## Title

Investigating the association between Nitrate in drinking water and the incidence of respiratory diseases in California, USA



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# University of California Merced

## Investigating the association between Nitrate in drinking water and the incidence of respiratory diseases in California, USA

A dissertation submitted in partial satisfaction of the requirements

for the degree Doctor of Philosophy

in

**Public Health**

by

Hamed Gharibi

**Committee in charge:**

**Prof. Ricardo Cisneros, chair**

**Prof. Mariaelena Gonzalez, and**

**Prof. Paul Brown**

**2022**

The Dissertation of Hamed Gharibi is approved, and it is acceptable

in quality and form for publication on microfilm and electronically:

**Mariaelena Gonzalez** **Date**

**Paul Brown** **Date**

**Ricardo Cisneros** **Date**

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2022

**List of Abbreviations**

| | |
|---|---|
| AT | An active source which is sampled after any treatment |
| COPD | Chronic Obstructive Pulmonary Disease |
| CSWCB | California States Water Control Boards |
| CT | Combined sources which are treated |
| DT | Sample point within the distribution system after treatment |
| ED | Emergency Department |
| EDT | Electronic Data Transfer |
| EPA | Environmental Protection Agency |
| GI | Gastrointestinal |
| GLM | Generalized linear model |
| ICD-9 | International Classification of Diseases, $9^{th}$ Revision |
| MCL | maximum contaminant level |
| OSHPD | California's Office of Statewide Health Planning and Development |
| PT | Purchased source water which is sampled after any treatment |
| PWSs | Public water systems |
| RR | Risk Ratio |
| WHO | World health Organization |

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**Acknowledgement**

I would like to thank those who helped me during my PhD program, especially my advisor Prof. Ricardo Cisneros, Prof. Mariaelena Gonzalez and Prof. Paul Brown.

**Curriculum Vita**

# Hamed Gharibi

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## CURRENT POSITION

**2021-Present: Epidemiologist II, Fresno County Department of Public health (FCDPH)**

## EDUCATION

**2016-2022 Ph.D. Public Health, University of California, Merced**

Dissertation: The association between Nitrate in drinking water and respiratory diseases in California

Committee: Ricardo Cisneros (Chair), Mariaelena Gonzalez, Paul Brown

**2016-2019 MSPH Public Health, University of California, Merced**

Dissertation: The association between Nitrate/Nitrite in drinking water and respiratory diseases in California

Committee: Ricardo Cisneros (Chair), Mariaelena Gonzalez, Paul Brown

**2009-2012 MS & MEng Environmental Health Engineering, Tehran University of Medical Sciences**

Dissertation: Developing a novel fuzzy logic based environmental index

**2007-2009 BS Environmental Health Engineering, Hamedan University of Medical Sciences**

**2005-2007 AS Environmental Health, Busher University of Medical Sciences**

## CERTIFICATES

**2021 University of California, Davis** SQL for Data Science

**2021 Imperial College London** Measuring disease in Epidemiology

**2021 Imperial College London** Study Designs in Epidemiology

**2021 Johns Hopkins University** Foundations of Health Equity Research

## SKILLS

Proficient in STATA, MATLAB, R, ArcGIS, SAS, SQL, Microsoft Excel, Microsoft word, Tableau

Expert in designing environmental epidemiological studies

Expert in writing grant for NIH and NSF

## RESEARCH AREAS

Environmental Epidemiology

Social Epidemiology

Environmental Justice

Environmental Health Inequity

Health Disparities

Health Equity

Air Quality

Water Quality

Respiratory Diseases (Asthma, COPD, Bronchitis, Pneumonia, URI)

Cancer and its relation to environmental pollutants

Multi-Pollutant Analytical Tools

## RESEARCH EXPERIENCE

I am highly experienced in working on CAL-OSHPD, SEER (Cancer), US Census, and environmental datasets. I have designed epidemiological studies (e. g. time stratified case crossover, bidirectional case crossover, and time series), and applied statistical techniques (e. g. conditional logistic regression, logistic regression, generalized linear model, oblique decision tree, and correlation analysis).

**PUBLICATIONS (Total citations: 1043; h-index:15 )**

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***Peer Reviewed Publications***

1. R Cisneros, D Schweizer, **H Gharibi**, P Tavallali, D Veloz, K Navarro. 2021. Air Quality Impacts during the 2015 Rough Fire in Areas Surrounding the Sierra Nevada, California. Fire 4 (3), 31.
2. R Cisneros, **H Gharibi**, MR Entwistle, P Tavallali, M Singhal, D Schweizer. 2021. Nitrogen dioxide and asthma emergency department visits in California, USA during cold season (November to February) of 2005 to 2015: A time-stratified case-crossover analysis. Science of The Total Environment 754, 142089.
3. **H Gharibi**, M Entwistle, D Schweizer, P Tavallali, R Cisneros. 2020. The association between 1, 3-dichloropropene and asthma emergency department visits in California, USA from 2005 to 2011: a bidirectional-symmetric case crossover study. Journal of Asthma 57 (6), 601-609.
4. P Tavallali, **H Gharibi**, M Singhal, D Schweizer, R Cisneros. 2020. A multi-pollutant model: a method suitable for studying complex relationships in environmental epidemiology. Air Quality, Atmosphere & Health, 1-13.
5. D Veloz, M Gonzalez, P Brown, **H Gharibi**, R Cisneros. 2020. Perceptions about air quality of individuals who work outdoors in the San Joaquin Valley, California. Atmospheric Pollution Research
6. **H Gharibi**, M Entwistle, S Ha, M Gonzalez, P Brown et al. 2019. Ozone Pollution and Asthma Emergency Department Visits in the Central Valley,

California, USA, During June to September of 2015: A Time-Stratified Case-Crossover Analysis. Journal of Asthma.

7. **H Gharibi**, MR Entwistle, D Schweizer, P Tavallali, C Thao, R Cisneros. 2019. Methyl-Bromide and asthma emergency department visits in California, USA from 2005 to 2011. Journal of Asthma, 1-10
8. MR Entwistle, **H Gharibi**, P Tavallali, R Cisneros, D Schweizer, P Brown. 2019. Ozone pollution and asthma emergency department visits in Fresno, CA, USA, during the warm season (June–September) of the years 2005 to 2015: a time-stratified case-crossover analysis. Air Quality, Atmosphere & Health 12 (6), 661-672
9. Mostafapour, FK., Jaafari, J., **Gharibi, H**., Sepand, MR., et al. 2018. Characterizing of fine particulate matter (PM1) on the platforms and outdoor areas of underground and surface subway stations. Human and Ecological Risk Assessment: An International Journal, volume 24, pages 1016-1029
10. Delvarianzadeh, M., Khosravi, F., **Gharibi, H**., Taghavi, N. 2017. "Factors Affecting Malnutrition and Failure to Thrive in Children Under 2 Years of Age in Shahroud, Iran, in 2015". Health Scope, Volume 6.
11. MRF Heravia, **H Gharibi**, J Jaafaric, A Mesdaghiniad, AH Mahvid. 2017. "Performance evaluation of sulfate reducing bacteria in removing lead, chromium and nickel by anaerobic packed bed reactor". DESALINATION AND WATER TREATMENT 59, 154-159
12. A Azari, **H Gharibi**, B Kakavandi, G Ghanizadeh, A Javid, AH Mahvi. 2017. "Magnetic adsorption separation process: an alternative method of mercury extracting from aqueous

solution using modified chitosan coated Fe3O4 nanocomposites". Journal of Chemical Technology and Biotechnology 92 (1), 188-200

13. E Ahmadi, B Kakavandi, A Azari, H Izanloo, **H Gharibi**, AH Mahvi, A Javid. 2016. "The performance of mesoporous magnetite zeolite nanocomposite in removing dimethyl phthalate from aquatic environments". Desalination and Water Treatment 57 (57), 27768-27782
14. M Hoseini, M Yunesian, R Nabizadeh, K Yaghmaeian, S Parmy, **H Gharibi**. 2016. "Biomonitoring of tobacco smoke exposure and self-reported smoking status among general population of Tehran, Iran". Environmental Science and Pollution Research 23 (24), 25065-25073
15. A Javid, A Mesdaghinia, S Nasseri, AH Mahvi, M Alimohammadi, **H Gharibi**. 2016."Assessment of tetracycline contamination in surface and groundwater resources proximal to animal farming houses in Tehran, Iran". Journal of Environmental Health Science and Engineering 14 (1), 4
16. AJ Jafari, B Kakavandi, RR Kalantary, **H Gharibi**, A Asadi, A Azari. 2016. "Application of mesoporous magnetic carbon composite for reactive dyes removal: Process optimization using response surface methodology". Korean Journal of Chemical Engineering 33 (10), 2878-2890
17. D Naghipour, **H Gharibi**, K Taghavi, J Jaafari. 2016."Influence of EDTA and NTA on heavy metal extraction from sandy-loam contaminated soils". Journal of Environmental Chemical Engineering 4 (3), 3512-3518
18. MH Ebrahimi, **H Gharibi**. 2016."A case study of a patient with diabetic retinopathy". Diabetes & Metabolic Syndrome: Clinical Research & Reviews 10 (3), 166-168

19. Javid, A., Hamedian, A., **Gharibi, H***., Sowlat, M. H. 2015. "Towards the application of fuzzy logic for developing a novel indoor air quality index (FIAQI)". International Journal of Public Health, Accepted Paper.

20. Sowlat, M.H., Abdollahi, M., **Gharibi, H**., Yunesian, M., Rastkari N. 2014. "Removal of VaporPhase Elemental Mercury from Stack Emissions with Sulfur-Impregnated Activated Carbon". Reviews of Environmental Contamination and Toxicology, volume 230, pages 1-34.

21. **Gharibi, H.**, Sowlat, M.H., Mahvi, A.H*., Keshavarz, M. 2012. “Performance evaluation of a bipolar electrolysis/electrocoagulation- (EL/EC) reactor to enhance the sludge dewaterability”. Chemosphere. 90 (4), 1487-1494.

22. **Gharibi, H**., Mahvi, A.H*., Nabizadeh, R., Arabalibeik, H., Yunesian, M., Sowlat, M.H., 2012. “A novel approach in water quality assessment based on fuzzy logic”. Journal of Environmental Management. 112, 87-95.

23. **Gharibi, H**., Sowlat, M.H., Mahvi, A.H*., Mahmoudzadeh, H., Arabalibeik, H., Keshavarz, M., Karimzadeh, N., Hassani, G., 2012. “Development of a dairy cattle drinking water quality index (DCWQI) based on fuzzy inference systems”. Ecological Indicators. 20, 228-237.

24. Mahvi, A.H*., Ebrahimi, S.J.A.D., Mesdaghinia, A., **Gharibi, H.**, Sowlat, M.H. 2011. “Performance evaluation of a continuous bipolar electrocoagulation/electrooxidation electroflotation (ECEO-EF) reactor designed for simultaneous removal of ammonia and phosphate from wastewater effluent”. Journal of Hazardous Materials. 192 (3), pp. 1267-1274.

25. Hassani, G., Mahvi, A.H*., Nasseri, S., Arabalibeik, H., Yunesian, M., **Gharibi, H**., 2012. "Designing Fuzzy-based ground water quality index". JOURNAL OF HEALTH AND HYGIENE, Vol. 3, No. 1 (8); 18-31.

26. Sowlat, M.H., **Gharibi, H*.,** Yunesian, M., Mahmoudi, M.T., Lotfi, S., 2011. "A novel, fuzzy-based air quality index (FAQI) for air quality assessment". Atmospheric Environment. 45 (12), 2050-2059.

27. Hassani, G., Nasseri, S., **Gharibi, H.**, 2011." Removal of Cyanide by Electrocoagulation Process". Analytical & Bioanalytical Electrochemistry. Vol. 3, No. 6, 625 – 634.

28. **Gharibi, H.**, Mahvi, A.H*., Chehrazi, M., Sheikhi, R., Sadat Hosseini, S., 2010." Phosphorous removal from wastewater effluent using electro-coagulation by aluminum and iron plates". Analytical & Bioanalytical Electrochemistry. Vol. 2, No. 3, 165- 177.

**Conference**

1. **H Gharibi**, M Entwistle, et al. Pesticide Air toxicants and Asthma Emergency Department Visits in California, USA in 2005 to 201: A bidirectional symmetric Case-Crossover Analysis. Environmental Epidemiology, Canada 2018

## RESEARCH SUPPORT/GRANTS

1. The association between nitrate in drinking water and colorectal cancer incidence in California. *Under review at NIH*
2. Determining the added human health impacts caused by smoke (PM2.5) from wildland fires. *Under review at NIH*

## TEACHING EXPERIENCE

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**2017- Present University of California, Merced**

PH-110-01-LECT: Environmental Health (Fall 2021)

PH-105-01-LECT: Health Policy (Spring 2021)

PH-181-01-LECT: Introduction to Public Health (Spring 2020)

PH-105-01-LECT: Health Policy (Fall 2020)

PH 110-02: Environmental Health (Fall 2019)

PH-100-01-LECT: Epidemiology (Spring 2018)

PH-110-01-LECT: Environmental Health (Fall 2017)

**2013-2016 Shahroud University of Medical Sciences, Shahroud, Iran**

Public Health

Water Quality

Environmental Health

### Workshop

**2011** How to Write a Scientific Article

**2012** How to Write a Patent

## OTHER RELATED WORK EXPERIENCE

**Feb/2021- July/2021 Air Pollution Specialist (AD paid and volunteer)**

Setting up and calibrating BAM and E-BAM devices; also, I collected and analyzed air quality data from the air samplers in Southern California.

## PROFESSIONAL SERVICE ACTIVITIES

Occasional Peer Reviewer: Elsevier “Journal of Hazardous Materials”, Pinnacle Journals, American Journal of Analytical Chemistry, Science Publications, Springer "Iranian Journal of Environmental Health and Engineering", International Journal of Public Health

## PROFESSIONAL AFFILIATIONS

1- College of Social Sciences, Humanities and Arts, Department of Public Health, University of California, Merced, USA
2- Center for Environmental Research, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3- Center for Solid Waste Research, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran
4- School of public Health, Shahroud University of Medical Sciences, Shahroud, Iran

## General Abstract

This research, titled as "Investigating the association between Nitrate in drinking water and the incidence of respiratory diseases in California, USA" is conducted by Hamed Gharibi under the supervision of Prof. Ricardo Cisneros in the University of California Merced in the year 2022. **This is the first study on the effect of Nitrate on the health of people with focusing on respiratory diseases.**

California, the leading US state in agricultural activity, produces nearly 400 commodities (more than 30% of the country's vegetables and 60% of the country's fruits and nuts); the abundance of agricultural activity in this state has resulted in the increased application of Nitrogen fertilizers. The application of such fertilizers over decades resulted in leaching of nitrate to the water resources in California. Previous studies reported that California has the highest mean concentration of Nitrate over the maximum contaminant level (MCL) in drinking water, compared to all other states in the US. In addition, it is found that the greatest average annual number of people served by systems in violation of nitrate standard level is in California. The MCL for nitrate in the public drinking water is 45 mg/l-NO3 or 10 mg/l-N; however, this MCL is aimed to protect infants from methemoglobinemia and it is not for protecting people from other possible diseases in relation with long-term exposure to nitrate.

