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## Nitrate in Groundwater and Excretion of Nitrate and Nitrosamines in Urine: A Review

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### Abstract

Nitrate is harmful to humans as it can form endogenous nitrosamines which can cause cancer. The major contribution of nitrate contamination in well water is largely from agricultural activities (e.g.; fertilizers and pesticide), wastewater treatment plant discharge, animal yard and manure storage lagoons. Biomarkers such as urine and saliva can be used to determine the occurrence and formation of nitrosamines in the human body. This paper provides an overview of nitrate occurrence in groundwater and the mechanism of nitrosamines formation and its excretion from human body via urine and saliva. The suitability of urine and saliva as biomarkers of endogenous nitrosamines formation were also discussed in this review.

**Keywords:** groundwater; nitrate; nitrosamines; urine; saliva

### 1. Introduction

Humans have altered the nitrogen cycle dramatically over the last century, and as a result, nitrate ( $\text{NO}_3^-$ ) is steadily accumulating in water resources such as in groundwater and surface water. Contamination of  $\text{NO}_3^-$  in groundwater is a worldwide problem [1] and fertilizers are the largest contributor of anthropogenic  $\text{NO}_3^-$  worldwide. Other major sources include animal and human wastes, nitrogen oxides from utilities and automobiles, and leguminous crops that fix atmospheric nitrogen [2]. These organic and inorganic sources of nitrogen are transformed to  $\text{NO}_3^-$  from the mineralization, hydrolysis, and bacterial nitrification process [3].

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This compound can migrate to surface water and groundwater when its not taken up by plants or denitrified [2]. Drinking groundwater contaminated with  $\text{NO}_3^-$  can cause adverse health effects such as the occurrence of methemoglobinemia and non-Hodgkin's lymphoma [4, 5]. Ingestion of  $\text{NO}_3^-$  also can increase the risk of cancer [6]. In the human body, can be endogenously reduced to nitrite ( $\text{NO}_2^-$ ), which can undergo nitrosation reactions in the stomach with amines ( $\text{RR}'\text{NH}$ ) to form a variety of *N*-nitroso compounds (NOCs), which are carcinogens [7, 8]. NOCs such as nitrosamines have been linked to cancer in animals but evidence of cancers in humans from these compounds remain inconclusive due to a variation of consistency of the association [4, 9, 10, 11]. Besides, study by [5] also have indicated the elevated  $\text{NO}_3^-$  levels in drinking water may be associated with spontaneous abortions.  $\text{NO}_3^-$  and nitrosamines (an endogenous nitrosation, indicator of cancer) in human body can be excreted in urine [8]. The other major route of  $\text{NO}_3^-$  and nitrosamines excretion is in saliva. The maximum contamination level (MCL) value for  $\text{NO}_3^-$  in drinking water established by U.S Environmental Protection Agency (USEPA) at 45 mg/L of or 10 mg/L in terms of  $\text{NO}_3\text{-N}$  to protect infants from methemoglobinemia [5, 12, 13].

This paper provides an overview of  $\text{NO}_3^-$  contamination in groundwater,  $\text{NO}_3^-$  and nitrosamines metabolism in the human body and the formation of endogenous nitrosamines excreted in human biomarkers such as urine and saliva. The information about the sources and occurrence of  $\text{NO}_3^-$  gained from this review is useful for reducing activities that contributed to  $\text{NO}_3^-$  contamination in groundwater. Besides, information regarding the mechanism of nitrate in the human body will be more understood. This review may also be useful for other researches especially for those exploring the uses of biomarkers such as urine and saliva for determining endogenous nitrosamines formation

## **2. Contamination of nitrate in groundwater**

Many regions all over the world depends entirely on groundwater resources such as developing countries such as in India and Pakistan and in developed countries such as Mexico and USA as potable water due to its easily accessibility and less microbiological contamination comparison to surface water [2, 14-16]. The demands for groundwater have been reported to continuously rise comes from rapid increases in population growth, industrial or agricultural expansion areas which are essential to increase the production of food, to compensate the deterioration in the quality of surface water and to fulfil the needs when low flow of water surface sources during prolonged periods of drought [1]. Table 1 shows the usage of groundwater in several countries.

