Agricultural Nitrogen Use & Its Environmental Implications

Agricultural Nitrogen Use & Its Environmental Implications provides a comprehensive, interdisciplinary description of problems related to the efficient use of nitrogen in agriculture, in the overall context of the nitrogen cycle, its environmental and human health implications, as well as various approaches to improve N use efficiency. The book has been divided into six sections and targets graduates, postgraduates, research scholars and policy makers in Agricultural and Environmental Sciences.

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Nitrate Toxicity and Human Health

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Contents

Summary: Nitrate and Nitrite are natural ions of the nitrogen cycle and are commonly found in groundwater and surface waters. The nitrate ion (NO_3^-) is the most stable form of nitrogen in oxygenated environments, thus all nitrogen-containing molecules can act as sources of nitrates. Under acidic conditions, nitrites (NO_2^-) are formed naturally from nitrates, and nitrites in turn may combine with amines or amides to form N-Nitroso compounds (nitrosamines).

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For human consumption, WHO report, 2005 permits up to 50 mg/L for short term exposure, whereas IS-10500 permits 45 mg/L (as NO_3^-) as desirable limit and 100 mg of NO_3^- per liter as maximum permissible limit in the absence of alternate source.

The problem of nitrates is globally endemic. The epidemiological data are scarce but some surveys indicate that in certain areas people consume water with 1000 mg/l nitrate due to absence of any alternate source. It is very difficult to remove nitrates from water because it is chemically non reactive in dilute aqueous solutions.

In human the sources of nitrate are drinking water, vegetables, preservatives of food and cooking in aluminum utensils. Main sources of nitrate contamination in water are improper disposal of sewage and industrial effluents; and indiscriminate and excessive use of manure/nitrogenous fertilizers in agriculture.

After ingestion the ingested nitrate gets converted to nitrite by micro flora in the oral cavity and in the gastrointestinal tract. This results in increased oxidation of hemoglobin to methemoglobin, leading to methaemoglobinemia. Simultaneously, increased production of free oxide radical and free radical nitric oxide occurs. These radicals, predispose cells for irreversible damage. Further this metabolic outcome predisposes a person for carcinogenic and other effects. The other effects observed were increased infant mortality, abortions, birth defects, recurrent diarrhea, 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. Recent ongoing studies indicated that high nitrate ingestion adversely effects the immune system of the body.

Recently some studies have indicated that an adaptation process (Cytochrome b_5 reductase adaptation) in the human body becomes active with increasing nitrate consumption to compensate for undesired effects of nitrate toxicity.

Ascorbic acid, methionine, alpha-tocopherol and methylene blue have been found to be effective in treating the nitrate toxicity.

For prevention denitrification of drinking water can be done, but these processes are difficult to implement and maintain. The other way of prevention is to protect most vulnerable age group from nitrate toxicity through simple interventions, e.g., breast-feed infants preferably up to an age of 4 months, avoid high nitrate containing food for weaning, avoid high nitrate water to pregnant women and avoiding WHO ORS preparation with locally available high nitrate water during diarrhea. Recent studies are in favor that long term use of anti acid secretary agents e.g. H_2 receptor inhibitors/proton pump inhibitors/antacids etc. specially in pregnant mothers and children may be hazardous. In case if it is necessary to use these drugs, they should be used cautiously and preferably with the antioxidants.

1. INTRODUCTION

Nitrate and Nitrite are natural ions that are a part of the nitrogen cycle. Naturally occurring nitrate levels in surface and ground water are generally a few milligrams per liter. In many ground waters, an increase in nitrate level has been observed due to water percolating through nitrate rich rocks, and farming practices of using chemical fertilizers.

WHO report, 2004, maintains that extensive epidemiological data support limiting the value of nitrate-nitrogen to 10 mg/l or as nitrate to 50 mg/l (WHO, 2004) for human consumption, whereas

IS-10500 prescribes maximum permissible limits in drinking water as 45 mg of NO_3 per liter (IS-10500, 1995).

The problem of nitrate contamination is endemic, Internationally, and Nationally, but so far no compiled data at national level is available. The data available are either of small areas or scattered zones/areas. Workers tried to compile the status of nitrate in drinking water in Rajasthan (Kumar *et al*., 2002; PHED survey, 1991-1993).

2. SOURCES

There are numerous sources in environment that contribute to the total nitrate content of natural waters, e.g., atmosphere, geological features, anthropogenic sources, atmospheric nitrogen fixation and soil nitrogen. However, detailed hydrogeological investigations conducted have indicated a heterogeneous pattern of nitrate distribution. In sandy soil with low water holding capacity and high permeability, movement of pollutants like chloride and nitrate is much quicker than in clayey soil. This is probably the main cause for high nitrates in areas with sandy soil.

Food is the most significant source of nitrates for adults, but water may play a more important role for infants. Vegetables account for more than 70% of the nitrates ingested in the human diet. The remainder of nitrate in a typical diet comes from drinking water (21%), meat, and meat products (6%).

2.1. Sources of Nitrate in Water

2.1.1. Atmosphere

The oxides of nitrogen are generated through lightning, and with rain water these are available in water. The atmospheric contribution was supposed to be the smallest, but recent reports on dry and wet depositions of pollutants suggested a contribution to the extent of 25% of total load of nitrate (WHO, 1985a).

2.1.2. Sewage and Industrial Effluent

Direct discharge of septic tanks, sewage, and industrial effluent causes pollution of surface water ground water, and wells through percolation.

2.1.3. Agriculture

This is considered as one of the main sources of nitrate in water. Nitrate is brought to field in large quantities by manure, especially in stockbreeding areas. Another source is use of chemical fertilizers (nitrogenous fertilizers) in intensive crop producing areas, especially when they are used in uncontrolled manner. It is reported that production of nitrogenous fertilizers increased in terms of N from 15.8 million tones in 1961-62 to 42.3 million tones in 1974-75 (United Nations, 1976).

2.1.4. Natural Sources

Natural sources are organic compounds containing nitrogen in soil.

2.2. Sources of Nitrate in Foods

2.2.1. Vegetables

The common nitrate rich vegetables are lettuce, spinach, beetroot, celery, eggplant, beets, banana, strawberry, tomatoes, and peas.

2.2.2. Preservative of Foods

Nitrate and nitrite are widely used in preservation of some food materials, especially meat and fish. Nitrite is used in meat curing, which gives pink color and flavor to cured meat. About 5 mg/kg of nitrite is sufficient to give satisfactory color for limited time, but people use up to 20-50 mg/kg for making it stable for more time. Such preservatives are also used for preserving cheese.

2.2.3. Top Feed Prepared with Water Containing High Nitrate

Bottle-fed infants are also exposed to high nitrates. As nitrate levels in breast milk are low, the probability of exposure to high nitrate in breast-fed infants is very low.

2.2.4. Cooking

Cooking in aluminum utensils increases the reduction of nitrates to nitrite (WHO, 1985a), which results in an increase in intake.

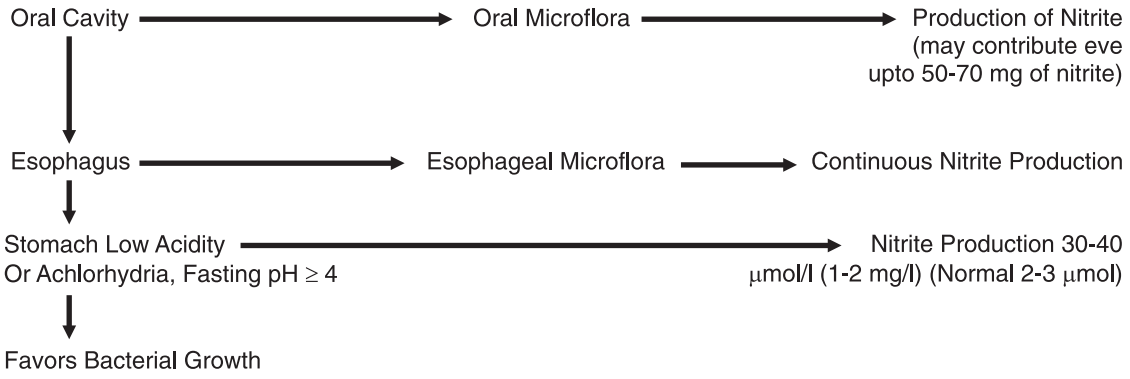
3. KINETICS AND METABOLISM

About 20% of ingested nitrate is reduced to nitrite by nitrate-reducing microflora present in saliva (Eisenbrand *et al.,* 1980) at the base of the tongue (Walker, 1995). The factors which influence the oral microflora and hence the reduction of nitrate are nutritional status, infection, environmental temperature, and age (more in elderly) (Eisenbrand *et al.,* 1980).

