# Health issues related to N pollution in water and air

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**Nitrates enter human body through drinking water, food and air. Ingested nitrates converted to nitrite by microflora lead to methaemoglobinemia, increased free oxide radicals that predispose cells to irreversible damage and effects like cancer, increased infant mortality, abortions, birth defects, recurrent diarrhoea, recurrent stomatitis, histopathological changes in cardiac muscles, alveoli of lungs and adrenal glands, deterioration of immune system of the body. When inhaled, $NO_x$ can cause unconsciousness, vomiting, mental confusion, congestion and inflammation of the respiratory tract, pulmonary oedema, genetic mutations, and adversely affect development of the foetus and decrease fertility.**

**Keywords:** Cytochrome $b_5$ reductase, methaemoglobinemia, nitrate, nitrite.

NATURALLY occurring nitrate levels in surface and groundwater are generally a few milligrams per litre. Higher nitrate levels are found in groundwater due to water percolating through nitrate-rich rocks and also due to an excessive use of chemical fertilizers. The WHO report of 2004 maintains that extensive epidemiological data support limiting the value of nitrate-nitrogen to 10 mg/l or as nitrate to 50 mg/l for human consumption[1], whereas IS-10500 prescribes[2] maximum permissible limits in drinking water as 45 mg of $NO_3$/l[2].

## Sources

The main sources contributing to nitrate content of natural waters are atmosphere, geological features, anthropogenic sources, atmospheric nitrogen fixation and soil nitrogen. Oxides of nitrogen are generated through lightning and reach the surface water with rain. Recent reports indicate that atmospheric contributions amount to 25% of the total load of nitrate[3]. Direct discharge from septic tanks, sewage and industrial effluent are other contributors. Excessive use of chemical fertilizers is one of the main sources of nitrate in water.

Out of the total human nitrate intake[3], fruit and vegetables account for 70%, drinking water 21%, and meat and meat products 6%. The common nitrate-rich vegetables are lettuce, spinach, beetroot, celery, egg plant, beet, banana, strawberry, tomatoes and peas. Preservatives used in the food industry are significant nitrate sources. Cooking in aluminum utensils enhances reduction of nitrates to nitrite[3], and hence increases the toxicity.

Nitrates known as endogenous nitrate are also produced in the body. A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by oxidation of the nitric oxide to nitrous anhydride, and then reaction of nitrous anhydride with water to yield nitrite. Gastrointestinal infections and non-specific diarrhoea increase endogenous (non-bacterial) nitrate synthesis, probably induced by activation of the mammalian reticuloendothelial system[3,4].

Hydrogeological investigations show that nitrate levels are high in sandy soil than in clayey soil, because of low water-holding capacity and high permeability of pollutants like chloride and nitrate.

## Kinetics and metabolism

About 20% of ingested nitrate is reduced to nitrite by nitrate-reducing microflora present in the saliva[5] at the base of the tongue[6]. The factors which influence oral microflora and hence reduction of nitrate are nutritional status, infection, environmental temperature and age (more in elderly)[5].

Ingested nitrate is reduced to nitrite by nitrate-reducing microflora in the stomach (under favourable conditions, viz. $pH \geq 4$) and upper part of the intestine. Conditions favouring high stomach pH are achlorhydria[7], atrophic gastritis[6,8], artificially fed infants, or patients using antacid or similar drugs, e.g. Omeprazole[9,10].

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## Nitrate intake by humans through leafy vegetables

Being a rich source of nutrients and antioxidants, leafy vegetables occupy an important place in the human diet. Some vegetable species are known to accumulate high concentration of nitrate under heavy fertilization. Vegetables are the major source of the daily intake of nitrate by human beings, supplying about 72–94% of the total intake. Part of this nitrate-N is converted to nitrite and N-nitroso compounds that have detrimental effects on human health.