According to the statistics released by State Water Resources Control Board, the highest number of violations happened in San Bernardino (1021 times), Los Angeles (945 times), Fresno (729 times), San Diego (173 times), Kern (147 times), Stanislaus (123 times), Riverside (115 times), Santa Clara (86 times) and Ventura (80 times). Based on the total

population served in each county, Los Angeles (population served: 6,972,171), San Diego (1,727,098), San Bernardino (1,477,920) and Fresno (731,215) has the greatest average number of people served by systems in violation. Most of the violations occurred after year 2000; in other words, 46.41%, 33.00% and 14.79% of the violations in the California, USA occurred between 2010 to 2015, 2005 to 2009 and 2000 to 2004, respectively.

There have been a few numbers of animal studies on the effect of nitrate on the lungs. In these studies, a correlation was found between nitrate in drinking water and the high methemoglobin levels, and pathologic changes in bronchi and lung parenchyma; namely, frequent dilation of bronchi with lymphocytic infiltration; atrophy of mucosa and muscles; frequent purulent bronchial exudates and interstitial round cell infiltration; and fibrosis at certain areas. The health effects of Nitrate is related to its concentration in the drinking water and also the formation of reactive free radicals of nitric oxide ($NO^{\cdot}$) and oxygen ($O^{\cdot}$).

There are few studies focusing on the association between Nitrate in drinking water and the incidence of respiratory diseases around the world. In this study, it is aimed to apply different methods, namely, generalized linear model (GLM) of Poisson family (a population-based study), and Decision Trees (DT) to evaluate the association between Nitrate in drinking water and the incidence of respiratory diseases in California from 2005 to 2015.

# Chapter 1: Introduction

## Introduction

California, the leading US state in agricultural activity, produces nearly 400 commodities (more than 30% of the country's vegetables and 60% of the country's fruits and nuts); the abundance of agricultural activity in this state has resulted in the increased application of Nitrogen fertilizers (CDFA, 2018). The application of such fertilizers over decades resulted in leaching of nitrate to the water resources in California (Harter et al., 2012; Pennino et al., 2017). Previous studies reported that California has the highest mean concentration of Nitrate over the maximum contaminant level (MCL) in drinking water, compared to all other states in the US (Pennino et al., 2017). In addition, it is found that the greatest average annual number of people served by systems in violation of nitrate standard level is in California (Pennino et al., 2017). The MCL for nitrate in the public drinking water is 45 mg/l-NO3 or 10 mg/l-N (USEPA, 2018); however, this MCL is aimed to protect infants from methemoglobinemia and it is not for protecting people from other possible diseases in relation with long-term exposure to nitrate (Ward et al., 2018). California, with a population around 39 million in 2015, is the most populated state in America.

Once nitrate entered the human body, it can be absorbed readily by upper gastrointestinal tract and end up in the blood stream and then saliva glands. The concentration of nitrate in the saliva glands is 20 times higher than that of plasma (Leach et al., 1987; Lv et al., 2012; Spiegelhalder et al., 1976; Tricker et al., 1989). Almost 6 to 7 percent of the nitrate in the saliva glands is reduced to nitrite by nitrate-reductive bacteria in the oral cavities; and then, it re-enters the gastrointestinal tract (Spiegelhalder et al., 1976, Eisenbrand et al., 1980; Eisenbrand et al., 1990). The acidic condition of stomach protonates nitrite to nitrous acid

($HNO_2$) which consequently yields dinitrogen trioxide ($N_2O_3$), nitric oxide (NO), and nitrogen dioxide ($NO_2$) (Hill et al., 1973). These derived metabolites are powerful nitrosating agents, which drive the endogenous nitrosation reactions in the gastrointestinal tract with amines and amides, resulting in the formation of N-nitroso compounds, which are proven to be some of the most potent known carcinogens (Tricker et al., 1989, Ward et al., 2009; Ward et al, 2018).

Long-term exposure to nitrate can jeopardize the health of people (Harter et al., 2012; Galloway et al., 2010; Ward et al., 2008; Schullehner et al., 2018). In previous works, nitrate has been found to be associated with carcinogenic outcomes due to the gastric nitrate conversion in the presence of amines and amides (Pennino et al., 2017; Ward et al., 2005). Upper gastrointestinal tract readily absorbs nitrate entered to the body via drinking water into the blood stream. The nitrate in the blood can then be transferred to the saliva glands; it is estimated that the concentration of transferred nitrate in these areas is 20 times higher than that of plasma (Leach et al., 1987; Lv et al., 2012; Spiegelhalder et al., 1976; Tricker et al., 1989). The nitrate-reductive bacteria in the oral cavities reduce 6% to 7% of the total nitrate in the cavities to nitrite and then the nitrate and formed nitrite can be swallowed up and re-enter the gastrointestinal tract (Spiegelhalder et al., 1976; Eisenbrand et al., 1980; Eisenbrand et al. 1990). These derived metabolites are powerful nitrosating agents which drive the endogenous nitrosation reactions in the gastrointestinal tract with amines and amides, resulting in the formation of N-nitroso compounds which are proven to be some of the most potent known carcinogens (Ward et al., 2005; Tricker and Preussman, 1991; Ward et al., 2018). In addition, there are exogenous N-nitroso compounds present in the public water systems which are measured by EPA (CSWCB, 2018) and can pose threat to human health (Jakszyn et al., 2006).

Based on the reports of California State Water Resources Control Board at Electronic Data Transfer (EDT) (CSWCB, 2018), the concentration of Nitrate (mg/l-NO3) has increased dramatically in drinking water systems in California, USA. This can be seen from Figure 1.

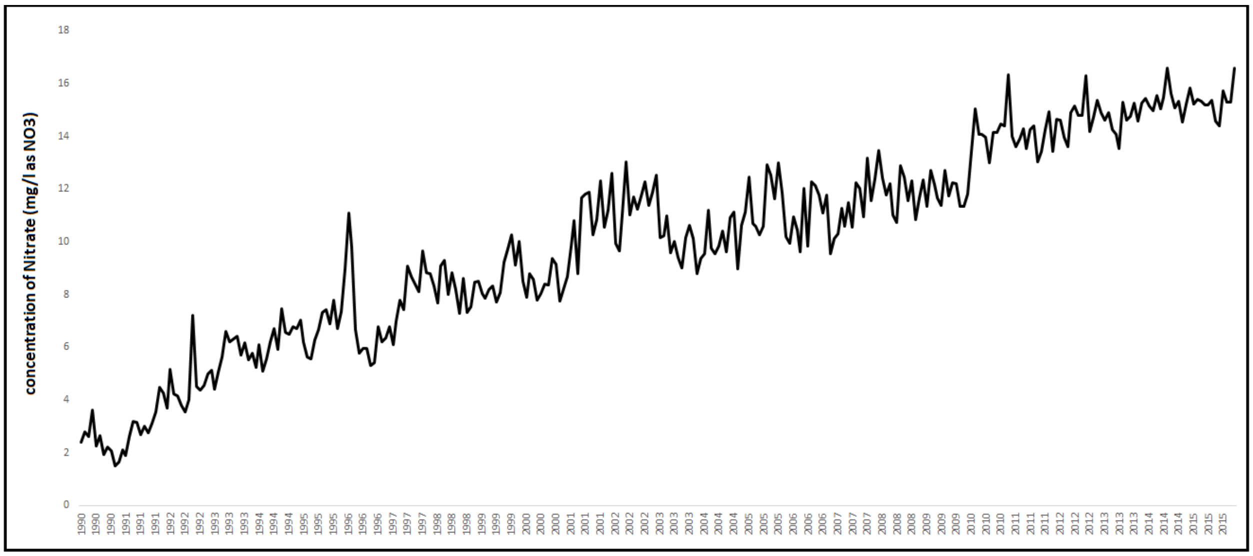


**Figure 1:** The monthly-average concentration of Nitrate (mg/l as NO3) in drinking water of California, USA from 1990 to 2015

**Source**: California States Water Control Boards (CSWCB). Electronic Data Transfer (EDT) Library and Water Quality Analyses Data and Download Page. Available online: https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/EDTlibrary.html

According to the statistics released by State Water Resources Control Board (CSWCB, 2018), the highest number of violations happened in San Bernardino (1021 times), Los

Angeles (945 times), Fresno (729 times), San Diego (173 times), Kern (147 times), Stanislaus (123 times), Riverside (115 times), Santa Clara (86 times) and Ventura (80 times). Based on the total population served in each county, Los Angeles (population served: 6,972,171), San Diego (1,727,098), San Bernardino (1,477,920) and Fresno (731,215) has the greatest average number of people served by systems in violation. As shown in Table 1, most of the violations occurred after year 2000; in other words, 46.41%, 33.00% and 14.79% of the violations in the California, USA occurred between 2010 to 2015, 2005 to 2009 and 2000 to 2004, respectively.

**Table 1:** The public water system characteristics in 25 counties in California, USA: trends in drinking water nitrate violations

| County | Connections to PWSs | Population served by Surface Water | Population served by Groundwater | Total population served | # violation of the standard level of 45 mg/l-$NO_3$ or 10 mg/l-N by each drinking water by the type of sampling station | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | AT | CT | DT | PT | Total |
| Alameda | 480993 | 1379040 | 396700 | 1775740 | 7 | 0 | 0 | 1 | 8 |
| Butte | 45994 | 41600 | 110698 | 152298 | 2 | 0 | 0 | 0 | 2 |
| Calaveras | 8197 | 4016 | 21397 | 25413 | 0 | 0 | 0 | 0 | 0 |
| Contra Costa | 127542 | 354148 | 68700 | 422848 | 16 | 0 | 0 | 0 | 16 |
| Fresno | 183556 | 647763 | 83452 | 731215 | 729 | 0 | 0 | 0 | 729 |
| Kern | 145523 | 316984 | 218076 | 535060 | 60 | 87 | 0 | 0 | 147 |
| Kings | 4960 | 12000 | 24813 | 36813 | 0 | 0 | 0 | 0 | 0 |
| Lake | 3007 | 6499 | 100 | 6599 | 0 | 0 | 0 | 0 | 0 |
| Los Angeles | 1221536 | 6409402 | 562769 | 6972171 | 662 | 281 | 2 | 0 | 945 |
| Marin | 82627 | 251110 | 1700 | 252810 | 0 | 0 | 0 | 0 | 0 |
| Nevada | 15673 | 45454 | 0 | 45454 | 0 | 0 | 0 | 0 | 0 |
| Placer | 52848 | 166797 | 30 | 166827 | 1 | 0 | 0 | 0 | 1 |
| Riverside | 327754 | 1049770 | 220765 | 1270535 | 68 | 46 | 0 | 1 | 115 |
| Sacramento | 246240 | 788483 | 13386 | 801869 | 0 | 0 | 0 | 0 | 0 |
| San Bernardino | 382221 | 1005466 | 472454 | 1477920 | 50 | 965 | 1 | 5 | 1021 |
| San Diego | 351836 | 1710828 | 16270 | 1727098 | 170 | 0 | 3 | 0 | 173 |
| San Joaquin | 116171 | 237039 | 254163 | 491202 | 2 | 0 | 0 | 0 | 2 |
| San Luis Obispo | 35582 | 14415 | 82125 | 96540 | 60 | 0 | 0 | 0 | 60 |
| San Mateo | 39950 | 168736 | 0 | 168736 | 18 | 1 | 0 | 2 | 21 |
| Santa Barbara | 65112 | 254798 | 80 | 254878 | 9 | 0 | 0 | 0 | 9 |
| Santa Clara | 11169 | 0 | 42262 | 42262 | 79 | 7 | 0 | 0 | 86 |
| Santa Cruz | 42208 | 96142 | 48229 | 144371 | 0 | 0 | 0 | 0 | 0 |
| Solano | 90236 | 315352 | 1455 | 316807 | 4 | 0 | 0 | 0 | 4 |
| Stanislaus | 11601 | 865 | 47902 | 48767 | 125 | 78 | 0 | 0 | 123 |
| Ventura | 96306 | 250962 | 142677 | 393639 | 70 | 7 | 0 | 3 | 80 |
| **Total** | **4,188,842** | **15,527,669** | **2,830,203** | **18357872** | **2132** | **1472** | **6** | **12** | **3542** |

Abbreviations: PWSs: Public water systems; AT: an active source which is sampled after any treatment; CT: Combined sources which are treated; DT: Sample point within the distribution system after treatment; PT: Purchased source water which is sampled after any treatment

There have been a few numbers of animal studies on the effect of nitrate on the lungs. In these studies, a correlation was found between nitrate in drinking water and the high methemoglobin levels, and pathologic changes in bronchi and lung parenchyma; namely, frequent dilation of bronchi with lymphocytic infiltration; atrophy of mucosa and muscles; frequent purulent bronchial exudates and interstitial round cell infiltration; and fibrosis at certain areas (Shuval et al., 1972; Gruener et al., 1970; Gupta et al., 1999; WHO, 1977). The health effects of Nitrate is related to its concentration in the drinking water and also the formation of reactive free radicals of nitric oxide (NO·) and oxygen (O·) (Winterbourn et al., 1976). Nitrites, resulted from reduction reaction in human body or from external sources, can cause oxidative stress within the lungs and lead to more inflammation within the body, which can worsen asthma. While nitrates and nitrites may worsen asthma, it is not certain that they create asthma.

The second chapter of this document is about the "investigating the effect of Nitrate in drinking water on asthma ED visit as an acute respiratory response in CA, USA between 2005 and 2015". In the second chapter, I used a generalized linear model of Poisson family to investigate the association between Nitrate in drinking water in CA and asthma ED visits during 2005 to 2015.

The third chapter of this document is about "Investigating the effect of Nitrate in drinking water on chronic respiratory diseases in CA, USA between 2005 and 2015". In the third chapter, I used a generalized linear model of Poisson family to investigate the association between Nitrate in drinking water in CA and chronic respiratory diseases, including COPD, bronchitis and pneumonia during 2005 to 2015.

Within the fourth chapter, I applied a decision tree model to investigate the level of Nitrate exposure among those who experienced COPD, asthma, bronchitis and pneumonia in their drinking water during 2005 to 2015. This model was applied to pin point those who were affected by exposure to Nitrate in drinking water; unlike the regression models, decision tree allows you to know which subjects contributed to the output and how many of them.

**Research Questions and Hypothesis**

**In this study, I tried to answer the following research questions and hypothesis:**

1. Is there an association between Nitrate in drinking water and asthma attacks in California, USA?
2. Is there an association between Nitrate in drinking water and COPD in California, USA?
3. Is there an association between Nitrate in drinking water and bronchitis in California, USA?
4. Is there an association between Nitrate in drinking water and pneumonia in California, USA?
5. Where, in California, is more affected by exposure to Nitrate in drinking water?
6. Which respiratory disease, namely Asthma, COPD, Bronchitis and Pneumonia, is more connected to the exposure to Nitrate in drinking water?