Ingested nitrate is reduced to nitrite by nitrate reducing microflora in stomach (in favorable conditions viz. pH $\geq$ 4) and upper part of intestine, and may be in other parts of the human gastrointestinal tract. In normal conditions, the reduction of nitrate to nitrite does not occur in stomach, but in situations where the stomach pH is high, such as achlorhydria (Ruddell *et al.,* 1978), atrophic gastritis (Walker, 1995; Mirvish, 1975), artificially-fed infants, or patients using antacid or similar drugs e.g., Omeprazole (Farinati *et al.,* 1996; Colbers *et al.,* 1996; Vermeer *et al.,* 2001), the conversion of nitrate to nitrite occurs even in stomach. The high pH of stomach favors the growth of nitrate reducing organisms. This nitrite is readily and completely absorbed from both the stomach and the upper small intestine. Approximately, 25% of ingested nitrate is actively secreted into saliva, where it is partly (20%) reduced to nitrite by the oral microflora; nitrate and nitrite are then swallowed and re-enter the stomach. Absorbed nitrite is then rapidly distributed throughout the tissues. It is rapidly oxidized to nitrate in the blood, with the formation of methemoglobin.

3.1. Fate of Nitrates Ingested

The following is the flow chart of the fate of ingested nitrate (Li *et al*., 1997; WHO, 1977a):



3.1.1. Metabolism in Mouth

Nitrate is converted to nitrite by microorganisms in the saliva. About 4-7% of ingested nitrate was detected as nitrite in the saliva (Eisenbrand *et al.,* 1980; Speijers *et al.,* 1987; Brüning-Fann and Kaneene, 1993). The major site for this reduction appears to be at the base of the tongue where a stable, nitrate-reducing microflora is established (Walker, 1995). Once nitrite is formed, it has a short biological half-life, being rapidly oxidized to nitrate in the blood. Nitrate undergoes active secretion in humans not only in the salivary duct cells but also in the gastric parietal cells, and is in passive equilibration with other intestinal secretions. This active secretion also occurs at a number of other sites leading to enterosystemic cycling of nitrate and nitrite.

Factors that may influence the oral microbial flora are nutritional status, infection, environmental temperature, and age (Eisenbrand *et al.,* 1980). Salivary nitrite levels were generally higher in older age groups, though considerable variation between individuals was noted (Eisenbrand *et al.,* 1980; Forman *et al.,* 1985).

3.1.2. Metabolism in Stomach

A low pH (1-2) in the fasting stomach is considered normal for adults, and under these conditions, bacterial nitrate reduction does not take place because of the poor bacterial growth. High gastric pH values and sometimes correspondingly high nitrite levels were observed in patients with achlorhydria, stomach cancer, gastric ulcer, atrophic gastritis (Mirvish, 1975; Walker, 1995), and patients treated with cimetidine, omeprazole, which are used to treat hyperacidity, and antacids (Farinati *et al*., 1996; Jaskiewicz *et al*., 1990; Farinati *et al*., 1989; Vermeer *et al*., 2001).

3.1.3. Metabolism in Intestine

Small intestine and lower part of the gut are rife with microorganisms, but nitrate and nitrite have not been found generally in the lower gut or feces. Studies on ileostomy patients given a conventional or high nitrate/nitrite meal indicated that the type of foodstuff ingested can significantly alter levels of nitrite and nitrate in the distal ileum and is a factor in determining nitrite/nitrate input into the proximal colon (Radcliffe *et al.,* 1989).

Wagner (1983a) observed that ascorbic acid did not affect nitrate plasma levels nor the amount of nitrate excreted in urine, feces, or saliva, indicating that ascorbic acid does not interfere with nitrate metabolism (Wagner *et al.,* 1983b).

In general, body sites containing both microflora and nitrate will generate nitrite.

3.2. Formation of N-Nitroso Compounds

Formation of N-nitroso compound is a multiple step process (Choi, 1985). First, nitrate is converted to nitrite after consumption. Then, the nitrite reacts with natural or synthetic organic compounds (known as secondary amines or amides) in food or water to form new combinations called N-nitroso compounds (either nitrosamines or nitrosamides). Many of these N-nitroso compounds have been found to be carcinogenic in all the animal species tested, though some of the most readily formed compounds, such as N-nitrosoproline, are not carcinogenic in humans. At least 75% of the 120 N-nitroso compounds have been found to be carcinogenic to animals (Gilli *et al*., 1984; Terblanche, 1991). The most common N-nitroso compounds are dimethylnitrosamine (DMN), N-methylmethanamine (DMA), trimethylamine (TMA), and trimethylamine oxide (TMAO). The N-nitrosocompounds are carcinogenic in animal species and are probably also carcinogenic to humans. The data from a number of epidemiological studies are at most, only suggestive, relating to carcinogenicity in humans, but it had been reported that a link between cancer risk and endogenous nitrosation as a result of high intake of nitrate and or nitrite and nitrosatable compounds is possible (RIVM, 1989; WHO, 1996).

3.3. Endogenous Synthesis of Nitrate and Nitrite

The excess nitrate excretion that has often been observed after low nitrate and nitrite intake originates from endogenous synthesis, which amounts, in normal healthy humans, to 1 mmol/day on average, corresponding to 62 mg of nitrate per day or 14 mg of nitrate-nitrogen per day. Gastrointestinal infections greatly increase nitrate excretion, as a result, at least in part, of increased endogenous (non-bacterial) nitrate synthesis, probably induced by the activation of mammalian reticuloendothelial system (WHO, 1985b, 1996; Speijers *et al*., 1987; Wishnok *et al*., 1995; Shephard, 1995). This endogenous synthesis of nitrate complicates the risk assessment of nitrate. Increased endogenous synthesis of nitrate, as reported in animals with induced infections and inflammatory reactions, was also observed in humans. Infections and non-specific diarrhea played a role in the increased endogenous synthesis of nitrate (Tannenbaum *et al*., 1978; Green *et al*., 1981; Bartholomew and Hill, 1984; Lee *et al*., 1970; Gangolli *et al*., 1994). These observations are all consistent with the induction of nitric oxide synthase by inflammatory agents, analogous to the experiments described in animals and macrophages.

A major pathway for endogenous nitrate production is the conversion of arginine by macrophages to nitric oxide and citrulline, followed by the oxidation of nitric oxide to nitrous anhydride, and then reaction of nitrous anhydride with water to yield nitrite. Nitrite is rapidly oxidized to nitrate through reaction with hemoglobin. In addition to macrophages, many other cell types can also form nitric oxide, generally from arginine.