The Joint Expert Committee on Food and Agriculture in the World Health Organization (WHO)[1] established the Acceptable Daily Intake (ADI) of nitrate as 0–3.7 mg $kg^{-1}$ body wt. The US Environmental Protection Agency's reference dose for nitrate is 7.0 mg $kg^{-1}$ body wt per day[2]. In 1995, the European Commission's Scientific Committee for Food (SCF) established the ADI of nitrate as 3.65 mg $kg^{-1}$ body wt (equivalent to 219 mg $day^{-1}$ for a person weighing 60 kg)[3]. Assuming a 60 kg body wt[4], the ingestion of 100 g of fresh vegetables with nitrate concentration of 2500 mg $kg^{-1}$ fr wt exceeds the ADI for nitrate by about 13%. For a real assessment, however, nitrate content as well as average daily consumption amount in other sources need to be considered. Interestingly, at least 50% of the nitrate can be removed by cooking vegetables in water (with low nitrate concentration).

The nitrate content in samples of chenopodium and spinach being sold to Indian consumers has been found to be as high as 4451 and 4293 mg $kg^{-1}$ fr wt[5]. Studies on genotypes of spinach have shown enormous intraspecific variation of nitrate content. One genotype at the three-week stage of plant growth, and six genotypes at the six-week stage, exceeded the ADI limit. Petioles possessed several times higher level of nitrate than leaf lamina. All of the genotypes studied showed diurnal variation in nitrate accumulation with a minimum at noon[4,5].

Nitrate content in leafy vegetables possesses a significant reverse relationship with nitrate reductase (NR) activity, the first enzyme in the nitrate assimilatory pathway. It is held that NR expression level is largely responsible for different nitrate accumulation patterns in different cultivars of a given species. Thus nitrate accumulation in plants can be significantly reduced by over expression of NR genes in high nitrate-accumulating genotypes. In addition, it is also important to enhance the expression of NR so that nitrate does not accumulate in the green tissue after converting into nitrite. Over expression of these genes will reduce the nitrate concentration in vegetables to safe limits for human consumption. Moreover, a careful selection of vegetable genotypes based on their relative levels of nitrate content and NR activity, harvesting young plants at noon time, and removal of petioles from leaves could minimize the dietary intake of nitrate through leafy vegetables.

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The nitrite is readily and completely absorbed from both the stomach and the upper small intestine. The absorbed nitrite is then rapidly distributed throughout the tissues. It is rapidly oxidized to nitrate in the blood, with the formation of methaemoglobin.

The fate of nitrate is also associated with a metabolic pathway, which produces N-nitroso compound. This is a multiple-step process[11]. First, nitrate is converted into nitrite after consumption. Second, the nitrite reacts with natural or synthetic organic compounds (such as secondary amines or amides) in the food or water to form new combinations, called N-nitroso compounds (either nitrosamines or nitrosamides). More than one hundred of these N-nitroso compounds have been tested for carcinogenicity in animals[12], and 75–80% of them have been found to be carcinogens[4,13,14]. IARC[15] concluded that 11 N-nitroso compounds were carcinogenic in man. The most common *N*-nitroso compounds are dimethylnitrosamine (DMN), *N*-methylmethanamine (DMA), trimethylamine (TMA) and trimethylamine oxide (TMAO). Nitrite has been shown to cross the placenta and cause the formation of foetal methaemoglobinemia in rats[16]. The half-life of nitrate in the body after ingestion[18] is approximately 5 h[17].

## Metabolism of ingested nitrate in human body at cellular level

Ingestion of inorganic or organic nitrates will result in increased oxidation of haemoglobin to methaemoglobin and increased production of nitric oxide[19,20] (Figure 1). The conversion of nitrite to nitric oxide is non-enzymatic[20,21]. The oxidation of haemoglobin to methaemoglobin results in the formation of the superoxide radical by the transfer of a single electron. The enzyme superoxide dismutase present in the erythrocytes, catalyses the conversion of superoxide radical (O) to $H_2O_2$ and $O_2$. $H_2O_2$ is decomposed by glutathion peroxidase or catalase, both also present in erythrocytes[22,23]. Once the rate of oxidation of haemoglobin increases sufficiently in erythrocytes and overwhelms the protective and reductive capacities (e.g. cytochrome $b_5$ reductase system, etc.) of the cells[24,25], there is increased production of reactive free radicals of nitric oxide ($NO^{\bullet}$) and oxygen (O)[22].