Groundwater is used for agricultural and industrial consumption as well as for drinking water. However, contamination of groundwater by various pollutants might render groundwater unsuitable for consumption and put human and animal life as well as the whole environment at greater risk [14]. The soil system above the groundwater acts as a 'protective shield', providing inertia to quality changes and a slowed propagation of contaminants. But for the same reason, however, groundwater is vulnerable to pollution because once groundwater is contaminated, the effects are often irreversible, or at least, difficult and expensive to remediate [17].

Table 1. The usage of groundwater in developing and developed countries

	Country	Annual groundwater use (km <sup>3</sup> )	Population depending on groundwater (%)	Activities depending on groundwater (%)
Developing country	Pakistan	45	60 – 65	NA : Not available
	India	150	55 – 60	60 – agriculture irrigation 85 – drinking water
	Malaysia	0.064	3.4	60 – domestic 35 – industry 5 – agriculture
	Bangladesh	21	NA	88 – irrigation /agriculture sector 10 – domestic 2 – industrial sector
	China	75	22 – 25	NA
Developed country	USA	100	<1-2	51 – domestic use 49 – irrigation, industry, livestock
	Japan	10.8	NA	34 – domestic use 35 – industrial use 31 – agricultural use
	Australia	3.5	80	60 – agriculture irrigation 12 – mining 17- industries 5 – household water supply 9 – potable water supply network

NO<sub>3</sub><sup>-</sup> is the most common pollutant in groundwater [8, 14, 18, 19]. High NO<sub>3</sub><sup>-</sup> levels in groundwater may originate from a number of sources, including leaking septic tanks, animal wastes, and overuse of nitrogen fertilizers [5, 20]. Table 2 summarized the sources of NO<sub>3</sub><sup>-</sup> contamination in groundwater from previous studies.

Table 2. Source and concentration of  $\text{NO}_3^-$  in groundwater

Sources	Range of $\text{NO}_3^-$ concentration (mg/L)	Study area	References
Fertilizer usage	3.0 – 252.0	Hamadan, Western Iran	[21]
Animal waste (poultry farms, cattle shed and leakage from septic tanks) and nitrogenous fertilizers	BDL – 450.0	Srikakulam district, Andhra Pradesh, India	[22]
Irrigation water	<10.0 – 202.0	Yellow River delta, China	[23]
Animal waste, extensive agricultural activities	1 – 166.0 ( $\text{NO}_3\text{-N}$ )	Kanpur district, Uttar Pradesh, India	[24]
Fertilizer usage	7.1 – 162.0	Rajasthan, India	[25]
Fertilizer usage	4.81 – 17.16	Kota Bharu, Kelantan, Malaysia	[26]
Manure from beef farm and long term soiled water irrigation	0.07 – 12.21 ( $\text{NO}_3\text{-N}$ )	South-east Ireland	[27]
Direct discharge of solid waste and wastewater on the ground	4.0 – 198.0	Southern part of Abidjan District	[28]
Improper disposal of sewage and solid waste and over application of fertilizers and pesticides	10.3 – 14.84	Charsadda district, Khyber Pakhtunkhwa, Pakistan	[29]
Fertilizer usage	< 10	Bachok, Kelantan, Malaysia	[30]

Leaching of  $\text{NO}_3^-$  from the unsaturated zone is a complex interaction consisting of many factors such as land use practices, on-ground nitrogen loading, groundwater recharge, soil nitrogen dynamics, soil characteristics, and depth to water table [1]. Once it reaches the groundwater,  $\text{NO}_3^-$  migrates in the aquifer via advection and dispersion.  $\text{NO}_3^-$  will most likely undergoes denitrification in the groundwater zone depending on the properties and prevailing conditions. Many sources can contribute to the contamination of  $\text{NO}_3^-$  in groundwater, however, nitrogen fertilizers are the largest contributor of anthropogenic nitrogen globally and it is used to enhance plant growth [3]. Nevertheless, when nitrogen-rich fertilizer application exceeds the plant demand and the denitrification capacity of the soil, nitrogen can leach into the groundwater in the form of  $\text{NO}_3^-$  which is highly mobile with little sorption [1, 2 18].  $\text{NO}_3^-$  from nitrogen fertilizers appear to be an important source of  $\text{NO}_3^-$  in drinking water in rural areas which have low depth wells due to shallow water table and low vertical gradients [6-8, 31, 32-33]. High  $\text{NO}_3^-$  in drinking water have been most often associated with privately owned wells, especially with shallow wells with depth < 15 m, bored or dug construction within unconfined aquifers [5, 34].