3.4. Predisposing Factors

A direct correlation between gastric pH, bacterial colonization and gastric nitrite concentration has been observed in healthy people (Mueller *et al*., 1986). In individuals with gastrointestinal disorders and achlorhydria, high levels of nitrite have been reported (Rudell *et al*., 1978; Dolby *et al*., 1984). Infections and non-specific diarrhea played a role in the increased endogenous synthesis of nitrate (Tannenbaum *et al*., 1978; Green *et al*., 1981; Hegesh and Shiloah, 1982; Bartholomew and Hill, 1984; Lee *et al*., 1970; Gangolli *et al*., 1994). These observations are all consistent with the induction of nitric oxide synthase by inflammatory agents. This induction in humans has been difficult to demonstrate directly, but administration of (^{15}N) arginine to two volunteers resulted in the incorporation of ^{15}N into urinary nitrate in both individuals, confirming the arginine-nitric oxide pathway in humans (Leaf *et al*., 1989).

3.5. Transplacental Cross

Nitrite has been shown to cross the placenta and cause the formation of fetal methaemoglobinemia in rats (El Nahas *et al*., 1984).

3.6. Half-Life

The half-life of nitrate in the body after ingestion is approximately 5 h (Wagner *et al.,* 1983a). Nitrite was not detected in any of the body fluids studied except saliva, where it appeared to increase as nitrate levels decreased (Cortas and Wakid, 1991).

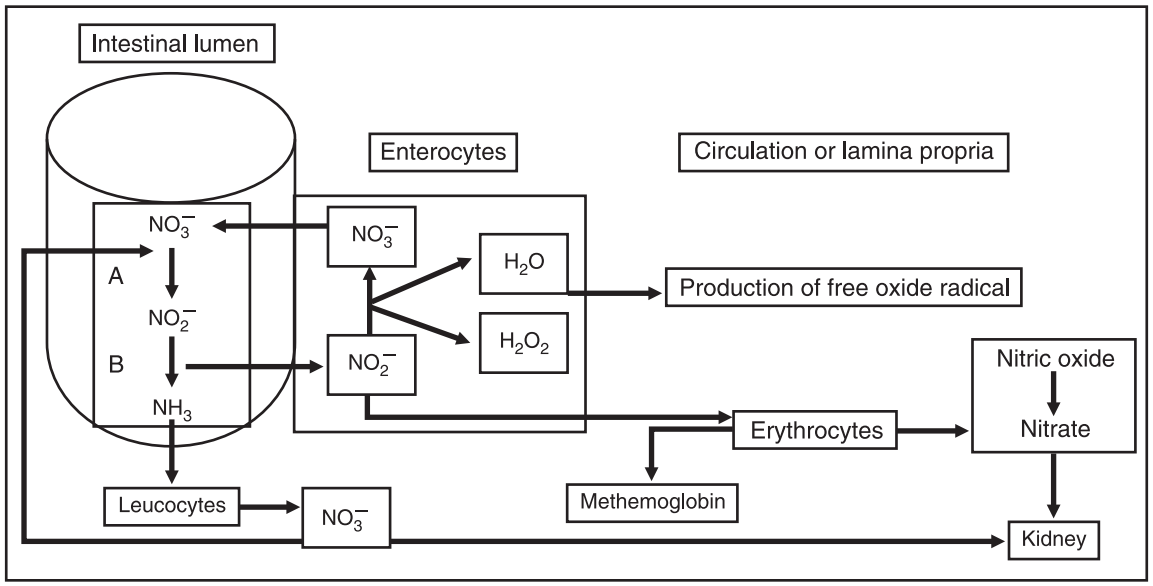


Figure 1: *Metabolism of ingested nitrate in human body at cellular level. a: Bacterial nitrate reductase; b: Bacterial nitrite reductase*

4. METABOLISM OF INGESTED NITRATE IN HUMAN BODY AT CELLULAR LEVEL

Ingested inorganic or organic nitrates will result in increased oxidation of hemoglobin to methemoglobin and increased production of nitric oxide (Murray *et al*., 1993; Waldman *et al*., 1987; Craven *et al*., 1978) **(Fig. 1)**. The conversion of nitrite to nitric oxide is non-enzymatic (Robertson, 1996; Smith *et al*., 1997; Vane *et al*., 1994; Lowestein *et al*., 1994). The oxidation of hemoglobin to methemoglobin results in the formation of superoxide radical by the transfer of single electron. The enzyme superoxide dismutase present in the erythrocytes catalyses the conversion of superoxide radical ($O^{\cdot}$) to H_2O_2 and O_2. The H_2O_2 is decomposed by glutathione peroxidase or catalase, both also present in erythrocytes (Winterbourn *et al*., 1976; Sutton *et al*., 1976). Once the rate of oxidation of hemoglobin increases sufficiently in erythrocytes and overwhelms the protective and reductive capacities (e.g., cytochrome b_5 reductase system etc.) of the cells (Bodansky, 1951; Jaffe, 1981), there is an increased production of reactive free radicals of nitric oxide ($NO^{\cdot}$) and oxygen ($O^{\cdot}$) (Winterbourn *et al*., 1976).

4.1. Fate of Free Radical Nitric Oxide ($NO^{\cdot}$)

Hemoglobin scavenges nitric oxide through the high affinity ferrous sites on heme to form S-nitrosothiol that has an affinity to nitric oxide, which is 8000 times higher than the affinity for oxygen (Hsia, 1998) by binding at β93 cysteine residue on the globin chain. As hemoglobin binds oxygen in the lungs, its binding affinity to S-nitrosothiol is increased. As hemoglobin releases oxygen at the periphery, its affinity for S-nitrosothiol is reduced and nitric oxide is released in the tissues (Hsia, 1998). 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, hemoglobin acts as a carrier of nitric oxide. The enhanced release of nitric oxide from S-nitrosohaemoglobin in hypoxic tissue in turn reduces regional vascular resistance.

Nitric oxide is a biogenic messenger, an endothelial derived relaxing factor (EDRF) (Jaffe, 1981; Hsia, 1998), and activates guanylyl cyclase system (Berger *et al*., 1997) [converts guanosine triphosphate (GTP) to 3′5′cyclic guanosine monophosphate (cGMP)], raising the cGMP pool and, therefore, inducing *inter alia* vasodilatation (Berger *et al*., 1997) by lowering intracellular calcium ion (Smith *et al*., 1997).

4.2. Fate of Free Oxide Radical ($O^{\cdot}$)

In a normal cell, O_2^- will be scavenged by the enzyme superoxide dismutase, and H_2O_2, which is a product of reaction, and by glutathione peroxidase and catalase (Roediger *et al*., 1986; Comly, 1945). 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 (Sutton *et al*., 1976; Hsia, 1998).

Thus, increased consumption of nitrate leads to:

- increased production of nitrite (Allison *et al*., 1984; Cole *et al*., 1980);
- enhanced absorption of sodium from the intestinal lumen (Roediger *et al*., 1986);

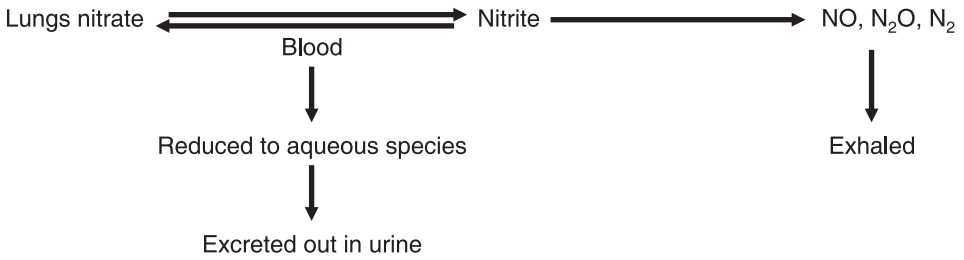
- excess $NO^{\cdot}$ (free radical nitric oxide) generation, having vasodilatory effect (Winterbourn *et al*., 1976; Hsia, 1998; Berger *et al*., 1997; Nitric Oxide, 1997);
- increased production of O_2^-, which will react with other cell constituents, possibly causing irreversible cell damage (Berger *et al*., 1997; Gupta *et al*., 1998) **(Fig. 1)**.