### *Fate of free radical nitric oxide*

Haemoglobin scavenges nitric oxide through the high-affinity ferrous sites on heme to form S-nitrosothiol, whose affinity to nitric oxide is 8000 times higher than that for oxygen[26] by binding at $\beta$-93 cysteine residue on the globin chain. As haemoglobin binds oxygen in the lungs, its binding affinity to S-nitrosothiol is increased. As haemoglobin releases oxygen at the periphery, its affinity for S-nitrosothiol is reduced and nitric oxide is released in the tissues[26]. The thiol group of S-nitrosothiol essentially protects nitric oxide from being scavenged by the binding site on heme. Thus in addition to carrying oxygen, haemoglobin acts as a carrier of nitric oxide. The enhanced release of nitric oxide from S-nitrosohaemoglobin in the hypoxic tissue in turn reduces regional vascular resistance.

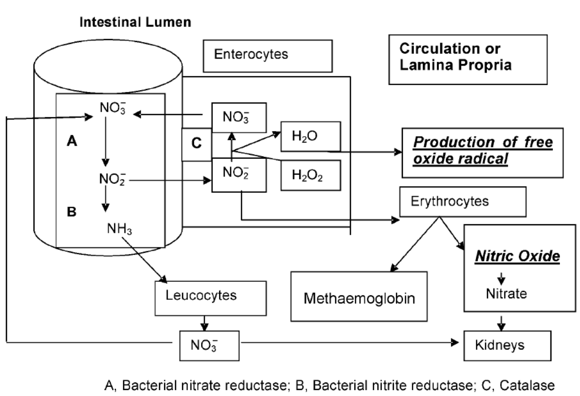


**Figure 1.** Metabolism of ingested nitrate in human body at cellular level.

Nitric oxide is a biogenic messenger, an endothelial-derived relaxing factor (EDRF)[25,26] and activates the guanylyl cyclase system[27] [converts guanosine triphosphate (GTP) to 3′,5′ cyclic guanosine monophosphate (cGMP)], raising the cGMP pool and therefore inducing *inter alia* vasodilatation[27] by lowering intracellular calcium ion[21].

### *Fate of free oxide radical*

In a normal cell, $O_2^-$ will be scavenged by the enzyme superoxide dismutase and $H_2O_2$, which is a product of the reaction, and by glutathion peroxidase and catalase[28,29]. Any $O_2^-$ that escapes this mechanism should react with other cell constituents, possibly causing irreversible cell damage. This mechanism is likely to become more significant if $O_2^-$ is produced in abnormally high amount (e.g. excessive nitrate ingestion) or if any of the protective mechanisms are defective[23,26]. Thus increased consumption of nitrate will lead to (a) increased production of nitrite[30], (b) enhanced absorption of sodium from the intestinal lumen[28], (c) excess $NO^{\bullet}$ (free radical nitric oxide) generation having vasodilatory effect[21,22,26,27] and (d) increased production of $O_2^-$, which will react with other cell constituents, possibly causing irreversible cell damage[27,31] (Figure 1).

## Excretion

The major part (70–75%) of the ingested nitrate is eventually excreted in the urine as nitrate, ammonia or urea within 24 h. The renal excretion is predominantly tubular[32] and occurs more in the first five hours[33]. Faecal excretion is negligible. A small amount is also excreted by exhalation through the lungs in the form of oxides of nitrogen (nitric oxide, nitrous oxide, nitrogen, etc.). Little nitrite is excreted[3,13] in the urine.

## Acute toxic effects

Toxic doses of nitrate ingestion in humans have been reported as 2–5 g of $NO_3$ (equivalent to 33–150 mg $NO_3^-$/kg body wt)[34]. Human lethal doses of 4–50 g $NO_3^-$ (equivalent to 67–833 mg $NO_3^-$/kg body wt) have been reported.

