1{,}254{,}754$)				
20–31 wk	7,191	484	14	7,689
32–36 wk	40,001	2,422	76	42,499
37–40 wk	1,134,150	68,020	2,396	1,204,566
Discordant sibling sets ($n = 188{,}564$)				
20–31 wk	598	715	50	1,363
32–36 wk	3,302	3,093	196	6,591
37–40 wk	92,046	82,608	5,956	180,610

Note: Sibling sample includes consecutive sibling births identified between 2000–2011. Concordant sibling sets are those for which all siblings were assigned to the same exposure category. Discordant sibling sets are those for which the exposure category differed for at least one sibling.

births varied by region; the San Joaquin Valley and Inland Empire had the highest prevalence of births in the medium and high categories, while Northern California and the San Francisco Bay Area had the lowest prevalence (Table S2). Exposed births had a higher percentage of Hispanic maternal ethnicity and lower percentage of non-Hispanic black and Asian maternal race relative to births with low exposure (Table S3). Fifty-three percent of births with high nitrate exposure were to Hispanic women, relative to only 41% of births with low exposure.

Within the sibling sample, 13% of sibling sets were discordant in nitrate exposure—that is, the mother's exposure changed during at least one pregnancy. As suggested by the demographic trends in exposure, Hispanic mothers were overrepresented among discordant sibling sets (Table 1).

Overall, 30% of observed IPIs involved a change in community water system (suggesting the mother moved between pregnancies). The rate of mobility was higher (55%) for intervals between consecutive siblings discordant in exposure. This suggests that just over half of maternal changes in nitrate exposure between pregnancies are accompanied by residential movement, while the remainder are because of changes in water quality within a given water system. There were 1,032,961 births in the subsample of births for which all siblings were assigned to the same water system. Of these, 88,618 belonged to sibling sets that were discordant in exposure.

We leveraged the presence of exposure-discordant sibling sets to evaluate the within-mother associations between nitrate exposure and preterm birth. In the full sibling sample, we estimated an elevated adjusted odds of early preterm birth (20–31 wk of gestation) associated with tap water nitrate concentrations during pregnancy (Table 3, "Within-mother analysis: all siblings"). The odds ratio (OR) for high exposure was 2.52 (95% CI: 1.49, 4.26) relative to low exposure, and for medium exposure, it was 1.47 (95% CI: 1.29, 1.67) relative to low exposure. We also

Table 3. Adjusted odds ratios (ORs) and 95% Wald confidence intervals (CIs) of preterm birth associated with gestational exposure to nitrate in tap water.

Exposure category	Term (n)	20–31 wk: Preterm (n)	20–31 wk: Adjusted OR (95% CI)	32–36 wk: Preterm (n)	32–36 wk: Adjusted OR (95% CI)
Within-mother analysis: all siblings					
Categorical					
Low (<5.0 mg/L)	92,042	598	Ref	3,302	Ref
Medium (5 to <10 mg/L)	82,607	714	1.47 (1.29, 1.67)	3,093	1.08 (1.02, 1.15)
High (≥10 mg/L)	5,955	50	2.52 (1.49, 4.26)	196	1.05 (0.85, 1.31)
Continuous					
Per mg/L	1,385,142	9,050	1.015 (1.008, 1.021)	49,089	1.003 (1.001, 1.006)
Within-mother analysis: siblings without maternal movement					
Categorical					
Low (<5.0 mg/L)	41,512	268	Ref	1,485	Ref
Medium (5 to <10 mg/L)	40,615	387	1.64 (1.37, 1.97)	1,563	1.15 (1.06, 1.25)
High (≥10 mg/L)	2,677	20	2.04 (0.83, 5.01)	87	1.10 (0.79, 1.54)
Continuous					
Per mg/L	991,832	6,337	1.031 (1.021, 1.042)	34,765	1.007 (1.003, 1.011)
Individual-level analysis					
Categorical					
Low (<5.0 mg/L)	3,404,894	25,359	Ref	123,240	Ref
Medium (5 to <10 mg/L)	404,732	3,669	1.49 (1.42, 1.56)	15,277	1.12 (1.09, 1.14)
High (≥10 mg/L)	22,464	179	1.34 (1.12, 1.60)	787	1.07 (0.97, 1.17)
Continuous					
Per mg/L	3,832,090	29,207	1.011 (1.009, 1.013)	139,304	1.003 (1.002, 1.004)

Note: Within-mother analyses were performed among matched consecutive siblings using conditional logistic regression analysis adjusted for maternal age, parity, and interpregnancy interval (IPI) less than 1 y (within mother-specific strata). The individual-level case control analysis used mixed-effects logistic regression adjusted for maternal age, parity, education, race, payer for delivery, and prenatal care initiation, with random intercepts for water system IDs. Ref, reference group for estimates.