**Hypothesis**

**H1.** There is an association between Nitrate in drinking water and asthma attacks in California, USA

**H2.** There is an association between Nitrate in drinking water and COPD in California, USA

**H3.** There is an association between Nitrate in drinking water and bronchitis in California, USA

**H4.** There is an association between Nitrate in drinking water and pneumonia in California, USA

**H5.** Communities of color are more affected by exposure to Nitrate

**H6.** Exposure to Nitrate in drinking water is more connected to Bronchitis than other respiratory diseases

# **Chapter 2:** Investigating the effect of Nitrate in drinking water on asthma ED visit as an acute respiratory response in CA, USA between 2005 and 2015

**Abstract**

***Background:*** The concentration of Nitrate in drinking water in CA, USA has increased drastically over the years. The standard level set for this pollutant is based on developing methemoglobinemia and therefore other diseases at the time were not included. Nitrate has been shown to be connected with damaging lung tissues leading to permanent lung damage among people.

***Objective:*** This study is aimed to investigate the association between the increase in the average concentration of Nitrate in drinking water from different sources between 2005 and 2015 and Asthma ED visits in California, USA through a time series study.

***Materials and Methods:*** In this study, a generalized linear model of Poisson family was applied to estimate the association between increase in $NO_3$ and increase in the count of ED visits due to asthma. The data for water quality was obtained from California State Water Resources Control Board and also OSHPD data was used to extract ED visits due to asthma in CA between 2005 and 2015.

***Results:*** In this work, 104701asthma ED visits were recorded by OSHPD during 2005 to 2015. Based on the results, the highest RR was found at lag 0-6; a 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-6 is associated with an asthma ED visits risk ratio of 3.2% [RR: 1.032 (95% confidence intervals: 1.017, 1.047)]. Also, the RR is higher among males, compared to that of females. Stratifying by race, the highest RR was found among Non-Hispanic Asians [RR: 1.240 (95% confidence intervals: 1.079, 1.424)] at lag 0-6. The highest RR was found among those who age 65 years or older [RR: 1.286 (95% confidence intervals: 1.162, 1.422)].

***Conclusion:*** This study indicated that there is a statistically significant association between Nitrate in drinking water in CA and asthma ED visits between 2005 to 2015.

***Keywords:*** Asthma; Nitrate; drinking water; California; generalized linear model

**Introduction**

The agricultural activity in California is far greater than that in other states, which means more usage of fertilizers (CDFA, 2018). The application of such fertilizers over decades resulted in leaching of nitrate to the water resources in California (Harter et al., 2012; Pennino et al., 2017). Previous studies reported that California has the highest mean concentration of Nitrate over the maximum contaminant level (MCL) in drinking water, compared to all other states in the US (Pennino et al., 2017). In addition, it is found that the greatest average annual number of people served by systems in violation of nitrate standard level is in California (Pennino et al., 2017). The MCL for nitrate in the public drinking water is 45 mg/l-$NO_3$ or 10 mg/l-N (USEPA, 2018); however, this MCL is aimed to protect infants from methemoglobinemia, and it is not for protecting people from other possible diseases in relation with exposure to nitrate (Ward et al., 2018).

Asthma, a clinical condition characterized by intermittent obstructive respiratory symptoms and airway hyperresponsiveness to a variety of stimuli may be worsened by exposure to environmental pollutants (Balmes, 1993). However, there has been very few studies on the acute association between Nitrate in drinking water and asthma onset. Nitrites can cause oxidative stress within the lungs and lead to more inflammation within the body, which can worsen asthma (Gosh and Erzurum, 2011).

Therefore, this study is aimed to investigate the association between the increase in the average concentration of nitrate in drinking water from different sources between 2005 and 2015 and Asthma ED visits in California, USA through a time series study. Generalized linear model of Poisson family is applied in this work. By stratifying for sex, race, and age on the outcome, the effects of these modifiers are investigated in this study.

## Material and Methods

### *Data and Participants*

The asthma ED visit data for the years of 2005 to 2015 in in California, USA was obtained from California's Office of Statewide Health Planning and Development (OSHPD). The asthma ED visits were identified using the International Classification of Diseases, 9$^{th}$ Revision (ICD-9) code 493 for asthma related visits (National Center for Health Statistics, 2011). From the OSHPD dataset, patient's date of visit, principal diagnosis, residential ZIP code, the patient's age, race, and sex were kept for the analysis. Multiple visits by a single person cannot be identified because the data is not linked by person longitudinally due to the lack of access to social security number; therefore, each valid observation was taken as an independent observation.

### *Study design*

This is time-series study, using generalized linear model of Poisson family, with the aim of investigating the association between exposure to increased concentration of Nitrate in drinking water in California from 2005 to 2015 and those who visited ED due to asthma attacks between 2010 to 2015.  The dependent variable is the number of ED visits due to asthma during 2005 to 2015. Figure 2 shows all the sampling stations within the study area and each county. As can be seen from the figure, the number and distribution of stations covers the residential areas within the counties. To handle the over dispersion (i. e. the variance of the outcome counts is higher than predicted under a Poisson distribution) in the data, the Pearson chi-square statistic divided by the residual degrees of freedom as a scale parameter is applied (McCullagh 1989).

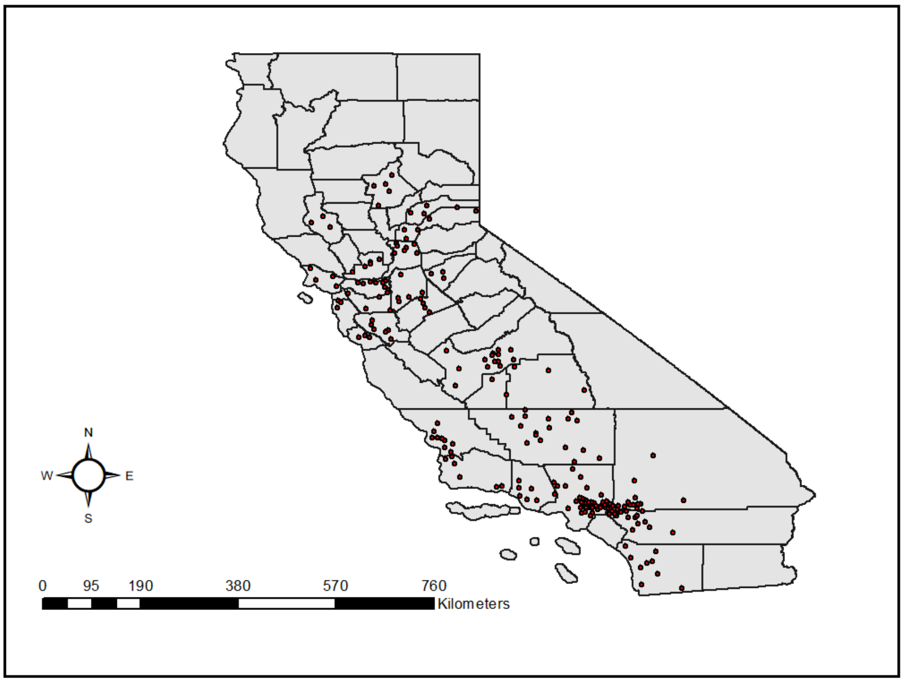


**Figure 2:** The water sampling stations within the study area (25 counties in California)

*Exposure Assessment*

The water quality data was obtained from California State Water Resources Control Board at Electronic Data Transfer (EDT) Library and Water Quality Analyses Data and Download Page (CSWCB, 2018). This dataset contains information on each water quality parameters (i. e. nearly 580 different parameters) among which Nitrate ($NO_3$) was extracted.

In this dataset, 19 different sources of sampling for water quality parameters are included, which only the samples from active treated (an active source which is sampled after any treatment), treated water in the distribution system (Sample point within the distribution system after treatment), combined treated (Combined sources which are treated), and purchased water (Purchased source water which is sampled after any treatment) are extracted for this study. The goal of choosing this type of samples were to only focus on the water treated for drinking purposes; in other words, these samples are taken from points where people in each county are more possible to drink from than the other sources.

The data for $NO_3$ from 1995 to 2015 was available only for 25 counties (Alameda, Butte, Calaveras, Contra Costa, Fresno, Kern, Kings, Lake, Los Angeles, Marin, Nevada, Placer, Riverside, Sacramento, San Bernardino, San Diego, San Joaquin, San Luis Obispo, San Mateo, Santa Barbara, Santa Clara, Santa Cruz, Solano, Stanislaus, and Ventura) in California. Due to this and the fact that nearly 30 million out of 37 million population of California residents live in these 25 counties, we only focused on this area.

*Statistical analysis*

In this study, generalized linear model of Poisson family was applied to estimate the association between increase in $NO_3$ and increase in the count of ED visits due to asthma. The model used is as follows:

$Y_t \sim Poisson\ (\mu_t)$

$\log(\mu_t) = \alpha + \beta_1(NO_3) + \ f(Time_t, 4) + Calendar\ Time$

where $t$ is the day of the observation, $Y_t$ is the count of asthma ED visits on year t, α is the intercept, $s(Time_t, 4)$ is a flexible spline function with 4 $df$ for seasonal and long-term patterns. *(NO₃)* stand for Nitrate. Furthermore, $\beta_1$ refers to the coefficient for $NO_3$.

To control for seasonal and long-term patterns in this work, periodic functions (Fourier series terms) was applied. Using this method, pairs of sine and cosine functions of time can be created to capture very regular seasonal patterns (Bhaskaran et al. 2013). Noted that long-term non-seasonal trends cannot be included in the model by using only Fourier terms; that is the reason of adding calendar time to the model (Bhaskaran et al. 2013). Four harmonics (4 sine/cosine pairs) was included in the model to capture seasonality, plus a linear function of time to capture broader trends over time. In this study, the annual count of Asthma ED visits in each community during 2005 to 2015 was merged with mean concentration of $NO_3$ (mg/l as $NO_3$); and, the effect of sex, age of diagnosis, and race/ethnicities were investigated to account for the probable modifiers. The reported risk ratios (RR) and 95% confidence intervals (CI) in this study are based on 1 (mg/l as $NO_3$) increase in the concentration of $NO_3$. All analyses were performed using STATA V. 14 (College Station, TX).

## Results

*Descriptive analysis of the hospital data*

In this work, 104701 asthma ED visits were recorded by OSHPD during 2005 to 2015 (Table 2). In addition, 56% of the study population were females, while 43% were males. 36.10%, 16.59%, 38.11% and 4.24% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. The mean age for the population in this study is 27 years old. 8.14%, 19.37%, 34.75%, 27.41%, and 7.77% of the visits were recorded from 2 to 5, 6 to 18, 19 to 40, 41 to 64, and 65+ years old, respectively.

**Table 2**: Characteristics of asthma ED visits during the years 2005 to 2015 in California, USA (n=104701)

| Characteristics | Asthma # (%) |
|---|---|
| **Sex** | |
| *Female* | 58905 (56.26) |
| *Male* | 45794 (43.74) |
| **Race** | |
| *White* | 37796 (36.10) |
| *Black* | 17370 (16.59) |
| *Hispanic* | 39904 (38.11) |
| *Asians* | 4438 (4.24) |
| *Others* | 5193 (4.96) |
| **Age** | |
| *2-5* | 8523 (8.14) |
| *6-18* | 20285 (19.37) |
| *19-40* | 36381 (34.75) |
| *41-64* | 28696 (27.41) |
| *>=65* | 8136 (7.77) |
| **Total Population** | **104701** |

*The descriptive analysis of Nitrate in drinking water*

The monthly-average concentration of $NO_3$ in drinking water has increased steadily from 1990 to 2015 in California, USA, as can be seen from Figure 2. In the 25 counties under study here, this trend can be seen.

An analysis into the number of populations served by either surface or groundwater resources and trends in drinking water nitrate violations across the study area was conducted (Table 3). As shown in the table, the highest number of violations happened in Los Angeles (779 times). Based on the total population served in each county through the four mentioned systems, Los Angeles (population served: 6,972,171), San Diego (1,727,098), San Bernardino (1,477,920) and Fresno (731,215) has the greatest average number of people served by systems in violation.

**Table 3:** The community water system characteristics in the study area: trends in drinking water nitrate violations across the study area

| County | Connections to PWSs | Population served by Surface Water | Population served by Groundwater | Total population served | # violation of the standard level of 45 mg/l-$NO_3$ or 10 mg/l-N |
|---|---|---|---|---|---|
| Alameda | 480993 | 1379040 | 396700 | 1775740 | 6 |
| Butte | 45994 | 41600 | 110698 | 152298 | 1 |
| Calaveras | 8197 | 4016 | 21397 | 25413 | 0 |
| Contra Costa | 127542 | 354148 | 68700 | 422848 | 14 |
| Fresno | 183556 | 647763 | 83452 | 731215 | 572 |
| Kern | 145523 | 316984 | 218076 | 535060 | 110 |
| Kings | 4960 | 12000 | 24813 | 36813 | 0 |
| Lake | 3007 | 6499 | 100 | 6599 | 0 |
| Los Angeles | 1221536 | 6409402 | 562769 | 6972171 | 779 |
| Marin | 82627 | 251110 | 1700 | 252810 | 0 |
| Nevada | 15673 | 45454 | 0 | 45454 | 0 |
| Placer | 52848 | 166797 | 30 | 166827 | 1 |
| Riverside | 327754 | 1049770 | 220765 | 1270535 | 70 |
| Sacramento | 246240 | 788483 | 13386 | 801869 | 0 |
| San Bernardino | 382221 | 1005466 | 472454 | 1477920 | 729 |
| San Diego | 351836 | 1710828 | 16270 | 1727098 | 170 |
| San Joaquin | 116171 | 237039 | 254163 | 491202 | 2 |
| San Luis Obispo | 35582 | 14415 | 82125 | 96540 | 56 |
| San Mateo | 39950 | 168736 | 0 | 168736 | 19 |
| Santa Barbara | 65112 | 254798 | 80 | 254878 | 9 |
| Santa Clara | 11169 | 0 | 42262 | 42262 | 84 |
| Santa Cruz | 42208 | 96142 | 48229 | 144371 | 0 |
| Solano | 90236 | 315352 | 1455 | 316807 | 4 |
| Stanislaus | 11601 | 865 | 47902 | 48767 | 111 |
| Ventura | 96306 | 250962 | 142677 | 393639 | 76 |
| **Total** | **4,188,842** | **15,527,669** | **2,830,203** | **18357872** | **5262** |

Abbreviations: PWSs: Public water systems

*Exposure to $NO_3$: a Poisson Regression Model*

Based on the results (Table 4), the highest RRs was found at lag 0-6; a 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-6, lag 0-5 and lag 0-2 is associated with an asthma ED visits risk ratio of 3.2% [RR: 1.032 (95% confidence intervals: 1.017, 1.047)], 1.3% [RR: 1.013 (95% confidence intervals: 1.000 1.025)], and 0.7% [RR: 1.007 (95% confidence intervals: 1.000, 1.013)], respectively.