The acute toxicity symptoms occur in the form of cyanosis, severe gastroenteritis with abdominal pain, blood in the urine and faeces, dyspepsia, mental depression, headache and weakness.

## Chronic toxic effects

The following effects of long-term exposure to non-lethal doses have been reported.

### *Methaemoglobinemia*

Nitrates in drinking water have been reported to cause methaemoglobinemia, mainly in infants up to 6 months of age. Nitrate, after conversion to nitrite, oxidizes ferrous ion of haemoglobin to ferric state, forming methaemoglobin. The methaemoglobin formed is normally reduced[35] by reduced cytochrome $b_5$:

$$Hb^{+3} + \text{Red cyt } b_5 \rightarrow Hb^{+2} + \text{oxy cyt } b_5.$$

This reaction thus forms oxidized cytochrome $b_5$, which is further regenerated by getting reduced by the enzyme cytochrome $b_5$ reductase in the presence of NADH to form red cyt $b_5$, making it again available for reducing methaemoglobin.

$$\text{Oxy cyt } b_5 + \text{NADH} \xrightarrow[\text{reductase}]{\text{cyt } b_5} \text{Red cyt } b_5 + \text{NAD}.$$

In conditions where either the consumption of nitrate is more than the tolerable limit or cytochrome $b_5$ reductase system is not fully developed (infants)[35], the cytochrome $b_5$ reductase system gets exhausted, causing methaemoglobinemia. It has been reported that most cases of methaemoglobinemia occur with 90 mg nitrate ion/l or more[25,29,36]. Recently, methaemoglobinemia has been reported in all age groups[37] and was more severe in infants and in older age groups (>45 years). Infants are more susceptible to nitrate toxicity because of relatively higher stomach pH (2.0–5.0), which permits growth of nitrate-reducing organisms such as coliforms, *E. coli*, *Pseudomonas fluorescence*, *B. subtilis*, *Staph. Albus*, etc., relatively higher consumption of water per unit weight of body, presence of foetal haemoglobin which readily gets oxidized to methaemoglobin and poorly developed cytochrome $b_5$ reductase system.

Repeated boiling of nitrate-rich water for feeding, high-nitrate water used for preparing dried milk powder, weaning with nitrate-rich vegetables, e.g. spinach and viral diarrhoea in children cause increase in nitrate toxicity. Toxicity to infants with pregnant mothers ingesting high nitrates is possible because of reported transplacental passage of nitrite. Methaemoglobin level in the blood more than 10% of the total haemoglobin, manifests as clinical cyanosis and causes cellular anoxia. When these levels exceed 25–50%, it causes cyanosis, dyspnoea, headache and disorientation. Levels more than 60% may be lethal[36].

### *Cytochrome $b_5$ reductase adaptation*

Gupta *et al.*[38] reported that methaemoglobinemia was more pronounced in infants and elderly persons, in comparison to children and adolescents. It was observed that cytochrome $b_5$ reductase activity increases with increased nitrate ingestion. This adaptation peaks at about 95 mg nitrate ion/l nitrate concentration and gets exhausted by 200 mg nitrate ion/l, thus making people more prone to toxic effects. This adaptation was more active in children and adolescents in comparison to infants and elderly.

### *Infant mortality rate*

A study on African mothers and other studies also[39,40] reported an increase in infant deaths with increasing exposure of pregnant mothers and infants to nitrate. This may have been either due to undetected toxic methaemoglobinemia or due to malformations and weaknesses in the infant caused by foetal nitrate exposure.

### *Nitrate, nitrite, nitrosamines and cancer*

Nitrate acts as a ‘procarcinogen’, meaning that it reacts with other chemicals (amines and amides) to form carcinogenic compounds (N-nitroso compounds). Formation of N-nitroso compounds depends upon the presence of nitrate, nitrate-reducing microbial population and conditions favourable for chemical nitrosation[41].