estimated modestly increased odds of near-term preterm birth (32–36 wk of gestation) with medium nitrate exposure (OR = 1.08; 95% CI: 1.02, 1.15) relative to low exposure. Although the association between high nitrate exposure and near-term preterm birth was also moderately elevated relative to low exposure, the OR did not reach statistical significance (OR = 1.05; 95% CI: 0.85, 1.31). The findings were largely unchanged in unadjusted models or models additionally adjusted for birth year, month of conception, and region of residence, and CIs generated through bootstrapping were slightly wider than Wald estimates, but the statistical significance and interpretation of the results remain unchanged (Table S4).

Secondary analyses using continuous measures of exposure suggested a modest but significant association between a linear increase in nitrate concentration and preterm birth at 20–31 wk, with an approximately 1.5% increase in odds with each mg/L increase in nitrate (OR = 1.015; 95% CI: 1.008, 1.021). There was a weaker linear association between nitrate and preterm birth at 32–36 wk (OR = 1.003; 95% CI: 1.001, 1.006).

Results were similar within the subsample of women who stayed in the same community water system for all pregnancies, with 95% CIs overlapping estimates from the full sibling sample (Table 3, "Within-mother analysis: siblings without maternal movement"). However, estimated associations between nitrate exposure and preterm birth were generally elevated relative to the results for the full sibling sample, with the exception of the association between high exposure and early preterm birth, which was attenuated. The linear association between nitrate and spontaneous preterm birth in both gestational age categories was approximately doubled among siblings in the absence of maternal movement.

Estimated odds of preterm birth were generally similar in the conventional individual-level analysis relative to within-mother analysis, with overlapping 95% CIs (Table 3, "Individual-level analysis"). However, there was a smaller estimated association between high nitrate exposure and early preterm birth in the individual-level analysis (OR = 1.37; 95% CI: 1.14, 1.65) relative to the within-mother analysis (OR = 2.52; 95% CI: 1.49, 4.26).

We did not find evidence of effect modification by maternal race/ethnicity in the sibling sample for preterm birth at 20–31 wk (Table S5). Although the p-value for the interaction between exposure and maternal race/ethnicity was statistically significant ($p = 0.01$) for preterm birth at 32–36 wk, the low power of stratified analyses makes it difficult to draw meaningful inferences related to the direction of effect modification.

Stratification by confidence in the exposure assessment yielded similar results relative to the individual-level analysis, though estimates of the association between medium nitrate exposure and early preterm birth were stronger among the births with higher uncertainty in exposure assessment (OR = 1.80; 95% CI: 1.65, 1.98) than for births with less uncertainty (OR = 1.54; 95% CI: 1.44, 1.65). (Table S6). The stratified results and interaction p-values do not suggest effect modification by infant sex, time period, or parity (Table S6). Although the estimates for first-parity births (which are not affected by time-dependent confounding) are uniformly lower than the estimates for the full individual-level sample, the differences were small, and the 95% CIs overlap.

Effect estimates differed by region with evidence of potential effect modification (Table S6). In general, associations between nitrate exposure and spontaneous preterm birth were elevated for births in the San Joaquin Valley and Inland Empire and attenuated in other regions, particularly the San Francisco Bay Area and South Coast.

ORs remained elevated when siblings with maternal comorbidities were included in the within-mother analysis (Table S7). The association between high nitrate exposure and early preterm birth was moderately attenuated (OR = 2.03; 95% CI: 1.30, 3.16), and the association between high nitrate exposure and near-term preterm birth was elevated but remained imprecise (OR = 1.16; 95% CI: 0.97, 1.40).

Discussion

Our study identified an association between elevated nitrate concentrations in tap water and increased odds of spontaneous preterm birth. The strongest association was between nitrate concentrations above 10 mg/L (present in 0.6% of the study population) and preterm birth at 20–31 wk of gestation relative to concentrations below 5 mg/L. Preterm birth at 20–31 wk was also associated with nitrate concentrations between 5 and 10 mg/L (present in 11% of the study population) relative to concentrations below 5 mg/L. Associations were weaker between nitrate in tap water and near-term preterm birth (32–36 wk of gestation), and they only reached statistical significance for nitrate exposure between 5 and 10 mg/L, relative to concentrations less than 5 mg/L. These associations were observed in within-mother and conventional individual-level analyses, were robust to model assumptions, and were not substantially modified by most observed covariates.