**Table 4**: The association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015

| | Total area | |
|---|---|---|
| | [a] **RR** | *95% CI* |
| Lag 0 | 1.001 | 0.999, 1.003 |
| Lag 0-1 | 0.981 | 0.977, 0.985 |
| Lag 0-2 | **1.007** | **1.000, 1.013** |
| Lag 0-3 | 0.996 | 0.988, 1.005 |
| Lag 0-4 | 0.991 | 0.981, 1.001 |
| Lag 0-5 | **1.013** | **1.000, 1.025** |
| Lag 0-6 | **1.032** | **1.017, 1.047** |
| Lag 0-7 | 1.004 | 0.987, 1.020 |
| Lag 0-8 | 0.966 | 0.948, 0.983 |
| Lag 0-9 | 1.002 | 0.991, 1.013 |

*[a] risk ratio (95% CI) per 1 mg/L as NO3 increase in $NO_3$*

*Stratification by sex*

Table 5 indicates the association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015 among females and males. As can be seen from the table, the RR is higher among males, compared to that of females. A 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-5 is associated with an asthma ED visits risk ratio of 5.6% [RR: 1.056 (95% confidence intervals: 1.026, 1.087) among males. Furthermore, A 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-6 is associated with an asthma ED visits risk ratio of 4.9% [RR: 1.049 (95% confidence intervals: 1.020, 1.078)] among females.

**Table 5**: The association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015 among females and males

| | Sex | | | |
|---|---|---|---|---|
| | Female | | Male | |
| | [a] **RR** | *95% CI* | [a] **RR** | *95% CI* |
| Lag 0 | 1.002 | 0.998, 1.006 | 0.998 | 0.993, 1.003 |
| Lag 0-1 | 0.985 | 0.977, 0.993 | 0.982 | 0.972, 0.992 |
| Lag 0-2 | 0.997 | 0.984, 1.009 | 1.014 | 0.999, 1.029 |
| Lag 0-3 | 1.002 | 0.985, 1.019 | 0.999 | 0.979, 1.019 |
| Lag 0-4 | 1.017 | 0.997, 1.038 | 0.956 | 0.933, 0.979 |
| Lag 0-5 | 0.979 | 0.956, 1.003 | **1.056** | **1.026, 1.087** |
| Lag 0-6 | **1.049** | **1.020, 1.078** | 1.008 | 0.975, 1.042 |
| Lag 0-7 | 1.016 | 0.984, 1.050 | 0.987 | 0.950, 1.026 |
| Lag 0-8 | 0.921 | 0.890, 0.954 | 1.020 | 0.978, 1.063 |
| Lag 0-9 | **1.028** | **1.007, 1.050** | 0.974 | 0.950, 0.999 |

*[a] risk ratio (95% CI) per 1 mg/L as NO3 increase in* $NO_3$

*Stratification by race*

Table 6 indicates the association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015 among different race/ethnicities. As can be seen from the table, a significant positive association was found for each race/ethnicity at different lags, however, the highest RR was found among Non-Hispanic Asians [RR: 1.240 (95% confidence intervals: 1.079, 1.424)] at lag 0-6. The highest RR among Non-Hispanic Whites was found at lag 0-6 [RR: 1.058 (95% confidence intervals: 1.016, 1.101)]. Furthermore, the highest RR among Non-Hispanic Blacks and Hispanics was found at lag

0-4 [RR: 1.093 (95% confidence intervals: 1.037, 1.152)] and lag 0-2 [RR: 1.054 (95% confidence intervals: 1.038, 1.071)], respectively.

**Table 6**: The association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015 among different race/ethnicities

| | Race | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-Hispanic Whites | | | Non-Hispanic Black | | | Hispanics | | | Non-Hispanic Asians | | |
| | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | |
| Lag 0 | 1.015 | 1.008 | 1.021 | 0.975 | 0.964 | 0.987 | 1.002 | 0.997 | 1.007 | 1.002 | 0.981 | 1.024 |
| Lag 0-1 | 0.976 | 0.965 | 0.987 | 0.999 | 0.977 | 1.021 | 0.990 | 0.980 | 1.000 | 0.995 | 0.954 | 1.037 |
| Lag 0-2 | 0.967 | 0.951 | 0.985 | 0.977 | 0.946 | 1.009 | **1.054** | **1.038** | **1.071** | 0.985 | 0.927 | 1.047 |
| Lag 0-3 | **1.028** | **1.004** | **1.053** | 0.970 | 0.930 | 1.011 | 0.963 | 0.943 | 0.983 | 1.016 | 0.936 | 1.104 |
| Lag 0-4 | **1.038** | **1.008** | **1.069** | **1.093** | **1.037** | **1.152** | 0.980 | 0.956 | 1.005 | 0.858 | 0.777 | 0.947 |
| Lag 0-5 | 0.954 | 0.923 | 0.987 | 0.983 | 0.922 | 1.048 | 1.008 | 0.979 | 1.038 | 1.034 | 0.922 | 1.161 |
| Lag 0-6 | **1.058** | **1.016** | **1.101** | 1.063 | 0.986 | 1.146 | 0.976 | 0.944 | 1.009 | **1.240** | **1.079** | **1.424** |
| Lag 0-7 | 0.991 | 0.945 | 1.038 | 1.006 | 0.920 | 1.099 | **1.039** | **1.000** | **1.080** | 1.171 | 0.992 | 1.384 |
| Lag 0-8 | 0.938 | 0.894 | 0.984 | 0.981 | 0.894 | 1.076 | 1.015 | 0.973 | 1.058 | 0.748 | 0.643 | 0.871 |
| Lag 0-9 | **1.053** | **1.024** | **1.084** | 0.920 | 0.871 | 0.973 | 0.978 | 0.953 | 1.004 | 0.969 | 0.897 | 1.046 |

*[a] risk ratio (95% CI) per 1 mg/L as NO3 increase in $NO_3$*

*Stratification by age*

Table 7 indicates the association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015 among different age groups. As can be seen from the table, no significant association was found among those who age from 2 to 5 years old and 19 to 40 years old. The highest RR was found among those who age 65 years or older [RR: 1.286 (95% confidence intervals: 1.162, 1.422)].

**Table 7**: The association between Nitrate in drinking water and asthma ED visits in California during the years 2005 to 2015 among different age groups

| | Age | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 to 5 years old | | | 6 to 18 years old | | | 19 to 40 years old | | | 41 to 64 years old | | | >=65 years old | | |
| | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | | **[a] RR** | *95% CI* | |
| Lag 0 | 0.999 | 0.986 | 1.012 | 1.004 | 0.996 | 1.012 | 1.002 | 0.996 | 1.008 | 0.994 | 0.987 | 1.001 | 1.012 | 0.998 | 1.026 |
| Lag 0-1 | 0.967 | 0.943 | 0.991 | 0.968 | 0.953 | 0.983 | 0.975 | 0.963 | 0.987 | 1.003 | 0.989 | 1.017 | 1.010 | 0.982 | 1.038 |
| Lag 0-2 | 1.012 | 0.975 | 1.051 | **1.034** | **1.010** | **1.059** | 1.006 | 0.988 | 1.025 | 0.983 | 0.962 | 1.004 | 0.993 | 0.953 | 1.035 |
| Lag 0-3 | 1.044 | 0.995 | 1.095 | 1.022 | 0.989 | 1.056 | 1.015 | 0.990 | 1.040 | 0.983 | 0.956 | 1.010 | 0.985 | 0.930 | 1.042 |
| Lag 0-4 | 0.945 | 0.890 | 1.004 | 0.939 | 0.902 | 0.977 | 0.975 | 0.946 | 1.004 | **1.079** | **1.043** | **1.116** | 0.964 | 0.903 | 1.028 |
| Lag 0-5 | 1.000 | 0.930 | 1.075 | 1.012 | 0.967 | 1.061 | 1.030 | 0.994 | 1.066 | 0.973 | 0.934 | 1.014 | 1.040 | 0.964 | 1.122 |
| Lag 0-6 | 0.984 | 0.906 | 1.068 | **1.096** | **1.040** | **1.156** | 1.013 | 0.972 | 1.055 | 0.999 | 0.953 | 1.049 | 0.863 | 0.790 | 0.943 |
| Lag 0-7 | 1.054 | 0.959 | 1.158 | 0.962 | 0.904 | 1.024 | 1.022 | 0.974 | 1.071 | 0.993 | 0.940 | 1.050 | **1.286** | **1.162** | **1.422** |
| Lag 0-8 | 1.024 | 0.935 | 1.122 | 1.070 | 0.998 | 1.147 | 0.957 | 0.910 | 1.007 | 0.957 | 0.906 | 1.012 | 0.891 | 0.810 | 0.980 |
| Lag 0-9 | 0.983 | 0.934 | 1.034 | 0.908 | 0.871 | 0.948 | 0.994 | 0.964 | 1.024 | 1.022 | 0.990 | 1.056 | 0.992 | 0.944 | 1.043 |

## Discussion

This study was aimed to investigate the association between the increase in the average concentration of nitrate in drinking water from different sources between 2005 and 2015 and Asthma ED visits in California, USA through a time series study. The result of this study showed that there is s statistically significant association between Nitrate in drinking water and asthma ED visits in California, USA. The association was found while stratifying for gender, race/ethnicity and age.

In animal studies conducted previously, it was found that there is correlation among drinking water nitrate concentration, high methemoglobin levels, and pathologic changes in bronchi and lung parenchyma (Shuval et al., 1972; Gruener et al., 1970). The presence of Nitrate in drinking water could cause frequent dilation of bronchi with lymphocytic infiltration (Shuval et al., 1972; Gruener et al., 1970); in addition, it can also lead to atrophy of mucosa and muscles; frequent purulent bronchial exudates and interstitial round cell infiltration; and fibrosis at certain areas (Gupta et al., 2000; Shuval et al., 1972; Gruener et al., 1970). In a study conducted by Gupta et al., (1999) on toxicological effects of nitrate ingestion on cardiorespiratory tissues in rabbit, it was found that ingestion of nitrate through drinking water in different concentrations leads to significant changes in lung parenchyma. In other words, the rabbits' lung indicated congestion, the presence of inflammatory cells and the breakdown of alveoli (Gupta et al., 1999). It should be noted that the authors found that increase in the concentration of nitrate in water resulted in the increase in damage to the tissues in rabbits' lungs. World health Organization (WHO) also reported that when the concentration of airborne nitrate increases, the frequency of asthmatic attacks increases as well (WHO, 1977).

Based on the results of previous studies, the concentration of Nitrate in drinking water and food is not the only factor affecting the lung, but also the conditions leading to the reduction of Nitrate to Nitrite in human body (WHO, 1993; Li et al., 1997). In other words, Nitrates, through bacterial activity in human body and mostly in intestine and oral cavity, are reduced to Nitrites, and reactive free radicals of nitric oxides.

The result of this study indicated that the concentration of Nitrate in drinking water in CA in most populated cities is in violation of its standard level, as discussed above. Constant exposure to Nitrate through drinking water and the reductive reactions by bacteria in intestine and oral cavities could increase the concentration of inflammatory compounds (free radicals of nitric oxides) in human body, leading to permanent damage to lungs. The issue of asthma attack has been approached mostly from the effect of air pollutants on lungs

(Gharibi et al., 2020; Gharibi et al., 2019, Gharibi et al., 2018; Tavallali et al., 2020), however there has not been more than few studies focusing on water pollutants and their effects on this issue; considering the fact that too much exposure to Nitrate through drinking water could lead to permanent damage to lungs.

**Limitations**

There was also potential for misclassification of asthma cases when only using the first diagnosis code, rather than the first 2-3. In previous studies, it has been shown that (Castner et al. 2018) other conditions (e. g. infection) may exacerbate asthma and using the primary billing code would exclude such visits. However, using the other diagnosing codes (i. e. second and third diagnosing codes for asthma) may sacrifice specificity in capturing asthma ED visits; in other words, there are ED cases (e. g. sprains and fractures) which are non-related visits with a secondary diagnosis of asthma.

In addition, the data regarding the Nitrate in drinking water is released every six months; this affects the analysis on the effect of Nitrate on an acute response (e. g. asthma attack). This should also be noted that the water quality data is not based on zip-code or blocks; the boundary of a community receiving water from water treatment plant is not fixed which makes it hard to match it with hospital data.

Furthermore, asthma attacks are mainly linked to exposure to air pollutants and controlling for exposure to such pollutants seems logical; however, due to the discrepancy between water quality data and air pollution data, it is not possible to control for air pollutants in this study. In other words, the concentration of air pollutants fluctuates daily and seasonally, and these fluctuations can be associated with acute responses, including asthma attacks. However, taking annual or six months average of air pollutants remove the fluctuation from the variation in the concentration of the pollutants which makes it ineffective in regression analysis.

**Conclusion**

This study was aimed to investigate the association between the increase in the average concentration of nitrate in drinking water from different sources between 2005 and 2015 and Asthma ED visits in California, USA through a time series study. The results indicated that there is a statistically significant association between Nitrate in drinking water in CA and asthma ED visits between 2005 to 2015. This association was found among different races and age categories. Although there are not enough studies to conclude that Nitrate worsen the asthma attacks among people, the pathological pathway of Nitrate affecting lung tissues suggest that the increase in the concentration of this pollutant in water in CA is of concern.

# **Chapter 3:** Investigating the effect of Nitrate in drinking water on chronic respiratory diseases in CA, USA between 2005 and 2015

**Abstract**

***Background:*** The growth in human population has resulted in ever increasing usage of Nitrogen Fertilizers by farmers around the world and in California, USA. This has caused an increase in the concentration of Nitrate in surface and ground water.

***Objective:*** This study is aimed to investigate the effect of Nitrate in drinking water in CA on the exacerbation of these chronic respiratory diseases, including COPD, pneumonia and bronchitis.

***Material and Methods:*** A generalized linear model was developed to study the association between Nitrate in drinking water in CA and exacerbation of COPD, pneumonia and bronchitis between 2005 and 2015. By stratifying for sex, race, and age on the outcome, the effects of these modifiers are also investigated in this study.

***Results:*** In this work, 82091, 63449, and 51190 ED visits due to COPD, bronchitis and pneumonia were recorded, respectively, by OSHPD during 2005 to 2015. The results indicate that there is a statistically significant association between Nitrate in drinking water and ED visits due to COPD, pneumonia, and bronchitis. The highest risk ratio (RR) for COPD, pneumonia and bronchitis was 2.2% [RR: 1.022 (95% confidence intervals: 1.000, 1.045)], 6.4% [RR: 1.064 (95% confidence intervals: 1.023, 1.106)], and 8.7% [RR: 1.087 (95% confidence intervals: 1.046, 1.130)], respectively. It was found that female Hispanics who were 6 to 18 and 41 to 64 years old and suffering from bronchitis were affected most by exposure to Nitrate in drinking water. For pneumonia, it was found that Non-Hispanic

Blacks who were 65 or older were affected by exposure to Nitrate in drinking water. Stratifying for gender, race and age, no association between those who suffer from COPD and exposure to Nitrate was found.