The contamination of  $\text{NO}_3^-$  are also influenced by soil types [34]. For example, a sandy soil can enhance

downward infiltration and transport of  $\text{NO}_3^-$  into groundwater once fertilizers have been applied [35]. Besides, high doses of fertilizers and the use of large amounts of irrigation water to permeable soils lead to the leaching of high quantities of  $\text{NO}_3^-$  that ultimately contaminate the groundwater [36]. A study by [37] stated that in clay soils, water containing  $\text{NO}_3^-$  movement is slower and pores lack oxygen which favors denitrification. Although water percolates slowly in clayed soils, cracks and pore abundance of soil horizon may change this rule. Preferential flow along soil cracks and macropores can lead to rapid transport of  $\text{NO}_3^-$  to the groundwater [37]. In addition, regions that receive high amounts of precipitation also contribute to higher concentrations of  $\text{NO}_3^-$  compared to drier areas. Dry areas have deeper water tables and lower amounts of precipitation to cause leaching. However, groundwater contamination can still occur in relatively dry regions with deep water tables and thick unsaturated zones due to preferential flow through fractures and cracks during rainfall or irrigation events [27].

### **3. Metabolism of nitrate and nitrosamines in human body**

The primary route of human exposure to  $\text{NO}_3^-$  is through ingestion [38]. When  $\text{NO}_3^-$  enters the human body  $\text{NO}_3^-$  is readily absorbed from the upper gastrointestinal tract. Up to 25% of  $\text{NO}_3^-$  is actively concentrated and excreted in saliva. The salivary  $\text{NO}_3^-$  concentration is approximately ten times higher than in plasma [39]. In the salivary gland, 20% of  $\text{NO}_3^-$  is reduced to  $\text{NO}_2^-$  (about 5 – 8% of total ingested  $\text{NO}_3^-$ ) by facultative anaerobic bacteria which are found on the surface of the tongue [15, 40]. These bacteria use  $\text{NO}_3^-$  in the absence of oxygen to act as a terminal electron acceptor and produce  $\text{NO}_2^-$  as a by-product [18, 41]. This conversion can occur at other sites including the distal small intestine and the colon [42]. Small amount of this  $\text{NO}_2^-$  will be swallowed and will react with stomach acid to produce intermediate nitrosating agent such as nitrous acid ( $\text{HNO}_2$ ), nitrous anhydride ( $\text{N}_2\text{O}_3$ ) and nitrous acidium ion ( $\text{H}_2\text{NO}_2^+$ ) [15, 18, 43, 44].  $\text{N}_2\text{O}_3$  is a powerful nitrosating agent capable of donating  $\text{NO}^+$  to secondary and tertiary amines to form potentially carcinogenic nitrosamines [41, 45-48].

Nitrosamines are a nitroso group ( $-\text{N}=\text{O}$ ) attached to a nitrogen atom ( $-\text{N}-\text{N}=\text{O}$ ) and these compounds have been classified as probable human carcinogens [43, 49, 50]. There are several nitrosamines detected endogenously formed in human body such as N-nitrodimethylamines (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR) and N-nitrosopiperidine (NPIP). The formation of nitrosamines is called the nitrosation process, a process where an addition of nitrosonium ion ( $\text{NO}^+$ ) via an electrophilic attack on organic compounds, primarily amine [51]. Other than acidic condition, the process also can take place at neutral pH via bacteria – catalyzed processes [43].  $\text{NO}_2^-$  can be reduced by bacterial activity to form NO, which can react with molecular oxygen to form nitrosating compounds,  $\text{N}_2\text{O}_3$  and nitrogen tetroxide ( $\text{N}_2\text{O}_4$ ). Then, the remaining nitrate and nitrosamines will be absorbed in the intestine and excreted by the kidney.