Nitrate is among the most common groundwater contaminants worldwide. The use of synthetic fertilizer, while contributing greatly to global food supply, has been the primary driver of increased nitrogen concentrations in the environment (Vitousek et al. 1997). In California, nitrogen inputs to groundwater have increased dramatically in the past century because of fertilizer application, concentrated animal feeding operations, wastewater effluent, and urban runoff (Rosenstock et al. 2014). Nitrate is also a naturally occurring nutrient in soils and is present in U.S. aquifers at a background level of around 1 mg/L (Dubrovsky et al. 2010). The current U.S. federal maximum contaminant level for nitrate in drinking water is 10 mg/L (as nitrogen); this limit was established to protect against methemoglobinemia in infants, or blue baby syndrome, the most widely recognized health consequence of nitrate exposure (Walton 1951).

Like infants, the developing fetus may be at risk of methemoglobin production because of nitrate exposure. Fetal hemoglobin is more susceptible to oxidation by nitrite (generated from nitrate ingestion) than adult hemoglobin, and levels of methemoglobin-reducing enzymes are low in the fetal and infant red blood cells (NRC 1981). Animal studies suggest that nitrate/nitrite can traverse the placenta, though placental transfer of nitrate in humans is not well understood (Fan et al. 1987). Tabacova et al. (1998) identified elevated cord blood methemoglobin levels and a higher prevalence of preterm birth among a small sample of women exposed to nitrate during pregnancy. Apoptosis (cell death) induced by hypoxia and oxidative stress has been found to confer risk of spontaneous preterm birth (Tarquini et al. 2018). Thus, the prooxidant properties of nitrate are a potential mechanistic explanation for the observed association between nitrate and spontaneous preterm birth. Other hypotheses related to the reproductive health effects of nitrate involve N-nitroso compound formation and thyroid and endocrine disruption (Ward et al. 2018; Poulsen et al. 2018).

Although exposures to higher levels of nitrate have been linked to some adverse birth outcomes (Ward et al. 2018; Manassaram et al. 2006), the epidemiologic literature on population-based exposure to low levels of nitrate in drinking water and preterm birth is sparse. Definitions of exposure and preterm birth differed among the few available studies, making it difficult to contrast results with those of the current study. Bukowski et al. (2001) found a significant dose–response relationship between median nitrate concentrations in groundwater and preterm birth in a case–control study of 336 preterm births in Prince Edward Island; births in regions with median groundwater nitrate concentrations of 3.1, 4.3, and 5.5 mg/L had increased odds of preterm birth relative to births in regions with median nitrate of <1.3 mg/L. Stayner et al. (2017) identified a linear association between county-wide nitrate in tap water and early preterm birth (<32 wk gestation) in four U.S. states, but only in counties with low domestic well use. The authors did not identify an association between overall preterm birth (<37 wk) and county-wide nitrate. A retrospective cohort study of over 13,000 women in France did not identify an association between nitrate concentrations of 3.6–6.0 mg/L or >6 mg/L in community water systems and preterm birth relative to concentrations below 3.6 mg/L (Albouy-Llaty et al. 2016).

Our study builds on this limited prior literature to add additional evidence for an association between nitrate in drinking water and spontaneous preterm birth. In both within-mother and individual-level analyses, we observed elevated and significant associations between nitrate exposure and spontaneous preterm birth. Like Stayner et al. (2017), we identified stronger associations between nitrate exposure and early preterm birth (20–31 wk gestation) relative to later gestational age classifications. In within-mother analyses, associations with early preterm birth were stronger for high exposure relative to medium; in the individual-level analysis, we identified stronger associations for medium exposure and both categories of preterm birth. Statistically significant linear associations were also identified for both outcomes. Additional research on this topic would clarify the shape of the exposure–response relationship between nitrate and preterm birth at different gestational ages.

The estimated associations between nitrate and preterm birth were not substantially modified by maternal or infant characteristics. However, sensitivity analyses suggest potential effect modification by region (defined by State Water Resources Control Board district management areas). Although statistical precision is limited in stratified analyses, effect estimates were generally highest in the San Joaquin Valley and Inland Empire. Notably, the San Joaquin Valley and Inland Empire are the regions with the highest prevalence of nitrate exposure, and the San Joaquin Valley is characterized by high reliance on groundwater for drinking water. Effect estimates were attenuated in the San Francisco Bay Area and South Coast—regions characterized by urban populations, reliance on surface water delivered by aqueducts, and large community water systems with complex distribution networks. The attenuated ORs in these regions may reflect exposure misclassification biasing estimates toward the null.