***Conclusion:*** The result of this study showed that there is a statistically significant association between Nitrate in drinking water and bronchitis, pneumonia and COPD in California, USA. The association with bronchitis and pneumonia was found while stratifying for gender, race/ethnicity and age but not for COPD.

***Keywords:*** COPD; Pneumonia; Bronchitis; California; Drinking water; Nitrate

## Introduction

There have been researchers around the world focusing on the effects of Nitrate on human health, particularly on its effect on respiratory systems (Gupta et al., 2000; Gupta et al., 1999; Shuval et al., 1972; Gruener et al., 1970). It was reported that exposure to Nitrate through drinking water could cause permanent damage to lung tissues and pathologic changes in bronchi and lung parenchyma (Shuval et al., 1972; Gruener et al., 1970). Considering these, there has been scarce number of studies on the effects of Nitrate in drinking water on respiratory system and its associated diseases, especially Chronic Obstructive Pulmonary Disease (COPD), pneumonia and bronchitis.

12 to 16 million people in the United States suffer from Chronic Obstructive Pulmonary Disease (COPD) (Mathers et al., 2003). It is speculated that the prevalence of COPD will increase in the near future (GOLD, 2015). Those who suffer from COPD experience intermittent exacerbation of the disease, resulting in worsening of health-related quality of life, hastened lung function decline, and reduced functional capacity (Donaldson et al., 2002; Seemungal et al., 1998). There are also reports indicating that the intermittent exacerbation of this disease results also in increased risk of death (Miravitlles et al., 2002; Connors et al., 1996; Donaldson and Wedzicha, 2006). Pneumonia, mostly prevalent among children, is reported to be the main cause of childhood death by 1.3 million deaths among children under five years (Walker et al., 2013). Bronchitis, characterized by persistent cough and sputum production, is reported to involve 9 million adults in 2018 (American Lung Association, 2022). Many people in CA and worldwide are suffering from

these diseases and the prevalence of these diseases is expected to increase in near future. However, most of the studies on these topics are from air pollution point of view. To the best of our knowledge, there has not been any study focusing on the role of Nitrate in drinking water on the exacerbation of these chronic respiratory diseases.

Therefore, this study is aimed to investigate the effect of Nitrate in drinking water in CA on the exacerbation of these chronic respiratory diseases, including COPD, pneumonia and bronchitis. A generalized linear model was developed to study this effect. By stratifying for sex, race, and age on the outcome, the effects of these modifiers are also investigated in this study.

## Materials and Methods

### *Data and Participants*

In this study, three chronic respiratory diseases were selected to be investigated. The ED visit data regarding COPD, pneumonia, and bronchitis for the years of 2005 to 2015 in California, USA was obtained from California's Office of Statewide Health Planning and Development (OSHPD). The ED visits were identified using the International Classification of Diseases, $9^{th}$ Revision (ICD-9) code for related visits (National Center for Health Statistics, 2011). From the OSHPD dataset, patient's date of visit, principal diagnosis, residential ZIP code, the patient's age, race, and sex were kept for the analysis. Multiple visits by a single person cannot be identified because the data is not linked by person longitudinally due to the lack of access to social security number; therefore, each valid observation was taken as an independent observation.

### *Study design*

In this time-series study, a generalized linear model of Poisson family was applied with the aim of investigating the association between exposure to increased concentration of Nitrate in drinking water in California from 2005 to 2015 and those who visited ED due to COPD, pneumonia, and bronchitis between 2010 to 2015. It should be noted that the investigation on each disease was conducted separately. The dependent variable is the number of ED visits due to COPD, pneumonia, or bronchitis during 2005 to 2015. Figure 2 shows all the sampling stations within the study area and each county. As can be seen from the figure, the number and distribution of stations covers the residential areas within the counties. To handle the overdispersion (i. e. the variance of the outcome counts is higher than predicted under a Poisson distribution) in the data, the Pearson chi-square statistic divided by the residual degrees of freedom as a scale parameter is applied (McCullagh 1989).

*Exposure Assessment*

The water quality data was obtained from California State Water Resources Control Board at Electronic Data Transfer (EDT) Library and Water Quality Analyses Data and Download Page (CSWCB, 2018). This dataset contains information on each water quality parameters (i. e. nearly 580 different parameters) among which Nitrate ($NO_3$) was extracted.

In this dataset, 19 different sources of sampling for water quality parameters are included, which only the samples from active treated (an active source which is sampled after any treatment), treated water in the distribution system (Sample point within the distribution system after treatment), combined treated (Combined sources which are treated), and purchased water (Purchased source water which is sampled after any treatment) are extracted for this study. The goal of choosing this type of samples were to only focus on the water treated for drinking purposes; in other words, these samples are taken from points where people in each county are more possible to drink from than the other sources.

The data for $NO_3$ from 1995 to 2015 was available only for 25 counties (Alameda, Butte, Calaveras, Contra Costa, Fresno, Kern, Kings, Lake, Los Angeles, Marin, Nevada, Placer, Riverside, Sacramento, San Bernardino, San Diego, San Joaquin, San Luis Obispo, San Mateo, Santa Barbara, Santa Clara, Santa Cruz, Solano, Stanislaus, and Ventura) in

California. Due to this and the fact that nearly 30 million out of 37 million population of California residents live in these 25 counties, we only focused on this area.

*Statistical analysis*

In this study, generalized linear model of Poisson family was applied to estimate the association between increase in $NO_3$ and increase in the count of ED visits due to COPD, pneumonia, and/or bronchitis. The model used is as follows:

$$Y_t \sim Poisson\ (\mu_t)$$

$$\log(\mu_t) = \alpha + \beta_1(NO_3) + \ f(Time_t, 4) + Calendar\ Time$$

where $t$ is the day of the observation, $Y_t$ is the count of ED visits due to COPD, pneumonia or bronchitis on year t, α is the intercept, $s(Time_t, 4)$ is a flexible spline function with 4 $df$ for seasonal and long-term patterns. $(NO_3)$ stand for Nitrate. Furthermore, $\beta_1$ refers to the coefficient for $NO_3$.

To control for seasonal and long-term patterns in this work, periodic functions (Fourier series terms) was applied. Using this method, pairs of sine and cosine functions of time can be created to capture very regular seasonal patterns (Bhaskaran et al. 2013). Noted that long-term non-seasonal trends cannot be included in the model by using only Fourier terms; that is the reason of adding calendar time to the model (Bhaskaran et al. 2013). Four harmonics (4 sine/cosine pairs) was included in the model to capture seasonality, plus a linear function of time to capture broader trends over time. In this study, the annual count of ED visits in each community during 2005 to 2015 was merged with mean concentration of $NO_3$ (mg/l as $NO_3$); and, the effect of sex, age of diagnosis, and race/ethnicities were investigated to account for the probable modifiers. The reported risk ratios (RR) and 95% confidence intervals (CI) in this study are based on 1 (mg/l as $NO_3$) increase in the concentration of $NO_3$. All analyses were performed using STATA V. 14 (College Station, TX).

## Results

### *Descriptive analysis of the hospital data*

In this work, as shown in Table 1, 82091, 63449, and 51190 ED visits due to COPD, bronchitis and pneumonia were recorded, respectively, by OSHPD during 2005 to 2015. The data was stratified for gender, race/ethnicity, and age (Table 8).

For COPD, 58% of the study population were females, while 42% were males. 54%, 10%, 26% and 4% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. 26%, 42%, and 30% of the visits were recorded from 19 to 40, 41 to 64, and 65+ years old, respectively. For bronchitis, 59% of the study population were females, while 41% were males. 44%, 9%, 38% and 4% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. 8%, 11%, 36%, 32%, and 13% of the visits were recorded from 2 to 5, 6 to 18, 19 to 40, 41 to 64, and 65+ years old, respectively. For pneumonia, 49% of the study population were females, while 51% were males. 47%, 8%, 34% and 6% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. 11%, 10%, 22%, 31%, and 25% of the visits were recorded from 2 to 5, 6 to 18, 19 to 40, 41 to 64, and 65+ years old, respectively.

**Table 8**: Characteristics of COPD, Bronchitis and Pneumonia ED visits during the years 2005 to 2015 in California, USA

| Characteristics | COPD<br># (%) | Bronchitis<br># (%) | Pneumonia<br># (%) |
|---|---|---|---|
| **Sex** | | | |
| *Female* | 47526 (57.89) | 37597 (59.26) | 25203 (49.23) |
| *Male* | 34565 (42.11) | 25851 (40.74) | 25987 (50.77) |
| **Race** | | | |
| *White* | 44972 (54.78) | 27859 (43.91) | 23918 (46.72) |
| *Black* | 8221 (10.01) | 5973 (9.41) | 3980 (7.77) |
| *Hispanic* | 21529 (26.23) | 23893 (37.66) | 17392 (33.98) |
| *Asians* | 3330 (4.06) | 2539 (4.00) | 2857 (5.58) |
| *Others* | 4039 (4.91) | 3185 (5.02) | 3043 (5.95) |
| **Age** | | | |
| *2-5* | - | 4995 (7.87) | 5840 (11.41) |
| *6-18* | - | 6838 (10.78) | 4953 (9.68) |
| *19-40* | 21859 (26.63) | 22801 (35.94) | 11358 (22.19) |
| *41-64* | 35116 (42.78) | 20444 (32.22) | 16003 (31.26) |
| *>=65* | 25116 (30.60) | 8371 (13.19) | 13036 (25.47) |
| **Total Population** | **82091** | **63449** | **51190** |

*Exposure to $NO_3$: a Poisson Regression Model*

The regression analysis was conducted using single and cumulative lags. The results, as shown in Table 9, indicate that there is a statistically significant association between Nitrate in drinking water and ED visits due to COPD, pneumonia, and bronchitis. Based on the results, 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-6 is associated with COPD ED visits risk ratio (RR) of 2.2% [RR: 1.022 (95% confidence intervals: 1.000, 1.045)]. The highest RR for pneumonia was found at lag 6 [RR: 1.064 (95% confidence intervals: 1.023, 1.106)]; the cumulative lag for pneumonia indicated that 1 mg/L as $NO_3$

increase in the concentration of $NO_3$ at lag 0-6 is associated with pneumonia ED visits risk ratio (RR) of 5.1% [RR: 1.051 (95% confidence intervals: 1.013, 1.089)]. The highest RR for bronchitis was found at lag 5 [RR: 1.087 (95% confidence intervals: 1.046, 1.130)]; the cumulative lag for bronchitis indicated that 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-6 is associated with bronchitis ED visits risk ratio (RR) of 10% [RR: 1.100 (95% confidence intervals: 1.061, 1.140)].

**Table 9**. The association between $NO_3$ exposure and ED visits due to COPD, pneumonia, and bronchitis in California during the years 2005 to 2015

| | COPD | | | Pneumonia | | | Bronchitis | | |
|---|---|---|---|---|---|---|---|---|---|
| | [a] **RR** | *95% CI* | | [a] **RR** | *95% CI* | | [a] **RR** | *95% CI* | |
| Lag 0 | 1.033 | 0.993 | 1.075 | 1.001 | 0.977 | 1.024 | **1.054** | **1.013** | **1.095** |
| Lag 1 | 0.980 | 0.949 | 1.013 | 0.991 | 0.968 | 1.015 | 1.000 | 0.974 | 1.026 |
| Lag 2 | 0.825 | 0.636 | 1.016 | 1.013 | 0.990 | 1.037 | 0.988 | 0.963 | 1.014 |
| Lag 3 | 1.017 | 0.994 | 1.041 | **1.062** | **1.021** | **1.104** | 1.012 | 0.989 | 1.036 |
| Lag 4 | 1.010 | 0.987 | 1.034 | 1.004 | 0.978 | 1.029 | 1.014 | 0.991 | 1.038 |
| Lag 5 | 1.013 | 0.988 | 1.040 | 1.015 | 0.990 | 1.041 | **1.087** | **1.046** | **1.130** |
| Lag 6 | 1.010 | 0.922 | 1.098 | **1.064** | **1.023** | **1.106** | 0.981 | 0.956 | 1.007 |
| Lag 7 | 1.027 | 0.987 | 1.069 | 1.003 | 0.978 | 1.028 | 1.022 | 0.999 | 1.046 |
| Lag 8 | 1.038 | 0.997 | 1.080 | 1.042 | 0.988 | 1.098 | 1.069 | 0.998 | 1.141 |
| Lag 9 | 1.041 | 0.998 | 1.084 | 1.021 | 0.998 | 1.045 | 1.005 | 0.979 | 1.031 |
| Lag 0-3 | 0.908 | 0.692 | 1.124 | 1.013 | 0.990 | 1.039 | **1.068** | **1.041** | **1.095** |
| Lag 0-6 | **1.022** | **1.000** | **1.045** | **1.051** | **1.013** | **1.089** | 1.016 | 0.976 | 1.057 |
| Lag 0-9 | 1.000 | 0.978 | 1.021 | 1.022 | 0.999 | 1.046 | **1.100** | **1.061** | **1.140** |

*[a] risk ratio (95% CI) per 1 mg/L as NO3 increase in* $NO_3$

*Cumulative lag models: stratification by sex, race, and age*

In this stud, cumulative lag 0-9 was applied to investigate sex, race and age. Based on the results, as shown in Table 10 below, no statistically significant association was found between Nitrate in drinking water and COPD in different genders, races and ages.

For bronchitis, it was found that 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ is associated with bronchitis ED visits risk ratio (RR) of 2.1% [RR: 1.021 (95% confidence intervals: 1.007, 1.035)]. Among different race/ethnicities, Hispanics were found to be affected by exposure to Nitrate in drinking water; no significant association was found among other race/ethnicities. It was found that 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ is associated with bronchitis ED visits risk ratio (RR) of 4.8% [RR: 1.048 (95% confidence intervals: 1.035, 1.061)]. Among different ages, statistically significant association was found between exposure to Nitrate and those who age 6 to 18 years [RR: 1.024 (95% confidence intervals: 1.011, 1.037)] and 41 to 64 years [RR: 1.044 (95% confidence intervals: 1.015, 1.073)].

For pneumonia, no significant association was found while stratifying for female and male. Among different race/ethnicities, Non-Hispanic Blacks were found to be affected by exposure to Nitrate in drinking water; no significant association was found among other race/ethnicities. It was found that 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ is associated with pneumonia ED visits risk ratio (RR) of 8.4% [RR: 1.084 (95% confidence intervals: 1.004, 1.164)]. Among different ages, statistically significant association was found between exposure to Nitrate and those who age 65 years old and/or older [RR: 1.036 (95% confidence intervals: 1.018, 1.054)].