5. EXCRETION

Nitrate excretion in urine generally reflects nitrate intake. The major part of the ingested nitrate is eventually excreted in urine as nitrate, ammonia, or urea, fecal excretion being negligible. Little nitrite is excreted (WHO, 1985b; RIVM, 1989) in urine. About 70-75% of ingested nitrate is excreted within 24 hours of ingestion, irrespective of the amount of intake. The excretion was noticed high in first 5 hours. (Bartholomew and Hill, 1984; Wagner *et al.,* 1983a).The mean nitrate clearance after an oral dose of $NaNO_3$ of 470 µmol/kg body weight was 25.8 ml/minute corrected for a body area of 1.73 m^2. The urinary nitrate/creatinine ratio increased 25 to 70 times after dosing. These results indicated a predominantly tubular excretion of nitrate (Ellen *et al.,* 1982). However, various authors have reported that urinary nitrate excretion may exceed nitrate intake even in infants if the latter is low, as a consequence of endogenous nitrate formation (Hegesh and Shiloas, 1982). Urinary nitrate excretion in infants was reported to be 80-100% of the average intake, but no specific data were given for exposure levels (Turek *et al.,* 1980).

Low levels of nitrate and nitrite were detected in the feces of humans (Saul *et al.,* 1981).

A small amount of ingested nitrate converted to nitrite, changes to oxides of nitrogen (nitric oxide, nitrous oxide, nitrogen etc.) in blood, and gets excreted by exhalation through the lungs (as shown in flow chart given below).



6. ACUTE TOXIC EFFECTS

Exposure to high nitrate may cause acute toxicity.

6.1. In Humans

Human lethal doses of 4-50 g NO_3^- (equivalent to 67-833 mgNO_3^-/kg bw) have been reported. Toxic doses with methemoglobin formation as a criterion for toxicity ranged from 2 to 5 g

(Corré and Breimer, 1979) of NO_3. These values are equivalent to 33-83 and 100-150 mg NO_3^-/kg body weight respectively.

Signs and symptoms of acute nitrate toxicity: Fassett (1973) reported a rapidly occurring severe gastroenteritis with abdominal pain, and blood in the urine and feces as symptoms of acute nitrate intoxication. Repeated doses gave rise to dyspepsia, mental depression, headache, and weakness. Additionally, cyanosis (bluish discoloration) is also observed.

6.2. In Animal Studies

The studies relating to acute exposure of nitrate were conducted in animals. The acute oral toxicity of nitrate to laboratory animals is low to moderate. LD50 values of 1600-9000 mg of sodium nitrate per kg of body weight have been reported in mice, rats, and rabbits. Ruminants are more sensitive to the effects of nitrate as a result of high nitrate reduction in the rumen; the LD50 for cows was 450 mg of sodium nitrate per kg of body weight. Nitrite is more toxic than nitrate: LD50 values of 85-220 mg of sodium nitrite per kg of body weight have been reported for mice and rats (RIVM, 1989; WHO, 1996).

7. CHRONIC TOXIC EFFECTS

It is caused due to long term exposure of non lethal doses. The following effects have been reported:

7.1. Methaemoglobinemia

Nitrates in drinking water have been reported to cause methaemoglobinemia in infants up to 6 months of age.

7.1.1. Mechanism of Methemoglobin Formation

The essential action in the formation of methemoglobin is an oxidation of the ferrous to ferric ion. This oxidation may be brought about in one of the following ways (Bodansky, 1951) by the direct action of the oxidants, or by the action of hydrogen donors in the presence of oxygen, or by auto oxidation. In the presence of nitrites, the ferrous ion of hemoglobin gets oxidized directly to ferric state. Normally, the methemoglobin that is formed is reduced by the following reaction (Benz, 2001):

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

Oxidised cytochrome b_5 (Red Cyt b_5) is regenerated by the enzyme cytochrome b_5 reductase:

$$\text{cyt } b_5\text{oxy cyt } b_5 + \text{NADH} \xrightarrow[\text{Reductase}]{cyt\ b_5} \text{Red cyt } b_5 + \text{NAD Reductase}$$

Thus, the enzyme cytochrome b_5 reductase plays a vital role in counteracting the effects of nitrate ingestion. However, permissible concentration (50 mg/L as nitrate as per WHO) normally present in water or food does not cause any health risk to adults, but the infants constitute a vulnerable group for the following reasons (WHO, 1977b):

- Relatively higher stomach pH (2.0-5.0), which permits the growth of nitrate-reducing organisms such as *coliforms, E. coli, Pseudomonas fluorescence, B. subtilis,* and *Staph. albus*.
- Relative higher consumption of water per unit weight of body.
- The presence of fetal Hb, which readily gets oxidized to meth. Hb.
- Poorly developed cytochrome b_5 reductase system.
- Nitrate gets concentrated by repeated boiling of water for feeding.
- Bacterial contamination of the water itself or dried milk powder.
- Early weaning on to nitrate rich vegetables e.g., spinach.
- Diarrhea causes an increase in stomach pH.

In India, breast-feeding is a common practice especially up to the age of 6 months, and protects the infants from nitrate toxicity. The probability of toxicity to fetus in pregnant women cannot be ruled out, as transplacental passage of nitrate metabolite viz. nitrite has been documented in animal experiment.

Once methemoglobin level in blood exceeds 10% of total hemoglobin, it manifests as clinical cyanosis and causes cellular anoxia. Effects of methemoglobin (in relation to %) and clinical presentation are given below (WHO, 1977c).

Meth-Hb	**Clinical presentation**
<10%	No signs and symptoms
10-25%	No symptoms
	Cyanosis present
25-50%	Cyanosis, dyspnoea, headache
50-60%	Dyspnoea even on lying, cyanosis, disorientation
60%	Lethal levels

While a few cases of methaemoglobinemia in infants have been reported to be associated with water nitrate levels of less than 50 mg nitrate ion/liter, most cases occur with nitrate level of 90 mg nitrate ion/liter or more (Comly, 1945; Cornblath *et al*., 1948; Jaffe, 1981; Marshall *et al*., 1945; Knotek *et al*., 1964; WHO, 1977a). Recently, methaemoglobinemia has been reported in all age groups (Gupta *et al*., 2000). It was observed that methaemoglobinemia was prevalent among all age groups, but was more severe in infants and older age groups (>45 years).

7.2. Cytochrome b_5 Reductase Adaptation

In several Indian villages, people have been consuming water containing high nitrate concentrations, at times up to 1200 mg nitrate ion/liter, which causes methaemoglobinemia in all age groups. (Gupta *et al*., 2000). It was observed that methaemoglobinemia was more in infants and elderly people (Gupta *et al*., 2000); children and adolescents have lower levels of methemoglobin. Adaptation to the reserve of cytochrome b_5 reductase activity with increasing water nitrate concentration to compensate methaemoglobinemia was reported to be a possible mechanism. The study (Gupta *et al*., 1999b) indicated that high nitrate concentrations are hazardous not only to infants but also to groups over 18 years of age. It was reported that the adaptation of cytochrome b_5 reductase activity

peaks at about 95 mg nitrate ion/liter nitrate concentration, and gets exhausted by nitrate level 200 mg nitrate ion/liter, hence making people more prone to toxicity.

7.3. Infant Mortality Rate

A study (Super *et al*., 1981) on African mothers and other studies also (Schwiede, 2005; Fewtrell, 2004; Spalding and Exner, 1993; CDC, 1996; Dorsch *et al*., 1984) reported an increase in infant deaths with an increasing exposure of pregnant mothers and infants to nitrate. This may have been either due to undetected toxic methaemoglobinemia, or malformation and weakness in the infant caused by fetal nitrate exposure.

It was further suggested that because of high IMR, there is a need to revise the nitrate standards for drinking water (Kumar *et al*., 2002).