This study has several notable strengths. We used a linked data set of birth certificate and hospital discharge records for over 6 million births, representing 98% of births in California during the study period, to identify a sample of over 1 million consecutive sibling births. Our sibling-matched design offers improved causal inference compared with ecological and case–control designs. The within-mother approach has the advantage of largely controlling for maternal structural genetic factors, as well as behavioral, socioeconomic, and psychosocial factors that are assumed to be more stable across pregnancies than between subjects. Similar designs have previously been applied to family cohort studies and epidemiologic analysis (Shachar et al. 2016; Lindstrom et al. 2011; Laugesen et al. 2013). However, to our knowledge, only one prior study has applied a sibling design to evaluate the reproductive health impacts of drinking water contamination (Currie et al. 2013). Somewhat surprisingly, the individual-level analysis among all births and first-parity births yielded similar results to the within-mother approach, though with smaller estimates of the association between high nitrate exposure and early preterm birth.

This study is also among the first population-based assessments of tap water nitrate concentrations among pregnant women. Balazs et al. (2011) used a similar methodology to estimate nitrate in tap water throughout California's San Joaquin Valley from 1999 to 2001. The authors found that 0.2% of residents were potentially exposed to nitrate in tap water above the regulatory limit (10 mg/L) and that Hispanic residents were more likely to have elevated tap water concentrations. We found that exposure to nitrate above the regulatory limit was higher among pregnant women from 1999–2011, with 0.6% exposed in the study population and 1.1% exposed in the San Joaquin Valley, though our definition of the San Joaquin Valley excludes Stanislaus and San Joaquin counties. However, we observed similar social disparities in exposure: there was a higher percentage of Hispanic mothers among those with high nitrate exposure during pregnancy relative to low exposure.

This study also has limitations. The vital statistics data do not include information on maternal smoking and body mass index, which are known risk factors for preterm birth. However, these covariates are unlikely to be associated with changes in nitrate exposure between sibling births, making them unlikely confounders in the sibling analysis. Though the timing of exposure to nitrate during gestation likely has biological importance, we were not able to evaluate exposure during different periods of pregnancy owing to infrequent nitrate monitoring in many water systems. We were also unable to investigate the potential influence of live birth bias (bias introduced by conditioning on live births) because of lack of data on spontaneous abortion and stillbirth in the study population. This source of bias is expected to lead to inverse or attenuated ORs for high levels of exposure (Raz et al. 2018). Bias may also be introduced because of the overrepresentation of longer gestational ages at the beginning of the study and shorter gestational ages at the end of the study. However, we expect the long study period to mitigate this bias: only 3% of births were conceived more than 20 wk before the start of the study period or less than 41 wk before the end of the study period.

Our analysis does not consider other environmental coexposures, including other contaminants in drinking water. Contaminants that have been found to co-occur with nitrate in public water sources include pesticides, volatile organic compounds (VOCs), and perchlorate (Squillace et al. 2002; Kimbrough and Parekh 2007). Few studies have explored the links between exposure to these contaminants and risk of preterm birth, with mixed results (Larsen et al. 2017; Shaw et al. 2018b; Ferguson et al. 2013; Rubin et al. 2017); even fewer have focused on exposures in combination or through drinking water (Stayner et al. 2017). Nevertheless, it is possible that exposure to co-occurring contaminants or their interactions could contribute to the observed elevated odds of preterm birth.

The greatest threat to inference in this study is potential misclassification in exposure assessment. We linked births to public water systems based on the maternal address of residence at time of birth, but we were not able to identify women who may have moved residences earlier in pregnancy at potentially relevant gestational time points. In addition, we assume that water system service areas were accurately mapped and static over time, likely introducing error in exposure assignment. Although some private well users may have been inaccurately assigned to water systems, most were excluded from the analysis. Private wells are often shallower than public supply wells and therefore more vulnerable to contamination by nitrate and other contaminants (Burow et al. 2010). Thus, the percentage of the population with high exposure is likely underestimated in the study.

Our estimates of tap water nitrate concentrations stem from monitoring records from public water systems, rather than from direct measurements. Although imperfect, water monitoring records have been widely used for exposure assessment (Brender et al. 2013; Nuckols et al. 2011; Villanueva et al. 2014). In a validation study in Washington State, Searles Nielsen et al. (2010) found correlation between water quality monitoring records and tap water nitrate levels (R^2 of 0.3–0.5). The authors suggest that studies reliant on public records incur largely nondifferential measurement error. Schullehner et al. (2017) found a high correlation ($R^2 = 0.98$) and nearly equivalent nitrate concentrations in samples collected from water supply systems and consumer taps in a nationwide study in Denmark from 2007 to 2016.