In animal or human studies, N-nitroso compounds have been associated with 15 different types of cancers, including tumours in the bladder, stomach, brain, esophagus, bone and skin, kidney, liver, lung, oral and nasal cavities, pancreas, peripheral nervous system, thyroid, trachea, acute myelocytic leukaemia, and T and B cell lymphoma[14,15,42]. More than one hundred of these N-nitroso compounds have been tested for carcinogenicity in animals, and 75–80% of them have been found to be carcinogens[14]. IARC[15] concluded that 11 N-nitroso compounds were carcinogenic in man. In humans, the organs thought to be more at risk from cancer are the stomach, esophagus, nasopharynx and bladder.

Human epidemiology studies reported an increase in stomach cancer rates with consumption of water with high nitrate, especially if exposed during the first ten years of life[43]. People having low gastric acidity are more prone to gastric cancer, as it favours formation of N-nitroso compounds[9,10,44]. Association of other cancers with nitrate ingestion are bladder cancer[45], non-Hodgkin’s lymphoma[46] and colon cancer[47]. It is interesting to note that patterns as observed with colon cancer have not been observed with rectal cancer. It is probably because the N-nitroso compounds, which are formed in the digestive tract, have less contact time in the rectum than in the colon[48].

### *Respiratory system*

An increase in asthmatic attacks[36] is reported with high air-borne nitrate concentrations. High percentages (40–82) of cases of acute respiratory tract infection with history of recurrence have been reported in children drinking high-nitrate water[49]. These findings were further substantiated[50] in animal experiments, significant changes in lungs were observed with congestion, presence of inflammatory cells, breakdown of alveoli, frequent purulent bronchial exudates, interstitial round cell infiltration and fibrosis at certain areas, when animals were fed with water containing >100 mg/l of nitrate.

### Cardiovascular system

Earlier onset of hypertension has been reported with high nitrate ingestion[51]. In an animal study it was reported that high nitrate ingestion is associated with changes in form of small foci of inflammatory cells and fibrosis in cardiac muscles. Diffuse interstitial cellularity with pronounced degenerative foci was also noted. Thinning and dilatation of intramural coronary arteries[50] has also been reported. The changes in cardiac tissue even in animal studies are important, in view of the side/adverse effects related to the use of nitrate-containing drugs for the management of cardiac disorders and increasing drug tolerance of these nitrate-containing drugs[52].

### Gastrointestinal system

Recurrent diarrhoea has been reported with high nitrate ingestion, especially in children[53]. It has been suggested[30] that increased consumption of nitrate leads to (a) increased production of nitrite, (b) enhanced absorption of sodium from the intestinal lumen, (c) excess $NO^{\bullet}$ generation, having vasodilatory effect and (d) increased production of $O_2^-$, which will react with other cell constituents, possibly causing irreversible cell damage. These changes in enteric mucosa cause hyperemia and oedema in the enteric mucosa and later on possibly cause irreversible mucosal damage and therefore, provide high-risk conditions favourable for recurrent diarrhoea. These observations were further substantiated by animal studies which revealed the pathological changes in intestine and colon, and the changes were progressive as the nitrate content of the ingested water increased[53]. These findings are of interest since infants and children are supplemented with ORS, which if prepared with nitrate-rich water, during diarrhoea will be an aggravating factor for nitrate toxicity[19]. Recurrent stomatitis[54] was another problem reported in people using high nitrate-containing drinking water. The recurrent stomatitis was well correlated with increased cytochrome $b_5$ reductase activity following high nitrate ingestion.

### Abortions

Health effects associated with ingestion of nitrate-contaminated water have included stillbirth, low birth weight and slow weight gain and even death of the animals affected[55]. Spontaneous abortions have been reported in animal studies[55], as well as in humans[39,40,56].

### Birth defects

The risk of birth defects could be due to a single high dose of nitrate early in the pregnancy that later, having profound effects on long-term foetal development. In studies on rats and hamsters multiple birth defects, including malformations of the eye, central nervous system and musculoskeletal system were observed when a single dose of *N*-ethyl-*N*-nitrosourea, a nitrosamine, was given to the mother[57]. In humans, increased rates of anencephaly[58], birth defects of the central nervous system and musculoskeletal system have been reported.