7.4. Nitrate, Nitrite, Nitrosamines and Cancer

Nitrate itself is not carcinogenic, but instead acts as a "procarcinogen", i.e., it reacts with other chemicals (amines and amides) to form carcinogenic compounds (N-nitroso compounds). The amount of N-nitroso compounds, which can be formed *in vivo*, depends in part on the availability of nitrite, which is itself dependent on the availability of nitrate, the presence of microbial population with nitrate reductase activity; and conditions favorable to chemical nitrosation (Tannenbaum, 1983). The physiological studies (Weisenberg *et al*., 1982; NAS, 1977; Moller 1989) provide strong support indicating the association between nitrate contamination of drinking water and increased cancer rates. It has been reported that endogenously formed N-nitroso compounds are important in human cancer (Michaud, 2001; Mirvish, 1995). Szaleczky *et al*. (2000) reported that endogenously formed nitrogen and oxygen-free radicals are believed to be involved in human cancer etiology. They reported that plasma nitrate/nitrite originates from endogenous nitric oxide production in fasting humans. Decrease in superoxide scavenger activity (SSA), and free sulfhydryl groups (SH) reflects the amount of superoxide anion generated, and nitrotyrosine is believed to be formed by the interaction of tyrosine and peroxynitrite.

Population ingesting larger amounts of nitrate might be expected to have a higher incidence of cancer of the relevant target organ (NAS, 1981). In addition, products of nitric oxide, generated by macrophages during inflammation, can react with water at neutral pH to form nitrite and nitrate, and with amines to form nitrosamines (Mirvish, 1995). Hence, the International Agency for Research on Cancer (IARC, 1978; Fraser *et al*., 1980) has classified nitrate/nitrite as possibly carcinogenic to humans.

In animal or human studies, N-nitroso compounds has been associated with 15 different types of cancers, including tumors in the bladder, stomach, brain, esophagus, bone and skin, kidney, liver, lung, oral and nasal cavities, pancreas, peripheral nervous system, thyroid, trachea, acute myelocytic leukemia, and T and B cell lymphoma. This group has been found to be carcinogenic in a wider range of tumors than any other group of carcinogens (NAS 1977, 1978; IARC, 1978; Mirvish, 1991, 1983). 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 (NAS 1977). IARC (1978) concluded that 11 N-nitroso compounds were carcinogenic in man. In humans, the

organs thought to be most at risk from cancers are the stomach, esophagus, nasopharyngeal cancer, and cancer of the bladder.

Still there are studies (RCEP, 1979; Fraser *et al*., 1980; Zaldivar *et al*., 1973; Armijo *et al*., 1975; Zaldivar *et al*., 1975; Zaldivar, 1977; Cuello, 1976; Hill *et al*., 1973), which have drawn no firm conclusion to prove nitrate as carcinogen.

7.4.1. Nitrate, Nitrite, Nitrosamines and Cancer in Infants and Children

A high cancer risk: A series of human and animal studies have indicated that infants, children, and even the fetus may face elevated risk later in life due to the effects of nitrate or nitrite exposure. Human epidemiology studies found that an increase in stomach cancer rates with the consumption of well water high in nitrate, and individuals exposed during the first ten years of life formed a high risk group (Gray *et al*., 1991; Cuello *et al*., 1976). Studies with N-nitroso compounds on both fetal and infant equivalent animals support this finding (Gray *et al*., 1991; Cuello *et al*., 1976). Animal studies have documented transplacental passage of nitrite and reported that a high dose of nitrate to the pregnant ham can cause subacute methaemoglobinemia in fetal rats (Shuval and Gruner 1972). Further animal studies with nitrosamine compounds showed that exposure during infancy increases the cancer risk due to N-nitroso compounds by a factor of six (Gray *et al*., 1991).

7.4.2. Nitrate and Gastric Cancer

Since 1976, a relationship between increasing rates of stomach cancer and increasing nitrate intake has been documented (Mirvish 1983; Armijo 1975, 1981; Cuello 1976; Xu, *et al*., 1992; Reed *et al*., 1981, 1983). An increased risk of gastric cancer in conditions associated with low gastric acidity is well-recognized, and lends support to the hypothesis that N-nitroso compounds may be involved in its development. High levels of gastric juice nitrites and elevated urine levels of nitrosamines have been reported in patients with chronic atrophic gastritis or high intragastric pH levels (Farinati *et al*., 1996; Jaskiewicz *et al*., 1990; Farinati *et al*., 1989; Vermeer *et al*., 2001; Dallinga, 1998). High nitrate intake with low vitamin C intake, high meat intake, or chronic bowel inflammation have been shown to be associated with increased cancer incidence. A study suggests that the *Cyp2E1 RsaI* polymorphism is associated with a reduced risk of gastric cancer (Nishimoto *et al*., 2000).

Reed *et al*. (1983) reported for the first time in humans a significant lowering of gastric juice and N-nitroso compounds by ascorbate treatment in 51 achlorhydric subjects. This may be an important observation for preventing gastric cancer in high-risk subjects (NAS, 1981; WHO, 1985b, 1996; ECETOC, 1988; RIVM, 1989).

7.4.3. Nitrate and Non-Hodgkins Lymphoma (NHL)

Epidemiological study of nitrate in well water in Nebraska showed an association between nitrate contamination and a different kind of cancer—non-Hodgkins lymphoma (Weisenburger, 1990). The authors concluded that these findings suggest that NHL in eastern Nebraska may be related to the use of pesticides and nitrogen fertilizers.

7.4.4. Nitrate and Colo Rectal Cancer

Roos *et al*. (2003) observed negligible overall associations of either cancer with average nitrate level and with the number of years with average nitrate-nitrogen level greater than 5 or 10 mg/L. They reported an increased risk of colon cancer associated with drinking water nitrate among certain subgroups expected to have high rates of nitrosation, but they did not observe the same patterns for rectum cancer. The colon and rectum have similar epithelial tissues, but these two cancer types have somewhat different risk factors. Although N-nitroso compounds formed in the digestive tract would be expected to pass through the rectum, contractile activities in the rectum cause fecal matter to pass through quickly, resulting in less contact time with the rectum than with the colon. (Vander *et al*., 1994).

7.4.5. Nitrate and Urinary Bladder Cancer

Recently, studies have pointed out the risk of bladder cancer by high nitrate ingestion (Michaud, 2004). In another epidemiological study, drinking water nitrate levels were associated with a significant elevation in bladder cancer risk (Weyer *et al*., 2001).

7.5. Respiratory System

A correlation between drinking water nitrate concentration, high methemoglobin levels, and pathological changes in bronchi and lung parenchyma has been reported in animals studies (Shuval *et al*., 1972; Gruener *et al*., 1970). Changes in lungs reported were frequent dilation of bronchi with lymphocytic infiltration of mucosa and muscles, frequent purulent bronchial exudates, interstitial round cell infiltration, and fibrosis at certain areas. WHO (1977d) reported an association of increased asthmatic attacks and high air-borne nitrate concentrations.

A high percentage (40-82%) of the cases of acute respiratory tract infection with history of recurrence has been reported in children drinking water high in nitrate. (Gupta *et al*., 2000). These findings were further substantiated (Gupta *et al*., 1999c) in an animal experiment on rabbits. Significant change in lungs were observed with congestion, presence of inflammatory cells, and breakdown of alveoli. These changes were absent when animals were fed water containing 45 mg/L of nitrate, as the concentration of nitrate increased, the changes were more pronounced and severe.

7.6. Cardiovascular System

Earlier onset of hypertension has been reported with high nitrate ingestion (Malberg, 1978).

Animal studies in early seventies (Shuval *et al*., 1972; Gruener *et al*., 1970) reported a correlation between drinking water nitrate concentration, high methemoglobin levels, and cardiac muscles (Shuval *et al*., 1972; Gruener *et al*., 1970). The changes reported in cardiac muscles were small foci of inflammatory cells and fibrosis. Diffuse interstitial cellularity with pronounced degenerative foci was frequent in the highest nitrate groups only. The intramural coronary arteries provided surprising results with some degree of thinning and dilation in comparison to the control group, which showed some degree of thickening, a marked hypertrophy, and narrowing. Further, Gupta *et al*. (1999c) in an animal experiment reported that high nitrate ingestion was associated with changes in cardiac muscles in form of branching of myosites, presence of inflammatory cells, and focal degenerative

changes in cardiac muscles. These changes were absent when animals were fed with water containing 45 mg/L of nitrate, but as the concentration of nitrate increased, the changes were more pronounced and severe.