Although intake of high drinking water  $\text{NO}_3^-$  is associated with biological mechanism formation of nitrosamines, there are also numerous effective inhibitors of nitrosation reaction in the human body. Ascorbic acid (Vitamin C) is one of the inhibitors of nitrosamines formation. Other compounds such as antioxidant, alpha-tocopherol (Vitamin E), also work as inhibitors of nitrosamines formation. Ascorbic acid, erythorbic acid

and alpha-tocopherol inhibit nitrosamines formation due to their oxidation-reduction properties. They inhibit nitrosation by reducing  $\text{NO}_2^-$  to  $\text{NO}$ , which is not a nitrosating agent [41, 43, 44, 51]. A study by [19] indicated that vegetables are a significant contributor to  $\text{NO}_3^-$  exposure in humans other than well water. Green and leafy vegetables have high concentrations of  $\text{NO}_3^-$  in their leaves, and plant grown in low light conditions tend to have higher  $\text{NO}_3^-$  concentrations as the  $\text{NO}_3^-$  is stored and not reduced to form amino acids. However, the nitrosation process in the human body is inhibited by the presence of inhibitors in the vegetables [4].

Metabolism of  $\text{NO}_3^-$  and nitrosamines as well as the development of cancers in the digestive systems are complex processes mediated by several factors. Individuals with increased rates of endogenous formation of carcinogenic nitrosamines are likely to be more susceptible. Known factors altering susceptibility to the development of cancers in the digestive system are inflammatory bowel disease, high red meat consumption, amine-rich diets, smoking, and dietary of inhibitors of endogenous nitrosation [15, 38, 49, 52]. Cross et al. hypothesized that individuals with higher daily  $\text{NO}_3^-$  intake from drinking water and lower intakes of nitrosation inhibitors may be at elevated risk of pancreatic cancer [4].

#### 4. Urine and saliva as biomarkers of nitrosamines formation

Approximately 65-70% of ingested  $\text{NO}_3^-$  is rapidly excreted in urine and less than 1% is excreted in faeces of humans. Besides,  $\text{NO}_3^-$  also be found in milk, gastric fluid, endotracheal secretion, saliva and sweat after  $\text{NO}_3^-$  ingestion [53]. Excretion of  $\text{NO}_3^-$  in urine is maximal about 5 hours post-exposure and is essentially complete after 24 hours [54]. Among infants, approximately 100% of  $\text{NO}_3^-$  is excreted in urine under normal conditions. In healthy humans, urinary excretion rates of  $\text{NO}_3^-$  is about 1200  $\mu\text{mol}$  nitrate/24h [55]. In urine,  $\text{NO}_3^-$  is stable and accessible to quantitative analysis [56]. Measurement of  $\text{NO}_3^-$  in urine turns out to be the most suitable and practical method to be used [55]. Besides, large quantity of urine is simple to obtain and appropriate to be measured by modern analytical methods which provide reliable data [56].

Other than urine,  $\text{NO}_3^-$  and nitrosamines are also excreted in human saliva. The amount of  $\text{NO}_3^-$  secreted by the salivary glands depends directly on the amount of  $\text{NO}_3^-$  ingested [57]. It is assumed that  $\text{NO}_3^-$  is absorbed in the upper gastrointestinal tract and concentrated from the plasma into the saliva by the salivary glands. Approximately 25% of exogenous  $\text{NO}_3^-$  has been estimated to be actively taken up by the salivary ducts from circulation. In many studies, the peak salivary  $\text{NO}_3^-$  level has been observed 1-2 hours after ingestion of  $\text{NO}_3^-$  [49]. The highest salivary  $\text{NO}_3^-$  level ingested by drinking is reported to be two to three times higher than ingestion of  $\text{NO}_3^-$  by food due to different absorption rate from the gastrointestinal tract which depending on the texture of dietary intake [49].

High  $\text{NO}_3^-$  intake have been associated with increases in excreted nitrosamines such as *N*-nitrosoproline (NPRO) levels in urine [15, 48], and  $\text{NO}_3^-$  administrated via drinking water was found to be directly related to concentration of total NOCs in faeces [6]. In addition, populations with high rates of esophageal, gastric, and nasopharyngeal cancers excrete high levels of urinary nitrosamines [58]. These results demonstrate a contribution of drinking water  $\text{NO}_3^-$  to nitrosation process in the human body and suggest that  $\text{NO}_3^-$  intake may be used as a surrogate for exposure of human to NOCs, particularly in combination with increased intake of

dietary nitrosatable precursors [6, 41, 56]. The NPRO test is the best reasonable indicator of the nitrosamines formation and perhaps of other NOC. Nitrosamines such as NDMA and NPYR have also been quantified in urine. Previous studies investigated relationships between drinking water  $\text{NO}_3^-$  ingestion in combination with fish meal rich in amines as nitrosatable precursors, and there were significantly increases in urinary excretion of NDMA [7]. The concentrations of NDMA, NMEA, and NPIP were also shown to be significantly higher in urine of smokers than in nonsmokers[59]. Nitrosamines such as NDMA and NPYR was also detected in saliva after nitrosation of  $\text{NO}_2^-$  with nitrosatable precursors [43].