**Table 10**. The association between cumulative $NO_3$ exposure and ED visits due to bronchitis, pneumonia, and COPD in California during the years 2005 to 2015

| | Bronchitis | | | Pneumonia | | | COPD | | |
|---|---|---|---|---|---|---|---|---|---|
| Characteristics | [a] RR | (95 % CI) | | [a] RR | (95 % CI) | | [a] RR | (95 % CI) | |
| **Sex** | | | | | | | | | |
| *Female* | **1.021** | **1.007** | **1.035** | 1.011 | 0.990 | 1.032 | 1.016 | 0.985 | 1.047 |
| *Male* | 1.012 | 0.995 | 1.029 | 1.015 | 0.988 | 1.042 | 1.025 | 0.999 | 1.051 |
| **Race** | | | | | | | | | |
| *White* | 1.031 | 0.994 | 1.068 | 1.041 | 0.998 | 1.084 | 1.019 | 0.977 | 1.061 |
| *Black* | 1.001 | 0.976 | 1.026 | **1.084** | **1.004** | **1.164** | 1.024 | 0.964 | 1.084 |
| *Hispanic* | **1.048** | **1.035** | **1.061** | 1.052 | 0.983 | 1.121 | 1.036 | 0.981 | 1.091 |
| *Asian* | 1.011 | 0.971 | 1.051 | 1.101 | 0.957 | 1.245 | 1.047 | 0.88 | 1.214 |
| **Age** | | | | | | | | | |
| *2-5* | 1.016 | 0.984 | 1.048 | 1.001 | 0.981 | 1.021 | - | - | - |
| *6-18* | **1.024** | **1.011** | **1.037** | 1.154 | 0.999 | 1.309 | - | - | - |
| *19-40* | 1.01 | 0.986 | 1.034 | 1.018 | 0.998 | 1.038 | 1.088 | 0.979 | 1.197 |
| *41-64* | **1.044** | **1.015** | **1.073** | 1.024 | 0.999 | 1.049 | 1.092 | 0.921 | 1.263 |
| *>=65* | 1.027 | 0.97 | 1.084 | **1.036** | **1.018** | **1.054** | 1.291 | 0.988 | 1.594 |

[a] *risk ratio (95% CI) per 1 mg/L as NO3 increase in* $NO_3$

## Discussion

This study was aimed to investigate the effect of Nitrate in drinking water in CA on the exacerbation of these chronic respiratory diseases, including COPD, pneumonia, and bronchitis. The result of this study showed that there is a statistically significant association between Nitrate in drinking water and bronchitis, pneumonia and COPD in California, USA. The association with bronchitis and pneumonia was found while stratifying for gender, race/ethnicity and age but not for COPD.

It was found that female Hispanics who were 6 to 18 and 41 to 64 years old and suffering from bronchitis were affected most by exposure to Nitrate in drinking water. For pneumonia, it was found that Non-Hispanic Blacks who were 65 or older were affected by exposure to Nitrate in drinking water. Stratifying for gender, race and age, no association between those who suffer from COPD and exposure to Nitrate was found. It should be noted that there is not any study available, to the best of our knowledge, to compare its result with the present study.

However, there has been animal studies indicating the role of Nitrate in drinking water on the respiratory system (Shuval et al., 1972; Gruener et al., 1970; Gupta et al., 2000). It has been shown in these studies that Nitrate could lead to permanent damage to respiratory systems (Shuval et al., 1972; Gruener et al., 1970; Gupta et al., 2000). From pathobiological point of view, it should be noted that the Nitrate by itself is harmless; in fact, the process of reducing Nitrate to reactive and toxic nitrites makes it poisonous to human body (Manassaram et al., 2006). Through release of Nitrite and NO in human body, Nitrite and Nitrite induce the generation of free radicals which in turn overwhelm the host antioxidant defense system (Azhipa et al.,1990; Rubenchik, 1990; Kashko et al., 1993). NO, in low concentrations (Nano-molars) is in fact a transmitter in brain and other tissues; however, when its concentration increases in body, from nano-molar to near-micromolar, NO is linked with toxicity and cell death (Moncada et al., 1991; Hryhorenko et al., 1995; Kerwin, 1995; Peranovich et al., 1995; Hall et al., 2009). Furthermore, Nitrate has an inhibitory effect on electron transport in the mitochondrial respiratory chain; this can result in blockade of cytochrome oxidase (Antonov et al., 1989; Babsky and Shostakovskaya, 1992; Oladele et al., 1997). In a study conducted by Shehata (2005) on rabbits indicated that exposure to Nitrate in drinking water in a short-term period resulted in a significant decrease in hemoglobin concentration, erythrocyte count, total protein, albumin and globulin concentrations.

As mentioned earlier, one of the ways in which Nitrate harm human body is through oxidative stress. This process occurs when the balance of prooxidant-antioxidant is ruptured, resulting in the changes of biomolecules of all types and tissue damage (Mircescu, 2008). In fact, when the amount of reactive oxygen species (ROS) increases drastically in human body or the antioxidant level decreases, oxidative stress occurs (Droge et al., 2006). Oxidative stress is involved in tissue injury, and it is associated with adult respiratory distress syndrome, diabetes mellitus, ischemia reperfusion injury of the heart, lung diseases, such as emphysema, pneumoconiosis, smoking-related lung diseases (Lu et al., 1999; Ayo et al., 1990; Sax et al., 1992; Akinwande and Adebule, 2003).

**Limitations**

One of the main limitations in this study is inability to include the reductive process of Nitrate in human body to Nitrite and NO. In other words, bacterial activity within the intestine and oral cavity is responsible for this reductive process which can be different in each person in terms of rate and reaction time.

Furthermore, respiratory diseases are mainly linked to exposure to air pollutants and controlling for exposure to such pollutants seems logical; however, due to the discrepancy between water quality data and air pollution data, it is not possible to control for air pollutants in this study.

**Conclusion**

This study was aimed to investigate the effect of Nitrate in drinking water in CA on the exacerbation of these chronic respiratory diseases, including COPD, pneumonia, and bronchitis. The result of this study showed that there is a statistically significant association between Nitrate in drinking water and bronchitis, pneumonia and COPD in California, USA. The association with bronchitis and pneumonia was found while stratifying for gender, race/ethnicity and age but not for COPD.  Considering the biological and physiological evidence of Nitrate being poisonous in human body and also the fact that the concentration of this pollutant has increased over the years in drinking water in CA, it is of utmost importance to have more research into this topic to be able to either confirm or disprove the role of Nitrate in exacerbation of respiratory diseases.

# **Chapter 4:** Nitrate in drinking water in California (CA) and respiratory diseases: a decision tree model

**Abstract**

***Backgrounds:*** The concentration of Nitrate in drinking water in CA has increased over the years. Rain and irrigation are the main ways through which Nitrate is leaching from agricultural lands to surface and groundwater resources, resulting in the increase in the concentration of Nitrate in water.

***Objective:*** This study is aimed to develop a decision tree model to investigate the level of Nitrate exposure among those who experienced COPD, asthma, bronchitis and pneumonia in their drinking water during 2005 to 2015.

***Materials and Methods:*** A decision tree model was developed to investigate the level of Nitrate exposure among those who experienced COPD, asthma, bronchitis and pneumonia in their drinking water during 2005 to 2015.

***Results:*** Based on the total population served in each county, Los Angeles, San Diego, San Bernardino and Fresno has the greatest average number of people served by systems in violation. 104701, 82091, 63449, and 51190 ED visits due to asthma attack, COPD, bronchitis and pneumonia were recorded, respectively, by OSHPD during 2005 to 2015. Based on the result of the decision tree model, there are 2114 and 2671 individuals, who were suffering from asthma and bronchitis, respectively, on average are exposed to the highest concentration of $NO_3$ in CA during 2005 and 2015.

***Conclusion:*** It was found that there are considerable number of people who suffer from bronchitis and asthma and live in areas where the concentration of Nitrate is in violation of its standard level. Considering the effects of Nitrate's by-product in human body on lung, the concentration of Nitrate should be lowered and also its standard level should be reconsidered and includes other diseases in setting up the level of Nitrate.

***Keywords:*** decision tree; Nitrate; California; respiratory diseases; bronchitis; asthma

## Introduction

The concentration of Nitrate in drinking water in CA has increased over the years (Pennino et al., 2017). this could be due to ever increasing use of nitrogen fertilizers in CA. In fact, CA is the leading US state in agricultural activity by producing nearly 400 commodities. The abundance of agricultural activity in this state has resulted in the increased application of Nitrogen fertilizers (CDFA, 2018). Rain and irrigation are the main ways through which Nitrate is leaching from agricultural lands to surface and groundwater resources, resulting in the increase in the concentration of Nitrate in water (Harter et al., 2012; Pennino et al., 2017).

There have been animal studies showing that exposure to Nitrate in drinking water could result in damage to lungs and different organs in human body (Shuval et al., 1972; Gruener et al., 1970; Gupta et al., 2000; Gupta et al., 1999). For example, in a study conducted by Gupta et al., (1999) on toxicological effects of nitrate ingestion on cardiorespiratory tissues in rabbit, it was found that ingestion of nitrate through drinking water in different concentrations leads to significant changes in lung parenchyma. Although the pathobiological pathway by which Nitrate can endanger human health is studied and also the fact that the greatest average annual number of people served by systems in violation of nitrate standard level is in California (Pennino et al., 2017), there has not been any study on the association between Nitrate in drinking water in CA and respiratory diseases.

Therefore, this study is aimed to develop a decision tree model to investigate the level of Nitrate exposure among those who experienced COPD, asthma, bronchitis and pneumonia in their drinking water during 2005 to 2015. Since respiratory diseases are linked to many different air pollutants as well, it is necessary to know if people who suffer from such respiratory diseases are exposed to Nitrate in their drinking water as well. And, if they are exposed, the level of exposure is also of high importance.

## Material and Methods

### *Data and Participants*

In this study, four respiratory diseases were selected to be investigated, namely, asthma, COPD, bronchitis and pneumonia. The ED visit data regarding these respiratory diseases for the years of 2005 to 2015 in California, USA was obtained from California's Office of Statewide Health Planning and Development (OSHPD). The ED visits were identified using the International Classification of Diseases, $9^{th}$ Revision (ICD-9) code for related visits (National Center for Health Statistics, 2011). From the OSHPD dataset, patient's date of visit, principal diagnosis, and residential ZIP code were kept for the analysis. Multiple visits by a single person cannot be identified because the data is not linked by person longitudinally due to the lack of access to social security number; therefore, each valid observation was taken as an independent observation.

### *Background in Conventional Trees*

A tree is a structure that drives the data down its structure and outputs the prediction. The prediction can be a class for classification task or a vector of real numbers for regression task. A tree consists of split nodes that drive data to its children and leaf nodes and denote the output (predict). The route of any input sample starts from the root node. At split nodes, split function determines what next child the input will follow. As a result, any input will start at the root and continues processing over internal nodes on path to a leaf node that denotes the output. A trainset is needed to construct the tree based on the trainset and minimize the error over the trainset. Figure 2 shows the structure of a tree.

A tree is a model that categorizes the data based on the similarity of the samples similar to a librarian that stores books (samples in the case of a decision tree) on the shelves based on the category of the book (or similarity of sample). Here, in a classification or regression, the category of a sample is represented by the output response of the sample. The tree further categorizes the samples and stores them in the tree's leaf nodes. This categorization is based on samples similarity to a specific pattern. The patterns are learned and exist at the split nodes of the tree. As a result, understanding of the patterns at the split nodes leads to further understanding of the dataset itself.

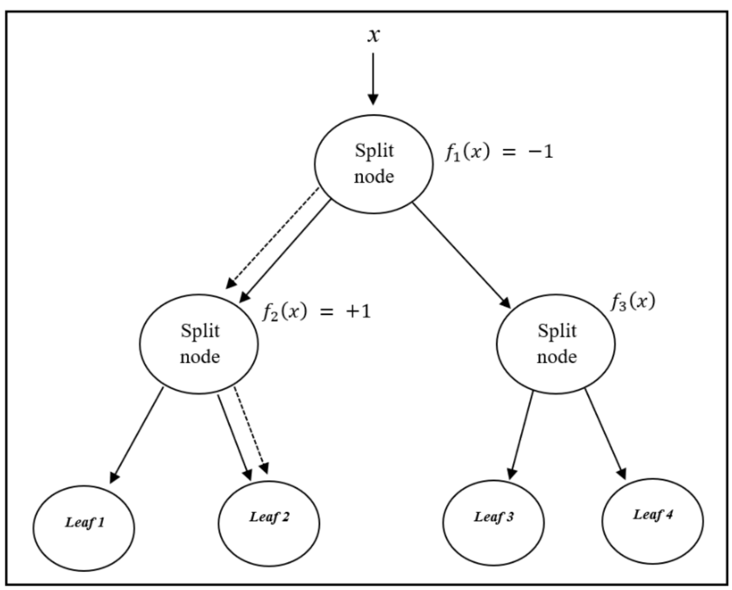


**Figure 3:** Decision tree model

Consider a trainset ($X$, $Y$) that contains $N$ observations ($x_i$, $y_i$), $i=1, \ldots\ldots N,$ where $x_i \in \mathbb{R}^p$ and $y_i \in \mathbb{R}^d$. Regression tree construction is cast as the following minimization problem by Breiman et al. (1984b)[17]:

$$\min_{T} \frac{1}{N} \sum_{i=1}^{N} L\left(y_i, T(x_i; F)\right) \ s.t. \begin{cases} N_j \geq N_{min} \forall_j \in \ leaves\ (T) \\ D \leq D_{\max} \end{cases} \quad [1]$$

Where $L\left(y_i, T(x_i; F)\right)$ is the error function (mean square error, absolute error, etc.), $T$ represents the tree function, leaves(T) returns the set of leaf nodes in T , and $F = \{f_1, \ldots\ldots,$ $f_{|T|}\}$is the decision functions at each node 1, ..., |T |. $D$ is the decision tree depth. $N_j$ is the number of samples at node $j$. The constraints in (1) show the limits on tree structure and minimum acceptable samples at each node. Finding optimal of (1) is NP-complete; hence, conventional algorithms approximate the solution to problem (1) using greedy algorithms. It is done by locally minimizing some cost function and recursively partitioning the data to grow the tree.

*Training decision tree*

Decision tree categorizes the patients for each disease based on the concentration of the input ($NO_3$), which was 1 mg/l in this study. After each disease was categorized by the primary model, 80% of the categorized data was selected to train the tree from which 60% was randomly selected for training. Then, the tree with the lowest error on validation set was selected.

## Results

*Descriptive analysis of the hospital data*

In this work, as shown in Table 1, 104701, 82091, 63449, and 51190 ED visits due to asthma attack, COPD, bronchitis and pneumonia were recorded, respectively, by OSHPD during 2005 to 2015. The data was stratified for gender, race/ethnicity, and age (Table 11).