### Diabetes

A positive correlation between high nitrate levels in drinking water and increased incidence of type-1 diabetes was observed[59,60]. High nitrate ingestion during pregnancy has been shown to be associated with increased incidence of type-1 diabetes in male offspring[61]. High nitrate ingestion causes increased production of free radicals which are toxic to pancreatic beta cells[49,54,60]. Some studies indicated no relationship between nitrites and nitrates in drinking water and increased incidence of type-1 diabetes[62]. Hence the association of dietary nitrites with diabetes remains tenuous and further research needs to be supported.

### Adrenal gland

In humans, high nitrate ingestion causes a decreased production of adrenal steroids as reflected by the decreased concentration of 17-hydroxysteroid and 17-ketosteroids in urine[63]. Results of animal studies on rabbits also reported the same results[64].

### Thyroid function and morphology

The thyroid gland contains an iodine-trapping transport mechanism which is accomplished by a membrane protein, the sodium–iodine symporter, which provides sufficient iodine substrate for hormone formation. This trapping mechanism for iodine is shared by other monovalent anions, including pertechnetate, perchlorate, thiocyanate and nitrate. It has been reported that relative potency of perchlorate for inhibiting iodine uptake is 15 and 240 times greater than that of thiocyanate and nitrate respectively, on a molar concentration basis in the serum[65]. Due to inhibition of this trapping mechanism, chronic nitrate exposure causes an inhibition in the accumulation of iodine in the thyroid gland. Consequently, it may result in thyroid malfunction causing higher relative risk of goiter[66] in children, more volume[67,68] and weight[69,70] of thyroid gland in children as well as in adults, and higher frequency of hypoechogenicity[68] of thyroid gland. Histomorphological changes reported are retention of lobular architecture, prominent vascular congestion, follicular hyperplasia, a vacuolization and an increase in the colloidal volume of the follicles[70]. Investigators have shown that a low dose or short-term nitrate intake causes a decrease in thyroid radioiodine uptake[71]. Whereas Eskiocak *et al.*[69,70] reported that high dose and long-term nitrate exposure results in an increase in the thyroid radioiodine uptake. These findings suggest that the effect of nitrate on thyroid iodine uptake is dose-dependent and the inhibition of thyroid iodine uptake may be stronger at higher amounts of nitrate.

Human studies[67] reported decrease in TSH levels, whereas others[68] reported an increase in TSH. A decrease in total and free $T_3$ and $T_4$ levels at high dose with long-term nitrate exposure[69,70] has been reported, whereas some studies[71] demonstrated that short-term nitrate administration may result in a significantly higher serum level of total $T_3$. These findings indicate that short or long-term nitrate exposure may be strongly responsible for the prominent change in thyroid hormone production.

### *Immunity*

Studies on human immune system indicated that nitrate ingestion does not affect lymphocyte growth, but nitrite decreases proliferation of lymphocytes[72]. Fibroblast growth remains unaffected. A decreased production of Th1 cytokines (interleukin-2, interferon-gamma and tumour necrosis factor-beta), which are responsible for resistance to a variety of infectious diseases was noted. No effect on the production of the Th2 cytokine interleukin-10, which is responsible for disease susceptibility, was noted. Because nitrate/nitrite shifted the balance from a Th1 to a Th2 response in some individuals, exposure to these compounds may decrease such a person's responsiveness to infectious diseases. Animal studies also reported an immune suppression due to high nitrate ingestion[73].

### *Air pollution and nitrate toxicity*

Inhalation of $NO_x$ causes a wide variety of health and environmental impacts because of various compounds and derivatives in the family of nitrogen oxides, including nitrogen dioxide, nitric acid, nitrous oxide, nitrates and nitric oxide. It reacts to form nitrate particles, acid aerosols and contributes to formation of acid rain.