Monitoring of nitrosamines excreted in urine appears to be a suitable procedure for estimating daily human exposure to endogenously formed nitrosamines compounds. Variability in  $\text{NO}_3^-$  ingestion may contribute greatly to the variability of urinary  $\text{NO}_3^-$  and nitrosamines excretion. Saliva provided several advantages as biomarker such that it can be collected non-invasively and there is no need for trained staff [60, 61]. Moreover, no special equipment is needed for the collection of the fluid and potentially valuable for children and older adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of other biomarker such as blood [61]. Furthermore, analysis of saliva may provide a cost-effective approach for the screening of large populations [60].

However, urine and saliva may also have disadvantages as biomarkers. For example, excreted metabolites may detoxify in detoxification pathways and this will leave very low levels of compounds of interest left to be detected in urine [62]. For saliva, numerous medications and radiation may affect salivary gland function and consequently the quantity and composition of saliva. Whole saliva may also contain proteolytic enzymes derived from the host and from oral microorganisms. These enzymes can affect the stability of certain diagnostic markers. Some molecules are also degraded during intracellular diffusion into saliva. Any condition or medication that affects the availability or concentration of a diagnostic marker in saliva may adversely affect the diagnostic usefulness of that marker [62].

## **5. Health effects of nitrate and nitrosamines**

Elevated  $\text{NO}_3^-$  levels in drinking water have been associated with several adverse health impacts such as methemoglobinemia, cancer, neural tube defects and thyroid dysfunction [4-6, 46]. Odds ratios (ORs) have been used to measure association between exposures and outcomes in such epidemiological studies. A study by [6], a matched case – control and  $\text{NO}_3^-$  ecology study was used to investigate the association between mortality attributed to rectal cancer and  $\text{NO}_3^-$  in drinking water in Taiwan. The results showed that individuals who resided in the municipalities with highest levels of nitrate in drinking water were at a statistically significant increased risk for development for rectal cancer compared to individuals living in municipalities with the lowest nitrate levels, after controlling for possible confounder. The adjusted ORs were 1.22 (95% Confidence Interval (CI) 0.98-1.52) for the group with nitrate ranging between 0.19 and 0.45 mg/L and OR 1.36 (95% CI 1.08-1.70) for the group with  $\text{NO}_3^-$  levels of 0.48 mg/L or more [6]. A study also has found a slightly association of  $\text{NO}_3^-$  exposure in drinking water with rectal cancer death [33]. The ORs for individuals who resided in municipalities served by drinking water with  $\text{NO}_3\text{-N}$  exposure to 0.38 ppm and greater were associated with risk of rectal cancer with OR 1.15 (95% CI 1.01-1.32). This study also has indicated the correlation between  $\text{NO}_3\text{-N}$  exposure

and the risk of rectal cancer development was influenced by calcium (Ca) in drinking water. A study by [46] also showed a positive association between  $\text{NO}_3^-$  in drinking water and bladder cancer. The results showed that adjusted ORs were 1.76 (95% CI 1.28 – 2.42) for  $\text{NO}_3^-$  levels between 0.19 mg/L and 0.45 mg/L and OR 1.96 (95% CI 1.41 – 2.72) for  $\text{NO}_3^-$  levels between 0.48 mg/L and more.