For asthma, 56% of the study population were females, while 43% were males. 36.10%, 16.59%, 38.11% and 4.24% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. The mean age for the population in this study is 27 years old. 8.14%, 19.37%, 34.75%, 27.41%, and 7.77% of the visits were recorded from 2 to 5, 6 to 18, 19 to 40, 41 to 64, and 65+ years old, respectively. For COPD, 58% of the study population were females, while 42% were males. 54%, 10%, 26% and 4% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. 26%, 42%, and 30% of the visits were recorded from 19 to 40, 41 to 64, and 65+ years old, respectively. For bronchitis, 59% of the study population were females, while 41% were males. 44%, 9%, 38% and 4% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively. 8%, 11%, 36%, 32%, and 13% of the visits were recorded from 2 to 5, 6 to 18, 19 to 40, 41 to 64, and 65+ years old, respectively. For pneumonia, 49% of the study population were females, while 51% were males. 47%, 8%, 34% and 6% of the visits were Non-Hispanic White, Non-Hispanic Black, Hispanic and Asian race/ethnicities, respectively.

11%, 10%, 22%, 31%, and 25% of the visits were recorded from 2 to 5, 6 to 18, 19 to 40, 41 to 64, and 65+ years old, respectively.

**Table 11**: Characteristics of asthma, COPD, Bronchitis and Pneumonia ED visits during the years 2005 to 2015 in California, USA

| Characteristics | Asthma<br># (%) | COPD<br># (%) | Bronchitis<br># (%) | Pneumonia<br># (%) |
|---|---|---|---|---|
| **Sex** | | | | |
| *Female* | 58905 (56.26) | 47526 (57.89) | 37597 (59.26) | 25203 (49.23) |
| *Male* | 45794 (73.74) | 34565 (42.11) | 25851 (40.74) | 25987 (50.77) |
| **Race** | | | | |
| *White* | 37796 (36.10) | 44972 (54.78) | 27859 (43.91) | 23918 (46.72) |
| *Black* | 17370 (16.59) | 8221 (10.01) | 5973 (9.41) | 3980 (7.77) |
| *Hispanic* | 39904 (38.11) | 21529 (26.23) | 23893 (37.66) | 17392 (33.98) |
| *Asians* | 4438 (4.24) | 3330 (4.06) | 2539 (4.00) | 2857 (5.58) |
| *Others* | 5193 (4.96) | 4039 (4.91) | 3185 (5.02) | 3043 (5.95) |
| **Age** | | | | |
| *2-5* | 8523 (8.14) | - | 4995 (7.87) | 5840 (11.41) |
| *6-18* | 20285 (19.37) | - | 6838 (10.78) | 4953 (9.68) |
| *19-40* | 36381 (34.75) | 21859 (26.63) | 22801 (35.94) | 11358 (22.19) |
| *41-64* | 28696 (27.41) | 35116 (42.78) | 20444 (32.22) | 16003 (31.26) |
| *>=65* | 8136 (7.77) | 25116 (30.60) | 8371 (13.19) | 13036 (25.47) |
| **Total Population** | **104701** | **82091** | **63449** | **51190** |

In order to have a better understanding of the trend of ED visits within the study area, the monthly number of ED visits due to asthma, bronchitis, COPD, and pneumonia in different regions under study between 2005 and 2015 was investigated (Fig. 3). As can be seen from Fig. 4, asthma is the prevalent respiratory disease within the study area and ED visits due to asthma increase mostly during the cold season and normally lowest around July. In Mojave Desert and Sacramento Valley, the ED visits due to asthma peak during the spring.

*Exposure to $NO_3$: A Decision Tree Model*

Decision tree, through classification based on the concentration of $NO_3$, categorized individuals into leaf nodes. In the following sections, each leaf node is explained.

*Decision Tree Analysis for Asthma Attack*

Figure 4 indicates the decision tree analysis for asthma attack and exposure to $NO_3$. In root node 1 “RN 1”, the population of people who were suffering from asthma attack during 2005 to 2015 are divided into two groups based on the concentration of $NO_3$ which they were exposed to at the time of visiting ED. The population which was exposed to the lowest concertation are sent to leaf node 1 “LN 1”. The average number of individuals per interval in the lowest section is 710, meaning that 710 individuals in average are exposed to the lowest concentration of $NO_3$ in CA during 2005 to 2015. In the split node, the rest of population is divided into leaf node 2 and 3 based on the concentration of $NO_3$ that they were exposed to. Those who were exposed to higher than 9 mg/L as $NO_3$ are put into leaf node “LN 3”. Based on the result, there are 2114 individuals in average who were suffering from asthma and exposed to highest concentration of $NO_3$ in CA during 2005 and 2015.

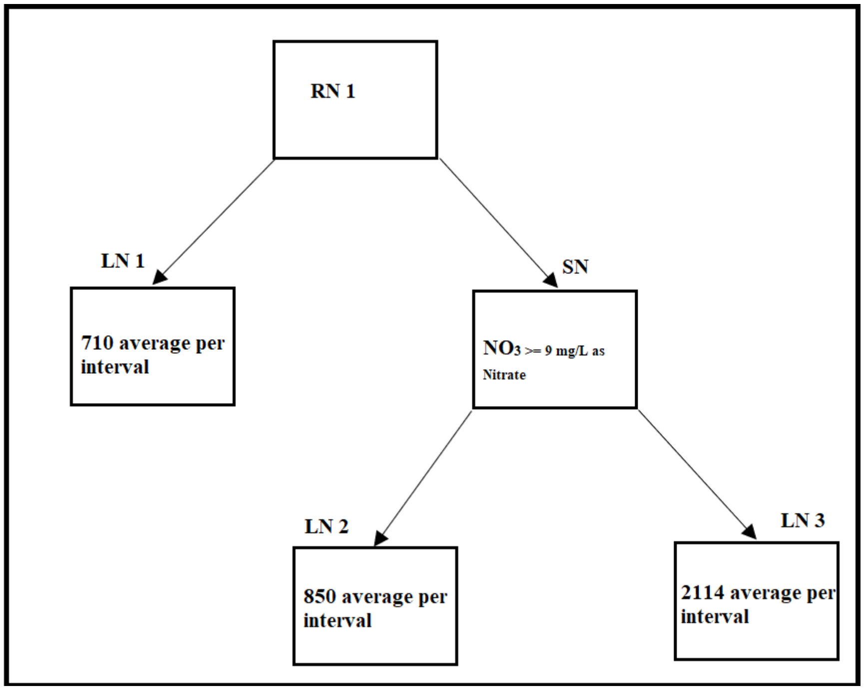


**Figure 4:** Decision tree analysis for asthma attack and exposure to $NO_3$ in CA, USA during 2005-2015

*Decision Tree Analysis for bronchitis Attack*

Figure 5 indicates the decision tree analysis for bronchitis and exposure to $NO_3$ in CA, USA during 2005-2015. As can be seen from the figure, those who were suffering from bronchitis and exposed to $NO_3$ are categorized based on the concentration of $NO_3$ which they were exposed to during 2005 to 2015. There are 640 individuals in average who are exposed to the lowest concentration of $NO_3$ in CA during 2005 to 2015. In split node 1 "SN 1", the population under study is divided into two groups based on the concentration of $NO_3$; those who are sent to leaf node 2, 780 individuals in average per interval, are exposed to less than 9 mg/L as $NO_3$. In split node 2, people are split again based on the concentration of $NO_3$ that they were exposed to; those who were exposed to between 9 and

12.5 mg/L as $NO_3$ are sent to leaf node 3. There are 896 individuals on average per interval who are exposed to between 9 and 12.5 mg/L as $NO_3$ at the time of their visit to ED due to bronchitis. 2671 individuals on average per interval are exposed to >12.5 mg/L as $NO_3$ at the time of their visit to ED due to bronchitis as shown in the figure (leaf node 4 "LN 4").

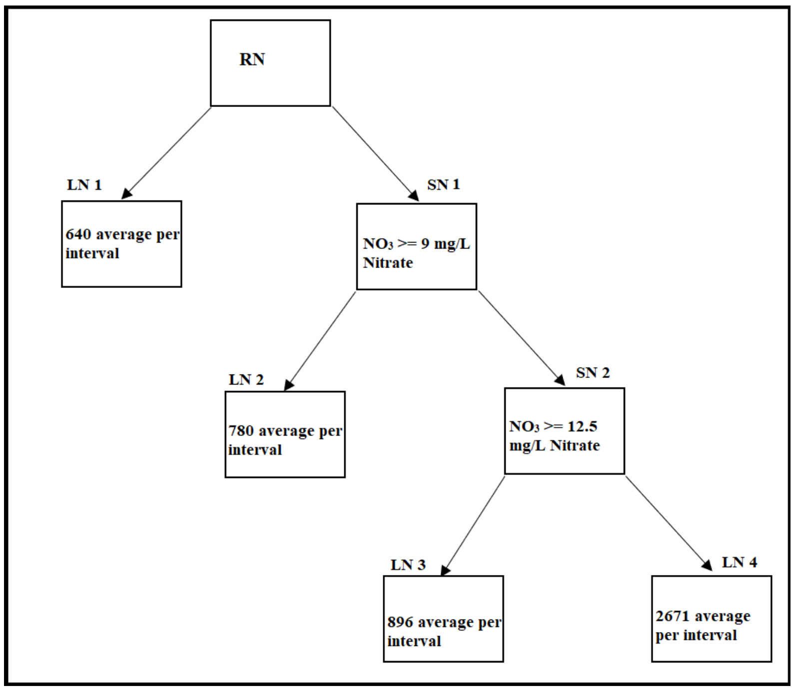


**Figure 5:** Decision tree analysis for bronchitis and exposure to $NO_3$ in CA, USA during 2005-2015

*Decision Tree Analysis for COPD Attack*

Figure 6 indicates the decision tree analysis for COPD and exposure to $NO_3$ in CA, USA during 2005-2015. For COPD, the decision tree was not able to go further than the first categorization. This means that the decision tree was not able to distinguish between those who were living in locations with highest and lowest concentration of $NO_3$. In fact, the decision tree was not able to find any association between visiting ED due to having COPD and concentration of $NO_3$ in drinking water in locations where they were living.

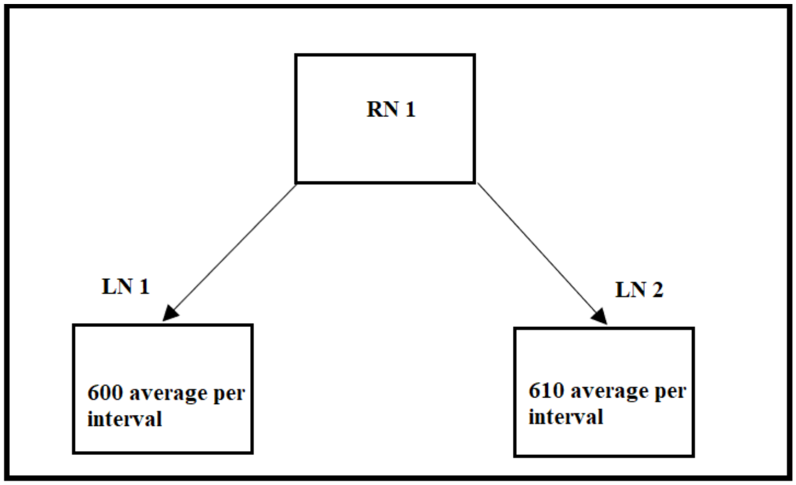


**Figure 6:** Decision tree analysis for COPD and exposure to $NO_3$ in CA, USA during 2005-2015

## Discussion

This study was aimed to investigate the association between $NO_3$ in drinking water in CA and visiting ED due to asthma, COPD, pneumonia, and bronchitis. The results indicated that there were on average 2114 and 2617 individuals who visited ED due to, respectively, asthma and bronchitis. No meaningful result was found for COPD and pneumonia in this study. These groups of people were living in the areas with the highest concentration of $NO_3$. To the best of our knowledge, there are no study using decision tree to find any possible association between $NO_3$ and respiratory diseases to compare the results.

Considering the fact that the standard level set for $NO_3$ is aimed to protect infants from methemoglobinemia, and it is not for protecting people from other possible diseases in relation with exposure to nitrate (Ward et al., 2018), having many people living in areas with high concentration of Nitrate in drinking water may result in having permanent health related issues. In a study conducted by Gupta et al., (1999), it was found that increase in the concentration of nitrate in water resulted in the increase in damage to the tissues in rabbits' lungs. Based on studies on animals, it is shown that exposure to Nitrate in drinking water can cause permanent damage to lung tissues and pathologic changes in bronchi and lung parenchyma (Shuval et al., 1972; Gruener et al., 1970; Gupta et al., 2000).

Exposure to Nitrate by itself is not harmful; in fact, the reductive process by which Nitrate turns to Nitrite and NO within human body is of importance here since Nitrite and nitric oxide are harmful to human body (Manassaram et al., 2006). The exposure to Nitrate could change the balance of prooxidant-antioxidant, resulting in the changes of biomolecules of all types and tissue damage (Mircescu, 2008). NO which is a byproduct of reducing Nitrate is linked to cellular damage and death (Moncada et al., 1991; Hryhorenko et al., 1995; Kerwin, 1995; Peranovich et al., 1995; Hall et al., 2009). One of the ways in which Nitrate affect human body is through creating free radicals, resulting in oxidative stress (Mircescu, 2008). When the amount of reactive oxygen species (ROS) increases drastically in human body or the antioxidant level decreases, oxidative stress occurs (Droge et al., 2006). Oxidative stress is involved in tissue injury, and it is associated with adult respiratory distress syndrome, diabetes mellitus, ischemia reperfusion injury of the heart, lung diseases, such as emphysema, pneumoconiosis, smoking-related lung diseases (Lu et al., 1999; Ayo et al., 1990; Sax et al., 1992; Akinwande and Adebule, 2003).

**Conclusion**

This study was aimed to investigate the association between $NO_3$ in drinking water in CA and visiting ED due to asthma, COPD, pneumonia, and bronchitis. The results indicated that there were on average 2114 and 2617 individuals who visited ED due to, respectively, asthma and bronchitis. No meaningful result was found for COPD and pneumonia in this study. The concentration of Nitrate in drinking water in CA has increased drastically over the years and more and more people are living in areas with higher concentrations of Nitrate in their drinking water. The by-products of reductive reactions of Nitrate in human body are proven to be very dangerous at cellular levels, causing cellular damages to lung. Considering this, the exposure to Nitrate in drinking water in CA should be taken into account from public health point of view.

# Chapter 5: General Conclusion

California has been suffering from different environmental pollutants, namely, air pollutants and water pollutants. The agricultural activity in this region has caused increase in the utilization of fertilizers leading to increase in the concentration of pollutants, including Nitrate, in surface and groundwater resources. Nitrate has been shown to cause oxidative stress, one of the main factors affecting the lung activity. It has also shown that this pollutant can permanently damage human cells within the body through different reactions. Due to its ability to reduce to Nitrite and then free radicals, it was speculated that this pollutant can worsen respiratory diseases through the process called oxidative stress. Therefore, we decided to study the association between Nitrate in drinking water and respiratory diseases in California. I applied two different models, a generalized linear model and decision tree, to study this possible association.