Nonetheless, water monitoring records in California have limitations that could introduce error or bias in our estimates. Underreporting may lead to missing data for some water sources. The historical statuses of sources were not available, so sources that were inactive or treated may be improperly included in the exposure assessment. Historical flow paths were also unavailable, so we relied on current water system flow paths to identify point-of-entry sources. We lack data on the flow contribution from each water source and therefore assume that all point-of-entry sources contribute equally to the distribution system. This assumption risks overestimating nitrate concentrations in water systems that down-regulated contaminated sources or where some sources (for example, purchased water) account for a large proportion of supply.

If high exposure is misclassified as medium, we risk positive bias in the ORs for medium exposure (Correa-Villaseñor et al. 1995). However, misclassification of medium or high exposure to the low category (or vice versa) would bias estimates for medium and high ORs toward the null. The analyses using categorical exposure metrics also assume no effect in the low exposure category. Indeed, some prior studies suggest that nitrate concentrations below 5 mg/L may be associated with preterm birth. To address these issues and avoid bias introduced by exposure categorization, we performed secondary analyses using a continuous exposure metric. These analyses also identified elevated ORs for early and near-term preterm birth associated with nitrate exposure.

Our exposure metric was nitrate concentrations in tap water. Although water treatment and consumption clearly affect exposure to nitrate in the study population, we were not able to measure these behaviors. We do not expect household treatment to be an important source of misclassification, as only sophisticated point-of-entry and point-of-use treatment systems using ion-exchange and reverse osmosis technologies remove nitrate from tap water (Lykins et al. 2018). However, water consumption among pregnant women likely varied widely, including by demographic and socioeconomic factors (such as race and education) that are independently associated with preterm birth (Drewnowski et al. 2013). The sibling design alleviates some of the confounding variability introduced by this data gap, as we assume that consumption will have greater between- than within-mother variation.

Even so, there may be a negative correlation between nitrate concentrations and water consumption. Water systems that receive official nitrate violations are required to notify customers, who may then avoid drinking tap water (Zivin et al. 2011). In this case, we would expect the estimated health risks of nitrate exposure above regulatory limits to be biased toward the null. However, this is an infrequent occurrence: only around 0.5% of births were assigned to a public water system with an active nitrate violation during the period of gestation.

The within-mother study design has potential drawbacks. The design reduces bias from confounding factors that are constant between pregnancies but can amplify bias by potential confounders that vary between pregnancies (Frisell et al. 2012). The sample is restricted to consecutive sibling births, and model estimates using categorical exposure metrics are drawn only from sibling sets with discordant exposure, resulting in sample size limitations for certain analyses, including stratifications and estimates for the rarest exposure and outcome categories. The selection of consecutive siblings also is potentially introducing selection bias that could reduce the external validity of the results. However, we intentionally target the high degree of internal validity achieved through the within-mother design. We believe this is appropriate given the conflicting findings from prior studies and lack of established connection between nitrate and preterm birth, despite biological plausibility. To complement the results of the within-mother analysis, we also present the findings of an individual-level analysis among over 4 million births. This design mitigates bias from maternal age and parity and prioritizes generalizability. Although some selection bias may be introduced by excluding births outside the boundaries of community water systems and without successful exposure assessment, this sample represents 88% of the study population and a majority of births in California during the study period. One of the more unexpected findings of these analyses was the robust association between medium nitrate concentrations (5 to <10 mg/L, relative to <5 mg/L) and elevated risk of early preterm and near-term birth. In contrast, high nitrate concentrations were associated only with early preterm birth (gestational length, 20–31 wk). This may be due in part to the scarcity of high nitrate exposures (present in 0.6% of the study population) and spontaneous preterm birth, leading to limited statistical power and larger CIs.

This research has potential implications for nitrate management and drinking water policy. The medium nitrate exposure category represents concentrations below the federal regulatory limit of 10 mg/L (as nitrogen) in drinking water. Given the potential for exposure misclassification discussed above, the exposure categories assigned will not always represent true nitrate concentrations in tap water, and cutoffs should be interpreted with care. Nonetheless, the findings suggest that future research should continue to probe associations between low-level nitrate exposure and reproductive health outcomes, including preterm birth.

In conclusion, we observed a robust association between nitrate concentrations in tap water and risk of spontaneous preterm birth at 20–31 wk in within-mother analyses. The study also identified modestly increased odds of spontaneous preterm birth at all gestational lengths with nitrate concentrations of 5 to <10 mg/L (relative to <5 mg/L)—below the federal regulatory limit. If future studies confirm the association between nitrate exposure below 10 mg/L and preterm births, the nitrate standard for drinking water may need to be reevaluated.

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