Previous studies have also showed evidence of positive association with other adverse health outcome. The study showed that exposure to  $\text{NO}_3^-$  in drinking water above 45 mg/L was associated with increased risk for anencephaly; a birth defect that leaves babies born without part of their brain or skull with OR was 4.0 (95% CI 1.0 -15.4) [63]. Besides, the results also showed statistically significant association of increased risk for anencephaly below than 45 mg/L among groundwater drinkers with ORs were 2.1 (95% CI 1.1 – 4.1) for 5 – 15 mg/L, 2.3 (95% CI 1.1 – 4.5) for 16 – 35 mg/L and 6.9 (95% CI 1.9 – 24.9) for 36 -67 mg/L [63]. A study by [45] showed that women whose drinking water nitrates measured 3.5 mg/L or greater were 1.9 times more likely (CI 0.8-4.6) to have neural tube affected pregnancy than women with lower levels of  $\text{NO}_3^-$  in their water but there was no statistically significant. A cross – sectional study by [53] showed positive association between thyroid dysfunction and  $\text{NO}_3^-$  among children with ORs 8.145, 95% CI 1.67 – 39.67. High  $\text{NO}_3^-$  concentration (16.4 mg/L,  $\text{NO}_3\text{-N}$ ) also was significantly associated with subclinical hypothyroidism in women, (OR 1.60, 95% CI 1.11 – 2.32) [20]. A cohort study by [19] have also showed a positive association between  $\text{NO}_3^-$  with age-related macular degeneration (AMD) (ORs 1.77, 95% CI 1.12 – 2.78) at level 5 – 9 ppm  $\text{NO}_3\text{-N}$  and 2.88 (95% CI 1.59 – 5.23) at level  $\geq 10$  ppm  $\text{NO}_3\text{-N}$ .

Another prospective study found a higher risk of breast cancer among women who used private well and have high total folate intake ( $\geq 400\mu\text{g/d}$ ) [64]. The results showed the women with high total folate intake ( $\geq 400\mu\text{g/d}$ ), breast cancer risk was statistically significant increase in women using public water with highest  $\text{NO}_3^-$  ( $\geq 33.5$  mg/2L) with hazard ratio (HR) was 1.40 (95% CI 1.05 – 1.87) and HR was 1.38 (95% CI 1.05 – 1.82) in those using private well [64]. A prospective study showed positive association for colon cancer with ORs were 1.53 (95% CI 1.09 – 2.16) when exposed to 0.36 to 1.00 mg/L  $\text{NO}_3^-$  and 1.54 (95% CI 1.08 – 2.19) when exposed to 1.01 to 2.46 mg/L  $\text{NO}_3^-$  [65]. The results also showed positive association with bladder cancer with OR was 2.83 (95% CI 1.11 – 7.19) for  $>2.46$  mg/L  $\text{NO}_3^-$ , lung and bronchus cancer with OR was 1.49 (95% CI 1.02 – 2.17) for 1.01 to 2.46 mg/L  $\text{NO}_3^-$ .

Although many studies have determined the association between  $\text{NO}_3^-$  and nitrosamine with various health effects, there were also few studies that showed no association. For example, [4] found no association between  $\text{NO}_3^-$  in drinking water with pancreatic cancer risk. This study suggested that long-term exposure to  $\text{NO}_3^-$  in drinking water at levels below than 45 mg/L  $\text{NO}_3^-$  were not associated with pancreatic cancer. A population-based case-control study by [31] also found no association of renal cell carcinoma with 10 year – consumption of  $>5\text{mg/L}$   $\text{NO}_3\text{-N}$  in drinking water with OR = 1.03, 95% CI 0.66-1.60. Table 3 summarized the health outcomes of  $\text{NO}_3^-$  exposure in drinking water from previous studies.