In my first study on the association between the increase in the average concentration of Nitrate in drinking water from different sources between 2005 and 2015 and Asthma ED visits in California, USA through a time series study (GLM), I found that there is a statistically significant association between Nitrate in drinking water in CA and asthma ED visits between 2005 to 2015. In this study, 104701asthma ED visits were recorded by OSHPD during 2005 to 2015. Based on the results, the highest RR was found at lag 0-6; a 1 mg/L as $NO_3$ increase in the concentration of $NO_3$ at lag 0-6 is associated with an asthma ED visits risk ratio of 3.2% [RR: 1.032 (95% confidence intervals: 1.017, 1.047)].

In my second study on the effect of Nitrate in drinking water and chronic respiratory diseases, I found that there is a statistically significant association between Nitrate in drinking water and bronchitis, pneumonia and COPD in California, USA. The association with bronchitis and pneumonia was found while stratifying for gender, race/ethnicity and age but not for COPD.  In my third study which I applied decision tree model, I found that there are considerable number of people who suffer from bronchitis and asthma and live in areas where the concentration of Nitrate is in violation of its standard level. Considering the effects of Nitrate's by-product in human body on lung, the concentration of Nitrate should be lowered and also its standard level should be reconsidered and includes other diseases in setting up the level of Nitrate.

The concentration of Nitrate has been increasing over the last two decades in California which is mostly due to the increase in the usage of Nitrogen Fertilizers. It has already shown that Nitrate in drinking water is connected to different types of cancer and this pollutant can harm people in many ways. The reduction process occurs within human body (i. e. anaerobic bacteria in oral cavities and intestines) turn this pollutant into known carcinogens and highly reactive compounds resulting in permanent damage to human cells. Considering these, there has not been any statewide plan to reduce the concentration of this pollutant in drinking water. We hope that conducting studies on this issue shed some light on this topic and bring this issue to the attention of policy makers and local public health jurisdictions.

## References

Akinwande, A.I. and Adebule, A. O. A. (2003). Ascorbic acid and beta-carotene alleviate oxidative effect of London King Size® cigarette smoke on tissue lipids. Nigerian Journal of Health and Biochemical Sciences. 2 (1): 12 – 15.

American Lung Association (ALA). Available online: https://www.lung.org/research/trends-in-lung-disease/copd-trends-brief/copd-prevalence (4/13/2022)

Antonov, B. I., Fedotova, V. I. and Sukhaya, N. A. (1989). Laboratory Investigation in Veterinary Medicine: Chemical and Toxicological Methods. Agroprdmizdat, Moscow, 320 pp.

Ayo, J. O. and Oladele, S. B. (1996). Natural antioxdiants and their potential uses in prophylaxis and therapy of disease conditions. West African Journal of Pharmacology and Drug Research. 12: 69 – 76.

Azhipa, Y. I., Reutov, V. P. and Kayushina, L. P. (1990). Ecological and medico-biological aspects of environmental pollution with nitrates and nitrites. Human Physiology. 16: 134 – 149.

Babsky, A. M. and Shostakovskaya, I. V. (1992). Effects of low concentration of sodium nitrite and nitrate on respiration and oxidative phosphorilation in the rat liver mitochondria. Ukrainian Biochemical Journal. 64: 71 – 74.

Balmes J. (1993). The Role of Ozone Exposure in the Epidemiology of Asthma. EnvironmentalHealthPerspectives , 101, 219-224.

Bhaskaran K, Gasparrini A, Hajat S, Smeeth L, Armstrong B (2013). Time series regression studies in environmental epidemiology. Int J Epidemiol 42(4): 1187–1195, PMID: 23760528, DOI: 10.1093/ije/dyt092.

CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE (CDFA). California agriculture production statistics. Available online: https://www.cdfa.ca.gov/statistics/ (accessed 12/31/2018)

California States Water Control Boards (CSWCB). Electronic Data Transfer (EDT) Library and Water Quality Analyses Data and Download Page. Available online: https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/EDTlibrary.html (accessed 12/31/2018)

Castner J, Guo L, Yong Y. (2018). Ambient air pollution and emergency department visits for asthma in Erie County, New York 2007-2012. Int Arch Occup Environ Health 91(2):205-214, PMID: 29043427, doi: 10.1007/s00420-017-1270-7.

Connors AF, Dawson NV, Thomas C, Harrell FE, Desbiens N, Fulkerson WJ, et al. (1996). Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments). Am J Respir Crit Care Med 1996; 154(4 Pt 1):959–967.

Donaldson GC, Wedzicha JA. (2006). COPD exacerbations—1: Epidemiology. Thorax; 61(2):164–168.

Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. (2002). Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax; 57(10):847–852.

Droge, W., Kinscherf, R., Hildebrandt, W.and Schmitt, J. (2006). The deficit in low molecular weight thiols as a target for anti-aging therapy. Current Drug Targets. 7 (11): 1505 – 1512.

Eisenbrand, G.; Spiegelhalder, B.; Preussmann, R. (1980). Nitrate and nitrite in saliva. Oncology, 37, 227–231.

Eisenbrand, G. The Significance of N-Nitrosation of Drugs; Nicolai, H.V., Eisenbrand, G., Bozler, G., Eds.; Gustav Fischer Verlag, Stuttgart: New York, NY, USA, 1990; pp. 47–69.

National Cancer Institute, The Surveillance, Epidemiology, and End Results. (https://seer.cancer.gov/data/) (accessed at 12/24/2018).

Galloway JN, Aber JD, Erisman JW, et al. (2010). The nitrogen cascade. Bioscience; 53:341–56.

Gharibi H, Entwistle MR, Schweizer D, Tavallali P, Cisneros R. (2019).The association between 1,3-dichloropropene and asthma emergency department visits in California, USA from 2005 to 2011: a bidirectional-symmetric case crossover study. J Asthma.

Gharibi H, Entwistle MR, Ha S, Gonzalez M, Brown P, Schweizer D, Cisneros R. (2018). Ozone pollution and asthma emergency department visits in the Central Valley, California, USA, during June to September of 2015: a time stratified case-crossover analysis. J Asthma;1–12.

Gharibi H, Entwistle MR, Schweizer D, Tavallali P, Thao C, Cisneros R. (2020). Methyl-bromide and asthma emergency department visits in California, USA from 2005 to 2011 J. Asthma, 57 (11), pp. 1227-1236

Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. 2015. Available from: http://www.goldcopd.org/guidelines-global-strategy-for-diagnosis-management.html (accessed 17 June, 2015).

Gruener N, Shuval Hi. Health aspects of nitrates in drinking water. In: Developments in Water Quality Research (Gruener N, Shuval HI, eds). Ann Arbor, Ml:Ann Arbor Humphrey Science Publishers, 1970;89-106.

Gupta et al., S.K. Gupta, R.C. Gupta, A.K. Seth, A.G. JK B. (2000). Recurrent acute respiratory tract infections in areas with high nitrate concentrations in drinking water Environ. Health Perspect., 108, p. 4

Gupta SK, Gupta RC, Seth AK, Gupta AB, Sharma ML, Gupta A. (1999). Toxicological effects of nitrate ingestion on cardiorespiratory tissues in rabbit. S Asian J Prev Cardiol 2(3):101-105

Hall, C. N., Robert, G., Keynes, R. G. and Garthwaite, J. (2009). Cytochrome P450 oxidoreductase participates in nitric oxide consumption by rat brain. Biochemical Journal, 419 (2): 411–418.

Harter, T., J. R. Lund, J. Darby, G. E. Fogg, R. Howitt, K. K. Jessoe, G. S. et al. (2012). Addressing Nitrate in California's Drinking Water with a Focus on Tulare Lake Basin and

Salinas Valley Groundwater. Report for the State Water Resources Control Board Report to the Legislature. Center for Watershed Sciences, University of California, Davis. 78 p. http://groundwaternitrate.ucdavis.edu.

Hill M, J Hawksworth G, Tattersall G.  (1973). Bacteria, nitrosamines and cancer of the stomach.  Brit J Cancer; 28: 562-567.

Hryhorenko, L. M., Woskowska, Z. and Foxthrelkeld, J. A. E. T. (1995). Nitric oxide (NO) inhibits release of nitric oxide. The Journal of Pharmacology and Experimental Therapeutics, 211: 918 – 926.

Kashko, N. F., Khokha, A. M., Antsulevich, S. N., Doroshkevich, N. A. and Voronov, P. P. (1993). The effect of ethanol and ethanol-induced lipid peroxidation on the steroidogenic activity of testes. Ukrainian Biochemical Journal. 65: 89-94.

Kerwin, J. F. Jr. (1995). Nitric oxide: A new paradigm for second messengers. Journal of Medical Chemistry. 38: 4343 – 4362.

Jakszyn P, Bingham S, Pera G, Agudo A, Luben R, Welch A, et al. Endogenous versus exogenous exposure toN-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. Carcinogenesisvol.27 no.7 pp.1497–1501, 2006. doi:10.1093/carcin/bgl019

Leach, S.A.; Thompson, M.; Hill, M. Bacterially catalyzed N-nitrosation reactions and their relative importance in the human stomach. Carcinogenesis 1987, 8, 1907–1912.

Li H, Duncan C, Townend J, Killham K, Smith LM, Johnston P, Dykhuizen R, Kelly D, Golden M, Benjamin N, et al. Nitrate-reducing bacteria on rat tongue. Appi Environ Microbiol 63(3):924-930 (1997).

Lu, C. Y., Lee, H. C., Fahn, H. J.and Wei, Y. H. (1999). Oxidative damage elicited by imbalance of free radical scavenging enzymes is associated with large scale MTDNA deletion in aging human skin. Mutation Research. 423 (1 – 2): 11 – 21.

Lv, J.; Neal, B.; Ehteshami, P.; Ninomiya, T.; Woodward, M.; Rodgers, A.; Wang, H.; MacMahon, S.; Turnbull, F.; Hillis, G.; et al. Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: A systematic review and meta-analysis. PLoS Med. 2012, 9, e1001293.

Manassaram, D. M., Backer, L. C. and Moll, D. M. (2006). A review of nitrates in drinking water: Maternal exposure and adverse reproductive and developmental outcomes. Environmental Health Perspective. 114 (3): 320 – 327.

Mathers CD, Bernard C, Moesgaard-Iburg K, Inoue M, Ma Fat D, Shibuya K, et al. Global Burden of Disease in 2002: data sources, methods and results. Geneva: World Health Organization 2003; 54.

McCullagh P, Nelder JA. Generalized Linear Models. 2nd edn. London: Chapman & Hall/CRC; 1989.

Miravitlles M, Murio C, Guerrero T, Gisbert R, Dafne Study Group. Pharmacoeconomic evaluation of acute exacerbations of chronic bronchitis and COPD. Chest 2002; 121(5):1449–1455.

Mircescu, G. (2008). Oxidative stress of chronic kidney disease. Acta Endocrinologica (Buc). 5 (4): 433 – 446.

Moncada, S., Palmer, R. M. J. and Higgs, E. A. (1991). Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacological Reviews. 43: 109 – 142.

Oladele, S. B., Ayo, J. O. and Adaudi, A. O. (1997). The emergence of nitrate and nitrite poisoning in humans and domestic animals. West African Journal of Pharmacology and Drug Research. 13: 50 - 58.

Pennino MJ, Compton JE, Leibowitz SG. Trends in Drinking Water Nitrate Violations Across the United States. Environ. Sci. Technol.2017, 51, 13450-13460

Peranovich, T. M. S., Da Silva, A. M., Fries, D. M., Stern, A. and Monteiro, H.P. (1995). Nitric oxide stimulates tyrosine phosphorylation in murine fibroblasts in the absence and presence of epidermal growth factor. Biochemical Journal. 305: 613 – 619.

Rubenchik, B. L., Kostyukovsky, Y. L. and Melamed, D. B. (1983). Prophylactics of Contamination of Food Products by Carcinogens, Zdorovya, Kiev, 160 pp.

Sax, V. A., Konorev, E. A., Grigoryantz, R.A. and Belenkov, K. N. (1992). Biochemistry of normal and ischaemic cardiac myocyte. Current research state. Cardiology. 32: 82 -91.

Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998; 157(5 Pt 1):1418–1422.

Schullehner J, Hansen B, Thygesen M, Pedersen CB, Sigsgaard T. Nitrate in drinking water and colorectal cancer risk: A nationwide population-based cohort study. Int. J. Cancer: 143, 73–79 (2018)

Shehata, S.A. (2005). Nitrate detoxification of drinking water by ascorbic acid in growing rabbits. World Rabbit Science. 13: 93 – 106.

Shuval HI, Gruener N. Epidemiological and toxicological aspects of nitrates and nitrites in the environment. Am J Public Health 62:1045-1052 (1972).

Spiegelhalder, B.; Eisenbrand, G.; Preussmann, R. Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to in vivo formation of N-nitroso compounds. Food Cosmet. Toxicol. 1976, 14, 545–548.

Tavallali P, Gharibi H, Singhal M, Schweizer D, Cisneros R. A multi-pollutant model: a method suitable for studying complex relationships in environmental epidemiology Air Qual. Atmos. Health, 13 (2020), pp. 645-657

Tricker, A.R.; Kalble, T.; Preussmann, R. Increased urinary nitrosamine excretion in patients with urinary diversions. Carcinogenesis 1989, 10, 2379–2382.

Tricker, A. R., and Preussmann, R. (1991). Carcinogenic N-nitrosamines in the diet: Occurrence, formation, mechanisms and carcinogenic potential. Mutat. Res.259,277-289.

USEPA. Regulated Drinking Water Contaminants: Inorganic Chemicals. Available online: https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants (accessed 12/31/2018).

C.L.F. Walker, I. Rudan, L. Liu, H. Nair, E. Theodoratou, Z.A. Bhutta, K.L. O'Brien, H. Campbell, R.E. Black Global burden of childhood pneumonia and diarrhoea Lancet, 381 (2013), pp. 1405-1416

Ward MH, Jones RR, Brender JD, de Kok TM, Weyer PJ, Nolan BT, Villanueva CM, van Breda SG. Drinking Water Nitrate and Human Health: An Updated Review. Int. J. Environ. Res. Public Health 2018, 15, 1557; doi:10.3390/ijerph15071557

Ward MH. Too much of a good thing? Nitrate from nitrogen fertilizers and cancer: president's cancer panel - October 21, 2008. Rev Environ Health 2009; 24:357–63.

Ward MH, Jones RR, Brender JD, de Kok TM, Weyer PJ, Nolan BT, Villanueva CM, van Breda SG. Drinking Water Nitrate and Human Health: An Updated Review. Int. J. Environ. Res. Public Health 2018, 15, 1557; doi:10.3390/ijerph15071557

WHO. Nitrate, Nitrite and N-Nitroso compounds. Environmental Health Criteria 5. Geneva: World Health Organization, 1977;77.

WHO. Guidelines for Drinking Water Quality, Vol. 1. Geneva: World Health Organization, 1993;52-82.