Health effects are related to levels of $NO_x$ as well as the duration of exposure[74,75]. Low levels of nitrogen oxides in the air irritate the eyes, nose, throat and lungs, leading to cough and shortness of breath, tiredness and nausea. Breathing high levels of nitrogen oxides can cause rapid burning, spasms and inflammatory swelling of tissues in the throat and upper respiratory tract. High exposures may lead to pulmonary oedema, leading to hypoxemia and even death. Industrial exposure to nitrogen dioxide may cause genetic mutations, damage a developing foetus and decrease fertility in women. Industrial exposure to nitric oxide can cause unconsciousness, vomiting, mental confusion, and damage to the teeth. So far, there is no evidence that nitrogen oxides are potential carcinogens.

A review of studies published in the last decade has shown urban pollution to be an environmental cardiovascular risk factor[76]. This link was significant for $NO_x$ and PM10. A study of short-term effects of nitrogen dioxide on total, cardiovascular and respiratory mortality in 30 European cities found significant association between the two[77]. Significant associations of daily changes in particle concentrations, nitrogen dioxide and carbon monoxide were found with hospitalization for respiratory diseases (COPD, pneumonia, asthma) and cardiovascular diseases[78]. Exposure to indoor $NO_2$ at levels well below the Environmental Protection Agency (EPA) outdoor standard (53 ppb) was associated with respiratory symptoms among children with asthma. Each 20 ppb increase in $NO_2$ increased both likelihood of any wheeze or chest tightness and days of wheeze or chest tightness[79].

The EPA has established that the average concentration of nitrogen dioxide in ambient air in a calendar year should not exceed 0.053 parts of nitrogen dioxide per million parts of air (0.053 ppm). The Occupational Safety and Health Administration (OSHA) has set a limit of 25 ppm of nitric oxide in workplace air during an 8-h workday, 40-h work week. OSHA has also set a 15-min exposure limit of 5 ppm for nitrogen dioxide in workplace air.

## Conclusion

Nitrate ingestion beyond permissible limit is toxic to human beings. The literature available on nitrate toxicity in humans is limited, except for reports documenting methaemoglobinemia in infants. Many studies indicated nitrate as a cause of cancer, but the finding is still controversial and no firm conclusions have been drawn. Other effects observed were increased infant mortality, abortions, birth defects, recurrent diarrhoea, recurrent stomatitis, early onset of hypertension, histopathological changes in cardiac muscles, alveoli of lungs and adrenal glands, recurrent respiratory tract infection in children, hypothyroidism and diabetes, and adverse effects on the immune system of the body. Recently, an adaptation system to nitrate ingestion has also been reported. This adaptation to an enzyme cytochrome $b_5$ reductase has been shown to be protective to human beings, but to a limited extent only.

Since denitrification of water is difficult and costly, it is recommended that some changes in habits and adoption of simple, preventive measures may be urgently introduced in Government campaigns, at least in high-nitrate belts. Indiscriminate use of nitrogenous fertilizers should be avoided. Promoting breast feeding up to the age of at least 6 months is an important strategy. Long-term use of antacids, $H_2$ receptor inhibitor or proton pump inhibitor should be avoided. If at all needed, it should be given with antioxidants. Health education system should be developed to make people aware of the toxic effects of nitrate ingestion and its prevention.

More detailed epidemiological health-related studies covering a large sample are required to provide insights to the nitrate toxicity on human beings, especially the exploration of pathophysiology, nitrate toxicity and role of free oxide radicals to yield better understanding of the various diseases caused by nitrates, and their prevention and treatment. It is highly desirable to study what can be the better acceptable standards for nitrate in drinking water and to develop an easy and cost-effective denitrification method.

Certain recommendations made by the Council on Scientific Affairs were adopted by the AMA House of Delegates, as AMA directives in 2004. (1) The AMA supports the current FDA and United States Department of Agriculture regulations, including current labelling requirements, for nitrites in food (Directive). (2) The AMA encourages continued research and surveillance of the safety of nitrite use in foods, with particular attention to its possible effects on type-1 diabetes (Directive).


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