Table 3. Health outcomes of NO<sub>3</sub><sup>-</sup> in drinking water

Health outcomes	Study design (Year)	Summary findings	References
Neural tube defects	Case – control study (1989 – 1991)	Significant association between risk of anencephaly and exposure above 45 mg/L NO <sub>3</sub> <sup>-</sup> with ORs was 4.0 (95% CI = 1.0 – 15.4).  Significant association were observed between increased risks for anencephaly at NO <sub>3</sub> <sup>-</sup> levels below 45 mg/L among groundwater drinkers with ORs; 2.1 (95% CI 1.1 – 4.1) for 5 – 15 mg/L 2.3 (95% CI 1.1 – 4.5) for 16 – 35 mg/L 6.9 (95% CI 1.9 – 24.9) for 36 -67 mg/L	[63]
Non-Hodgkin lymphoma, leukemia, colon, rectum, pancreas, kidney, bladder, breast, ovary, uterine corpus, lung and bronchus, ,melanoma	Prospective cohort study 1986 – 1998	Significant association with RRs were; Colon cancer; 1.53 (95% CI 1.09 – 2.16) for 0.36 – 1.00 mg/L NO <sub>3</sub> <sup>-</sup> 1.54 (95% CI 1.08 – 2.19) for 1.01 – 2.46 mg/L NO <sub>3</sub> <sup>-</sup> Bladder cancer; 2.83 (95% CI 1.11 – 7.19) for >2.46 mg/L NO <sub>3</sub> <sup>-</sup> Lung and bronchus cancer; 1.49 (95% CI 1.02 – 2.17) for 1.01 – 2.46 mg/L NO <sub>3</sub> <sup>-</sup>	[65]
Bladder cancer	Population-based case-control 1986 – 1989	No significant association between risk of bladder cancer and increasing average NO <sub>3</sub> <sup>-</sup> levels in drinking water; Women; ORs was 0.8 (95% CI 0.4-0.8) for ≥3.09 mg/L NO <sub>3</sub> -N Men; ORs was 0.5 (95% CI 0.4-0.8) for ≥3.09 mg/L NO <sub>3</sub> -N	[66]
Neural tube defects (NTDs)	Case – control study	No significant association between NTDs and women whose drinking water NO <sub>3</sub> <sup>-</sup> (≥3.5 mg/L) with ORs; 1.9 (95% CI 0.8-4.6)  Significant association between NTDs and women who took nitrosatable drugs during the periconceptional period and whose drinking water source (≥3.5 mg/L NO <sub>3</sub> <sup>-</sup> ) with ORs; 14 (95% CI 1.7 – 660)	[45]
Pancreatic cancer	Population-based case control study 1985 – 1987	No significant association between pancreatic cancer risk and years of exposure to community water supplies with ORs; 1.1 (95% CI 0.66 – 1.9) for 1 to 4 years (7.5 mg/L NO <sub>3</sub> <sup>-</sup> ) 1.2 (95% CI 0.79 – 1.9) for >4 years (7.5 mg/L NO <sub>3</sub> <sup>-</sup> ) 1.5 (95% CI 1.0 – 2.2) for 1 to 2 years (10 mg/L NO <sub>3</sub> <sup>-</sup> ) 0.58 (95% CI 0.18 – 1.9) for >2 years (10 mg/L NO <sub>3</sub> <sup>-</sup> )	[4]

		<p>No significant association between pancreatic cancer risk and average <math>\text{NO}_3^-</math> level in drinking water with ORs;                      1.2 (95% CI 0.79 – 1.8) for 0.6 - &lt;1.3 mg/L                      0.54 (95% CI 0.33 – 0.89) for 1.3 – 2.8 mg/L                      0.99 (95% CI 0.64 – 1.5) for &gt;2.8 mg/L</p> <p>No significant association between pancreatic cancer risk and years of well use overall or years using a shallow well. The ORs for shallow well use greater than 10 years was;                      0.6 (95% CI 0.4 – 1.0)</p>	
Urological malignancies	Community – based cohort study 1986 - 1997	<p>Significance association between risk of urinary tract tumours and two groups, who were exposed to 60 mg/L and 10 mg/L <math>\text{NO}_3^-</math>;                      RR, 1.98 (95% CI 1.10-3.54)</p> <p>No significant association with RR (95% CI) were ;                      0.87 (0.34 – 2.22) for renal tumours                      0.66 (0.14 – 2.88) for penile cancer                      1.06 (0.76 – 1.48) for prostate cancer.</p> <p>No significant inverse association for testicular tumours, with RR; 0.43 (95% CI 0.21 – 0.90) for 25 mg/L <math>\text{NO}_3^-</math></p>	[9]
Thyroid dysfunction (goiter)	Cross – sectional study 2006	<p>Significant association between thyroid dysfunction and children exposed to high <math>\text{NO}_3^-</math> levels in drinking water with ORs;                      8.145 (95% CI 1.67 – 39.67) for mean annual <math>\text{NO}_3^-</math> concentrations (<math>89.7 \pm 0.5</math> mg/L)</p>	[53]
Bladder cancer	Case – control study 1999 – 2003	<p>Significant association between bladder cancer and high <math>\text{NO}_3^-</math> in drinking water with adjusted ORs;                      1.76 (95% CI 1.28 – 2.42) for 0.19mg/L and 0.45 mg/L <math>\text{NO}_3^-</math>.                      1.96 (95% CI 1.41 – 2.72) for 0.48 mg/L <math>\text{NO}_3^-</math> and more.</p>	[46]
Rectal cancer	Matched case – control study 1999 - 2003	<p>No significant association between rectal cancer death and high <math>\text{NO}_3^-</math> levels in drinking water with adjusted ORs;                      1.36 (95% CI 1.08 – 1.70) for 0.48 mg/L <math>\text{NO}_3^-</math> and more                      1.22 (95% CI 0.98 – 1.52) for 0.19 – 0.45 mg/L <math>\text{NO}_3^-</math></p>	[6]
Renal cell carcinoma	Population-based case-control study 1986 - 1989	<p>No significant association between renal cell carcinoma with 10 years consumption of &gt;5 mg/l <math>\text{NO}_3^-</math>-N in drinking water with ORs; 1.03 (95% CI 0.66-1.60)</p>	[31]