The changes in cardiac tissue even in animal study may be of importance, 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 (USP DI, 1990).

At the same time, Morton (1971) reported an inverse relationship between cardiovascular mortality and nitrate concentration in water supplies.

7.7. Gastrointestinal System

Gupta *et al*. (2001) reported a problem of recurrent diarrhea in children upto 8 years of age. They suggested that increased consumption of nitrate leads to:

- increased production of nitrite (Allison *et al*., 1984, Cole *et al*., 1980);
- enhanced absorption of sodium from the intestinal lumen (Roediger *et al*., 1986);
- excess $NO^{\cdot}$ (free radical nitric oxide) generation having vasodilatory effect (Winterbourn *et al*., 1976; Hsia 1998; Berger *et al*., 1997; Nitric oxide, 1997);
- increased production of O_2^{-}, which will react with other cell constituents, possibly causing irreversible cell damage (Berger *et al*., 1997; Gupta, 1998).

These changes in enteric mucosa cause hyperemia and edema in the enteric mucosa, and later on possibly cause irreversible mucosal damage, and therefore provide high-risk conditions suitable for recurrent diarrhea.

These findings were correlated well with histopathological studies conducted on rabbits (Gupta *et al*., 2001). It was observed that the histopathological changes observed in the intestinal mucosa on rabbit study revealed that the degree of damage in colon was progressive, as the nitrate content of the ingested water increased.

These findings are of interest, since infants and children consume nitrate rich water, especially during diarrhea, which is an aggravating factor for nitrate toxicity (Murray *et al*., 1993), where the use of WHO oral rehydration solution (ORS) is a normal routine. The use of WHO ORS could be of grave concern if prepared with water containing high nitrate.

Recurrent stomatitis (Gupta *et al*., 1999a) was another problem reported in people using high nitrate containing drinking water. This finding was observed in all groups and was well correlated with an increased cytochrome b_5 reductase activity following high nitrate ingestion.

7.8. Abortions

Health effects associated with ingestion of nitrate-contaminated water include stillbirth, low birth weight, slow weight gain, and even death of the animals affected (Committee on nitrate accumulation, 1972). Spontaneous abortions were also observed in laboratory animals and livestock (FDA, 1972; Sund *et al*., 1957). In 1959, for the first time in humans, spontaneous abortions were reported to have an association with increased methemoglobin levels due to high nitrate ingestion (Muhrer, 1959). In subsequent years, some studies (Schmitz, 1961; CDC, 1996; Fewtrell, 2004) found an increased risk of spontaneous abortion or certain birth defects if the mother drank water high in nitrate. Therefore, it has been suggested that women who are pregnant or are trying to become pregnant should not consume water containing high levels of nitrate.

7.9. Birth Defects

The risk of birth defects due to nitrate exposure is a particular concern because of the fact that risk could be due to a single high dose of nitrate early in the pregnancy that later has profound effects on long-term fetal development. Animal studies have indicated that there is a transplacental transfer of N-nitroso compounds to the fetus (Shuval and Gruener, 1972) and this fetal exposure can cause cancer later in life (Druckrey, 1966). It was also reported that a single dose of nitrosamide given to pregnant rats on day 15 of the pregnancy was reported to cause birth defects in the offspring. In rats and hamsters studies, 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 (Druckery, 1966; Givelber, 1969).

Extending these finding on human being, a number of human epidemiology studies were conducted (Dorsch, 1984; Knox, 1972; Super, 1981). A link was found between anencephaly rates and intake of cured meat containing high levels of nitrite (Knox, 1972). This study provided the first suggestive evidence in humans that nitrite consumption in food could have adverse impacts on the fetus. A 1984 study (Dorsch *et al*., 1984) found statistically significant dose response relationships between birth defects of the central nervous system and musculoskeletal system and increasing nitrate concentration of drinking water. Arbuckle *et al*. (1988) found the evidence for an association between nitrate and birth defects to be weaker. Fan (1987) reported that nitrate contamination of 45 ppm nitrate ion or 10 ppm nitrate-nitrogen adequately protects the very young from nitrate-induced toxicity, both pre- and postnatally. The adverse reproductive effects reported occurred at doses that were about one thousand times higher than the estimated human intake. Croen *et al*. (2001) found that exposure to nitrate in ground water at concentrations above 45 mg/liter maximum contaminant level was associated with increased risk for anencephaly (Odds ratio 4.0; confidence interval 1.0-15.4). Risk for anencephaly increased for mothers with the highest nitrate exposure, 36-67 mg/liter in ground water compared with nitrate exposure less than 5 mg/litre (Odds ratio 6.9; confidence interval 1.9-24.9).

7.10. Diabetes

A positive correlation between high nitrate levels in drinking water and increased incidence of type 1 diabetes was observed (Kostraba, 1992; Parslow *et al*., 1997; Van Maanen *et al*., 2000). Further studies indicated that consumption of high levels of NOCs (N-nitroso compounds) by human mothers may result in an increased incidence of type 1 diabetes in male offspring (Helgason *et al*., 1981). In animal studies, NOCs have been reported to be toxic to pancreatic beta cells, providing the rationale for these observations (Wilson *et al*., 1983). Kostraba (1992) postulated that exposure to nitrate in drinking water causes increased production of free radicals, which may play a role in the aetiopathogenesis of IDDM. The production of free oxide radicals following high nitrate ingestion has been further supported by the studies conducted by Gupta *et al*. (1999a, 2000).

Further, it is to be noted that there are studies, which indicated no relationship between nitrites and nitrates in drinking water and increased incidence of type 1 diabetes. (Dahlquist *et al*., 1990; Virtanen *et al*.,1994; Verge *et al*., 1994). Thus, while some studies suggest an association between intake of nitrate/nitrites and risk of type 1 diabetes, the data remain limited and inconsistent (Longnecker *et al*., 2001). Hence, the association of dietary nitrites with diabetes remains tenuous and further research needs to be supported.

7.11. Thyroid

Nitrate competitively inhibits iodine uptake. If dietary iodine is available at an adequate range (corresponding to a daily iodine excretion of 150-300 μg/day), the effect of nitrate is weak, with a tendency to zero. The nitrate effect on thyroid function is strong if a nutritional iodine deficiency exists simultaneously (Höring *et al*., 1991; Höring, 1992). An association between high nitrate concentrations in drinking water and goiter has been (Höring *et al*., 1991; Höring, 1992; Van Maanen *et al*., 1994) described by various workers. A dose-response relationship could be demonstrated by Höring *et al*. (1991) (nitrate in drinking water *vs.* incidence of goiter) as well as by Van Maanen *et al.* (1994) (nitrate in drinking water *vs.* thyroid volume). Both the experimental and epidemiological studies give the impression that nitrate in drinking water has a stronger effect on thyroid function than nitrate in food.

7.12. Adrenal Gland

High nitrate ingestion affects the adrenal gland. In addition to the effect of nitrite on the adrenal zona glomerulosa in rats, a study in humans indicated that sodium nitrite (0.5 mg of sodium nitrite per kg of body weight per day, during 9 days) causes a decreased production of adrenal steroids, as reflected by the decreased concentration of 17-hydroxysteroid and 17-ketosteroids in urine (Til *et al*., 1988; Kuper and Til, 1995). Similar results were also found in rabbits (Violante *et al*., 1973). Although the mechanism is not clear, the effects of nitrite seen in rats seem relevant for the hazard assessment in humans.

7.13. Immunity

Few studies (Ustyugova *et al.,* 2002; Kozliuk *et al*., 1989; CDFA, 1989) reported the effect of nitrate/nitrite ingestion on immune system. The effect of nitrate ingestion (Ustyugova *et al.,* 2002) on human immune system indicated that nitrate had no effect on lymphocyte growth, but nitrite decreases the proliferation of lymphocytes. Fibroblast growth remain unaffected. A decreased production of Th1 cytokines (interleukin-2, interferon-gamma, and tumor necrosis factor-beta), which is 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 Th1 to Th2 response in some individuals, exposure to these compounds may decrease the person's responsiveness to infectious diseases. The levels of nitrate used in this study are relevant to human health because they are present in the liquid portion of the diet (non breastfed) of some 2-month-old infants in rural Romania. Animal studies also reported an immune suppression due to high nitrate ingestion. (Porter *et al*., 1999).

7.14. Animal Experiments

Clinical manifestations of nitrate experiments on different systems are as follows:

7.14.1. Central Nervous System

Experiments on mice indicated that nitrites had some form of sedative effects. The development of sensory-motor functions and adult learning behavior was studied in rats exposed to nitrate. The

results indicated a nitrate-induced deviation in behavioral development, and an impairment in learning behavior, particularly of the discriminative type (Markel *et al.,* 1989).

7.14.2. Gastrointestinal System

In a 19-month study, effects of nitrate on gastric epithelium were studied (Ptashekas, 1990). Ultrastructural examination showed that sodium nitrate alone or in combination with saphrol caused atypical changes in the gastric epithelium.

7.14.3. Cardiovascular System

Animal studies (Shuval *et al*., 1972; Gruener *et al*., 1970) reported on increased cardiac muscle contraction, which correlated directly to meth Hb levels. At 10-15% Meth. Hb, ECG shows shortening of Q-T interval, reduction in T wave.

7.14.4. Embryo Toxicity

Experiments on rats showed anemia, increased meth Hb, and increased mortality in offsprings. Nitrite appeared to cause fetotoxicity in rats at drinking-water concentrations equivalent to 200 and 300 mg of sodium nitrite per kg of body weight per day, causing an increased maternal meth. Hb levels. However, after similar doses in feed in other studies, no embryo toxic effects were observed in rats. In a reproductive toxicity study in guinea-pigs at dose levels of 0, 50, or 60 mg of sodium nitrite per kg of body weight per day given by subcutaneous injection, fetal death followed by abortion occurred at the highest dose level. Teratogenic effects were not reported in studies on mice and rats (RIVM, 1989; WHO, 1996).

7.14.5. Genotoxicity

In an animal experiment on mice, an increase in chromosome aberrations was found in a group of animals, who were consuming nitrate more than 707 mg/kg BW. It was observed that the number of micronuclei was enhanced at 79 and 236 mg/kg BW. The cytotoxicity occurred in the bone marrow as shown by a concomitant depression of the bone marrow. According to the authors, it cannot be excluded that formation of N-nitroso compounds was responsible for the bone marrow damage (Luca *et al.,* 1985).

7.14.6. Reproductive Behavior

Reproduction in female guinea pig (RIVM, 1989; WHO, 1996) was grossly impaired by high nitrate ingestion (30,000 mg of potassium nitrate per litre). In rabbits, dose levels of 250 or 500 mg of nitrate per litre administered during 22 weeks revealed no detrimental effects on reproductive performance after successive gestations. In sheep and cattle, no abortions were observed at dose levels causing severe methaemoglobinemia (RIVM, 1989; WHO, 1996).

7.14.7. Mutagenicity and Related End-Points

Nitrite is mutagenic: It causes morphological transformations in *in vitro* systems; mutagenic activity was also found in a combined *in vivo–in vitro* experiment with Syrian hamsters. The results of *in vivo* experiments were controversial (RIVM, 1989; WHO, 1996).

7.14.8. Serum Somatomedin Activity

A decrease in serum somatomedin activity due to nitrate administration was also observed (Jahreis *et al.,* 1987).

8. TREATMENT AND PREVENTION

8.1. Treatment

Supplementation of ascorbic acid, methionine, alpha-tocopherol, methylene blue has been found to be effective in overcoming the problem of nitrate toxicity. These substances are effective in inhibiting nitrosation if present in gastric juices at all time in significant concentrations.

8.2. Prevention

8.2.1. At Environmental Level

Avoid water pollution:

- Minimize the contamination of water supplies by nitrates originating from agricultural practice—avoid inadvertent and excessive use of nitrogenous fertilizers.
- Avoid water pollution.
- Avoid the habit of open air defecation.
- Avoid the stagnation of waste water around the source of water.
- Avoid the sewage disposal directly to ground water table.

Removal of nitrate from drinking water—denitrification: The removal of nitrate from water is costly and difficult to implement and maintain both at domestic as well as community level. The following are the known processes of denitrification:

- *Physiochemical process:* The commonly used processes under this category are:
  - — Reverse osmosis
  - — Ion exchange
  - — Electrodialysis
- *Biological treatments:* The details of only biological denitrification have been given in appendix 1. This process is important since it bears similarity with the metabolism of nitrates in living organisms.

8.2.2. At Human Level

- Breast feeding only, at least up to the age of 4 months.
- If top feed is necessary then preferably use cow milk (of course unadulterated) or dry milk preparations reconstituted with water containing low level of nitrate.

- Avoid the use of high nitrate containing food for weaning.
- Avoid use of nitrate and nitrite as preservative to the minimum extent possible especially to cured and canned meats and fish.
- Use of drinking water with low level of nitrates, especially to pregnant mothers.
- Avoiding WHO ORS preparation with locally available high nitrate water during diarrhea.
- Avoid long term use of anti-acid secretory agents especially in pregnant mothers and children. In case if it is necessary to use these drugs, they should be used cautiously and preferably with the use of antioxidants.

9. POLICY ISSUES AND RECOMMENDATIONS

For human consumption, WHO report, 2004, permits up to 50 mg/L but only for short term exposure, whereas IS-10500 permits 45 mg/L (as NO_3) as desirable limit and 100 mg of NO_3 per liter as maximum permissible limit in the absence of alternate source.

Since the literature available on nitrate toxicity in human beings is limited and does indicate the grave effects of nitrate ingestion e.g., methemoglobinemia (effecting all systems of body due to lack of oxygen carrying capacity), cancer, diabetes (especially in children), damage to lungs and heart muscles, increased infant mortality, abortions, birth defects, recurrent diarrhea etc., the reports are by large, non conclusive.

Considering these toxicities and limited role of the protective system (adaptation of enzyme cytochrome b_5 reductase) available in human body against nitrate toxicity, probably the present WHO guidelines for short exposure may be adhered to. Looking to the ground reality that available technology options are not affordable for public supplies, it is recommended that:

- simple policy changes to minimize the toxicity of nitrate may be urgently introduced in government campaign, at least in high nitrate belt.
- more detailed epidemiological health related studies covering a large sample are carried out to arrive at new acceptable standards.
- a simple and cost effective method for nitrate removal should be developed.
- health education system should be developed to make people aware about the toxic effects of nitrate ingestion and the ways of its prevention.
- certain recommendations made by the Council on Scientific Affairs were adopted by the AMA House of Delegates, as AMA directives at the 2004 AMA Annual Meeting can be adopted in other countries facing the problem of nitrate toxicity:
  - — The AMA supports the current Food and Drug Administration and United States Department of Agriculture regulations, including current labeling requirements for nitrites in food. (Directive).
  - — 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).