Methemoglobinemia	Cross-sectional study	Significant association between methemoglobinemia and children who drinking well water with a NO <sub>3</sub> <sup>-</sup> concentration >50 mg/L than those drinking well water with a NO <sub>3</sub> <sup>-</sup> concentration <50 mg/L ( <i>p</i> = 0.001 at 95% CI 1.22-2.64.	[67]
Adenocarcinoma of stomach	Population-based case-control study 1988 - 1993	No significant association between adenocarcinoma of stomach and high NO <sub>3</sub> <sup>-</sup> levels (<0.5 mg/L to 67 mg/L) in private wells with ORs; 4.7 (95% CI 0.5–41) for 0.5–4.5mg/L 5.1 (95% CI 0.5–52) for >4.5mg/L  No significant inverse association between esophagus cancer and exposed to more than 4.5mg/ L NO <sub>3</sub> <sup>-</sup> with ORs; 0.5 (95% CI 0.1–2.9).	[42]
Rectal cancer	Matched case – control study 2003 - 2007	Significant association of rectal cancer and individuals who resided in municipalities served by drinking water with NO <sub>3</sub> -N exposure ≥ 0.38 ppm with adjusted ORs; 1.15 (95% CI 1.01 – 1.32  Findings showed that the correlation between NO <sub>3</sub> -N exposure and risk of rectal cancer development was influenced by Ca in drinking water.	[33]
Non-Hodgkin lymphoma (NHL)	Matched cancer case-control	No significant association for NHL deaths for those with high nitrate levels in their drinking water with adjusted ORs as compared to lowest tertile; 1.02 (95% CI 0.87-1.2) and 1.05 (95% CI 0.89-1.24), respectively.	[48]
Hypothyroidism	Cohort Study 1995 - 2008	Significant association of subclinical hypothyroidism in women and high NO <sub>3</sub> (16.4 mg/L, NO <sub>3</sub> -N) with ORs; 1.60 (95% CI 1.11 – 2.32)	[20]
Breast cancer	Prospective cohort study 1986 - 2008	Among those with folate intake ≥ 400 µg/d, breast cancer was significantly increased in public water users with the highest nitrate quintile (HR = 1.40, 95% CI 1.05-1.87) and private well users (HR = 1.38, 95% CI 1.05 – 1.82)	[64]
Age-related Macular Degeneration (AMD)	Cohort study 1994 - 2009	Significant association with ORs; 1.77 (95% CI 1.12 – 2.78 for level 5 – 9 ppm NO <sub>3</sub> -N 2.88 (95% CI 1.59 – 5.23) for level ≥ 10 ppm NO <sub>3</sub> -N	[19]

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OR is odd ratio; HR is hazard ratio; RR is relative risk; CI is confident interval

## 6. Conclusion

Contamination of  $\text{NO}_3^-$  in groundwater comes from many sources, but the most common contributor is nitrogen fertilizers that are used in agricultural area. Nitrogen fertilizers leach into groundwater in the form of  $\text{NO}_3^-$ . The occurrence of  $\text{NO}_3^-$  in well water has the potential to produce negative health impacts to the population. Nitrosamines, a carcinogenic compound is formed in the human body from the interaction of nitrosating agents with the amines by nitrosation process. Excretion of nitrosamines in urine and saliva is one of the indicators of  $\text{NO}_3^-$  toxicity in human body.

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