10. BIOLOGICAL DENITRIFICATION

The biological reduction of nitrate to nitrite and subsequently to dinitrogen gas requires a suitable electron donor. The electron donor is usually an organic molecule, and methanol is the most commonly

used carbon source. Equations 1 and 2 represent the reduction of nitrate to nitrite and nitrite to nitrogen gas respectively, and the equation 3 represents the overall reaction using methanol as the electron donor (McCarty *et al*., 1969).

$$NO_3^- + 0.33\ CH_3OH \rightarrow NO_2^- + 0.67\ H_2O + 0.33\ CO_2 \quad ...(1)$$

$$NO_2^- + 0.5\ CH_3OH \rightarrow 0.5\ N_2 + 0.5\ H_2O + 0.5\ CO_2 + OH^- \quad ...(2)$$

and overall:

$$NO_3^- + 0.833\ CH_3OH \rightarrow 0.5\ N_2 + 1.16\ H_2O + 0.833\ CO_2 + OH^- \quad ...(3)$$

Other organic carbon sources such as ethyl alcohol, sucrose, acetone, brewery waste, chemical process waste, corn starch waste, molasses, wharf, sulfide liquor and winery residue etc. are taken as inexpensive carbon substrates.

Autotrophic denitrification has been studied using hydrogen or various sulfur compounds. Autotrophic denitrifying bacteria use molecular hydrogen or other inorganic compounds as the reductants and carbondioxide as the source of carbon as shown in equation below:

$$5\ H_2 + 2H^+ + 2\ NO_3 \rightarrow N_2 + 6\ H_2\ O \quad ...(4)$$

Some researchers have evaluated the use of reduced sulfur compounds such as sulfide and thiosulfate for the denitrification of water and domestic and industrial wastewater (Sutton *et al*., 1979; Amminudin and Nicholas, 1973). Sulfate is a by-product of denitrification using sulfur compounds as given in following equations:

Thiosulfate (Claus and Kutzner, 1985).

$$5\ S_2\ O_3^{2-} + 8\ NO_3^- + H_2O \rightarrow 4\ N_2 + 10\ SO_4^{2-} + 2H^+ \quad ...(5)$$

Sulfide (Barrenstein *et al*., 1986).

$$5\ S^{2-} + 8\ NO_3^- + 8\ H^+ \rightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O \quad ...(6)$$

Though oxygen inhibits denitrification, there have been periodic reports of aerobic denitrification (Marshall *et al*., 1953; Krul, 1976). Recently, under controlled conditions in homogeneously suspended bacterial cultures at D.O. concentrations ranging from 10% to twice air saturation, persistent denitrification has been reported by many workers (Meiberg *et al*., 1980, Gupta and Kumar, 1999). Robertson *et al.* (1988) not only detected the presence of appropriate enzyme but also demonstrated the production of nitrogen containing gases from nitrate by *Thiosphaera pantotropha* (isolated from desulphurizing, denitrifying waste water treatment systems) at dissolved oxygen concentrations up to 90% of air saturation.

Biological denitrification has a limited role for treatment of drinking water because introduction of carbon source and bacteria to water for denitrification would increase the post-treatment cost significantly. Several investigators have evaluated the injection of various substrates and nutrients into aquifers in an effort to simulate *in situ* denitrification. Liquid substrates such as acetic acid, ethanol and treated wastewater have been used and gaseous substances have been evaluated (Kruithof *et al*., 1988; Soares *et al*., 1988; Gayle *et al*., 1989). Application of *in situ* ground water denitrification depends on the prevention of well clogging, and biomass accumulation is thought to be the main

cause of *in situ* clogging. In shallow aquifers, accumulation of gas may represent a major contributor to the clogging of wells. It is important to take certain precautions while designing an *in situ* denitrification scheme, e.g., complete utilization of carbon sources should be ensured since no organic carbon is allowed to be present in drinking water; clogging of aquifer must be anticipated particularly in soil that provide small pore size; release of N_2 gas must be ensured; movement of bacteria in porous media should be controlled by giving due consideration to factors like ratio of cell size and pore size, shape of microorganisms, flow velocity, injection concentration of bacteria etc. A proper balance of above factors should be made to ensure uniform dispersion of bacteria in sub-soil. Biological denitrification has been extensively used for the removal of nitrates from domestic and industrial wastewaters, which can help prevent nitrate contamination of groundwater, however, only certain advancements in this process have been summarized in the subsequent paragraph.

Gupta (1997) gave a comprehensive review of the literature pertaining the enzyme system of *Thiosphaera pantotropha,* which is responsible for the nitrification and denitrification properties and its potential applications for wastewater treatment. Many laboratory studies were carried out in different suspended growth and fixed film systems to bring out low cost options for the simultaneous removal of carbon and nitrogen from different synthetic samples simulating domestic and industrial waste waters (Gupta and Gupta 1999, 2001; Gupta *et al*., 1994).

11. FUTURE RESEARCH AREAS FOR NITRATE TOXICITY

Nitrate is perhaps the most extensively studied anion in water for its chronic toxic manifestations, as it is one of the most common pollutants of drinking water all across the world. Despite the availability of vast literature on nitrate toxicity, the studies on humans are limited because of ethical and other considerations, and the most toxic manifestations are available in animal studies only. Many epidemiological studies have been conducted to establish certain cause-effect relationships for many clinical manifestations of nitrate toxicity, and the results of such studies are normally inconclusive or the correlations are statistically non significant. This has led to a very insufficient knowledge generation among researchers and practicing doctors about the preventive and curative aspects of different manifestations.

- Metabolism of nitrate indicated that there is a marked similarity in manifestations on oral mucosa, stomach and intestines that can be correlated with the conditions prevailing in those parts of the body, which govern the progress of biological denitrification to nitrite, nitric oxide, nitrous oxide or dinitrogen level. Such similarities needs to be studied by taking simultaneous samples from these body parts after subjecting test animals to high nitrate ingestion and analyzing for different nitrogen species.
- The effect of long term use of antacids, H_2 receptor inhibitor, proton pump inhibitor with high nitrate ingestion should be studied among human volunteers with adequate control. Microbiological analysis of salivary samples as well as the swab samples can help monitor the progress of biological conversion of ingested nitrate in these localized areas. Further it should be seen that Is there any improvement with addition of antioxidant.
- Role of ingested and endogenous nitrate is also important to delineate in human.
- Understanding the nitrate metabolism and its relation to free oxide radical production with their role in carcinogenesis may give rise to an insight to the pathophysiology of cancer development in human beings. This may provide a guideline in treatment and prevention of cancer.

12. CONCLUSION

Nitrate and Nitrite are natural ions that are a part of the nitrogen cycle. Naturally occurring nitrate levels in surface and ground water are generally a few milligram per liter. In many ground waters, an increase of nitrate level has been observed due to water percolating through nitrate rich rocks and owing to the farming practices of using chemical fertilizers recommends.

For human consumption, WHO report, 2004, permits up to 50 mg/L but for only short term exposure, whereas BIS-10500 permits 45 mg/L (as NO_3^-) as desirable limit and 100 mg of NO_3^- per liter as maximum permissible limit in the absence of alternate source.

Not much literature on human studies is available on nitrate toxicity except reports documenting methaemoglobinemia in infants due to high nitrate ingestion. Apart from methaemoglobinemia, few studies indicated nitrate as a cause of cancer, but it is still controversial and no firm conclusions have been drawn so far. The other effects observed were increased infant mortality, abortions, birth defects, recurrent diarrhea, 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. Recent studies have indicated that high nitrate ingestion adversely effects 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 being, but to a limited extent only.

More detailed epidemiological health related studies covering a large sample are required to provide insight to the nitrate toxicity on human beings. It is possible that a detailed study on patho-physiology of nitrate metabolism and its effect on human being would yield a better understanding of the various diseases caused by nitrates, and their prevention and treatment.

Since it is very difficult and costly to remove nitrate from water because it is chemically non reactive in dilute aqueous solutions, it is recommended that a simple change in habits and adoption of simple preventive measures may be urgently introduced in government campaign, at least, in high nitrate belts. Indiscriminate use of nitrogenous fertilizers should be avoided. The most important strategy is to promote breast feeding up to the age of at least six months.

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