# Recurrent Acute Respiratory Tract Infections in Areas With High Nitrate Concentrations in Drinking Water

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A review of the literature indicated an association among high nitrate ingestion, methemoglobinemia, and pathologic changes in bronchi and lung parenchyma. The present study examined a possible correlation among drinking water nitrate concentration, methemoglobin levels, cytochrome $b_5$ reductase activity, and acute respiratory tract infection with a history of recurrence (RRTI). Our study was conducted in five village units in the state of Rajasthan, India, with nitrate concentrations of 26, 45, 95, 222, and 459 mg $NO_3$ ion/L. We randomly selected 88 children. The children were up to 8 years of age, age matched, and represented 10% of the total population of these areas. We obtained detailed RRTI histories and conducted medical examinations. Methemoglobin levels and cytochrome $b_5$ reductase activity were estimated biochemically. The data collected were statistically analyzed using spreadsheet software on a personal computer. We observed strong interdependence between methemoglobin levels and RRTI in children up to 8 years of age. Methemoglobin levels alone explained 80% of the variation in the RRTI cases. This study indicates that methemoglobinemia, secondary to high nitrate ingestion in drinking water, causes RRTI. Increased production of methemoglobin and free radicals of nitric oxide and oxygen due to nitrate metabolism in the body lead to alveolar damage and mismatching of ventilation and perfusion, which may be the reason for high mortality in children due to RRTI. *Key words*: cytochrome $b_5$ reductase, drinking water, methemoglobinemia, nitrate, recurrent acute respiratory infection (RRTI). *Environ Health Perspect* 108:363–366 (2000). [Online 6 March 2000]
*http://ehpnet1.niehs.nih.gov/docs/2000/108p363-366gupta/abstract.html*

Acute respiratory infection (ARI) is a common disease. ARI contributes to approximately 20% of mortality in children younger than 5 years of age (*1–4*). An Indian Council of Medical Research multicentric study (*5*) indicated that 15–30% of childhood deaths occur because of ARI. A review of the literature reveals no reports on the high incidence of ARI in children who live in areas with high concentrations of nitrate in the drinking water.

Some animal studies have reported correlation among drinking water nitrate concentration, high methemoglobin levels, and pathologic changes in bronchi and lung parenchyma; namely, frequent dilation of bronchi with lymphocytic infiltration; atrophy of mucosa and muscles; frequent purulent bronchial exudates and interstitial round cell infiltration; and fibrosis at certain areas (*6,7*). A study conducted on rabbits to observe the effects of ingestion of different nitrate concentrations (0–500 ppm) on lungs indicated significant changes in lung parenchyma; namely, congestion, the presence of inflammatory cells, and the breakdown of alveoli (*8*). The degree of damage in these tissues was progressive as the nitrate content of the ingested water increased. An association of increased asthmatic attacks with high airborne nitrate concentrations has also been reported (*9*).

Excessive nitrate in drinking water causes methemoglobinemia in infants up to 6 months of age (*10–12*). The World Health Organization permissible limit in drinking water is 50 mg $NO_3$ ion/L (*10*). In several developing countries, especially in India, the consumption of water containing up to 500 mg/L nitrates is not uncommon.

Nitrates are reduced to nitrites mostly by bacterial action in the intestine, although this conversion also partially takes place in the oral cavity by oral microflora (*13*) and after absorption reacts with hemoglobin, oxidizing it to methemoglobin (*10*). The health risks from exposure to nitrates are therefore related not only to their concentration in drinking water and food, but also to the conditions conducive to their reduction to nitrites.

The increased nitrate ingestion increases the formation of methemoglobin, which creates hypoxia and causes vasoconstriction of the pulmonary arterioles, but simultaneously increases the production of reactive free radicals of nitric oxide ($NO^{\bullet}$) and oxygen ($O^{\bullet}$) (*14*). Nitric oxide is a biogenic messenger—an endothelial-derived relaxing factor (EDRF) (*15,16*). Nitric oxide induces, among other things, vasodilatation (*17*) by lowering intracellular calcium ions (*16*). $O^{\bullet}$ reacts with other cell constituents, possibly causing irreversible cell damage. Therefore, the vasoactivity of the pulmonary arterioles depends on the balance between constricting and dilating stimuli (*17*). Once the pulmonary arteriolar dilatation predominates, it will lead to congestion, the presence of inflammatory cells, and the breakdown of alveoli. These changes in pulmonary circulation and alveoli lead to mismatching of ventilation and perfusion, and therefore provide high-risk conditions suitable for recurrent respiratory infections.

Cases of ARI with a history of recurrence (RRTI) were noted during the epidemiologic investigations for the evaluation of nitrate toxicity (*18,19*). We wanted to find any correlation, if it exists, among drinking water nitrate concentration, cytochrome $b_5$ reductase activity, methemoglobinemia, and cases of RRTI.

An appropriate institutional review board with members from the SMS Medical College, Jaipur, India, and the Environmental Engineering Research Institute, Nagpur, India, approved this project, and we obtained informed consent for blood draws from both guardians and/or children.

## Methodology

We selected five village units where the drinking water source average nitrate concentrations were 26, 45, 95, 222, and 459 mg nitrate ion/L. The village units are in the state of Rajasthan, India. Village units were congruous areas located within a distance of approximately 2 km; the units were inhabited by people of similar socioeconomic status and similar food habits, and the entire population used drinking water from the same single source. There were no industries in the region and the areas were away from the highways; therefore, there were no significant sources contributing to air pollution.

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This study was carried out in collaboration with the National Environmental Engineering Research Institute, Nagpur, India. We thank the director of the institute and S.P. Sharma for all of the help and support. We acknowledge the Sookshm Vikas Sansthan, Jawanpura, Viratnagar, Rajasthan, India, for help in conducting the field studies.

This study was conducted at the Department of Physiology, SMS Medical College, Jaipur, and Zonal Laboratory, National Environmental Engineering Research Institute, Jaipur. The study was partially funded by a grant from the Department of Human Resource Development Group and Extramural Research, Council of Scientific and Industrial Research, New Delhi, India.

Received 1 September 1999; accepted 10 November 1999.

We obtained details about family members (name, age, sex, weight, etc.). We randomly selected 88 children for detailed clinical and biochemical examination. The children were age matched and were up to 8 years of age (prepubertal), representing 10% of the total population from these village units. The purpose of age matching was to select children that were in the same age ranges in all of the village units.

A detailed ARI and RRTI history was taken in the selected population. The children who presented with ARI at the time of clinical evaluation and who had a history of recurrence were selected for detailed examination and biochemical evaluations. Most of the time it is difficult to differentiate between upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI) because in most respiratory illnesses both the upper and lower portions of the tract are affected simultaneously or sequentially (*20*). Therefore, we used the terms respiratory tract infection (RTI) and RRTI in the text. In cases where a child had more than six RTI attacks in 1 year, where a child seemed to recover from one attack only to enter another, or where in a child there was more or less persistent RTI and general failure to do well , we considered those children to have RRTI (*20*).

The history of present and past illness included questions about the LRTI symptoms such as cough, stridor, noisy breathing, inability to drink, excessive drowsiness, increased respiratory rate, chest indrawing, and any treatment received previously for such complaints, with or without symptoms of URTI such as nasal congestion, nasal secretions, and sore throat (*20,21*).

We conducted medical examinations to confirm the acute attack of RTI; we examined for the presence of LRTI signs such as respiratory grunting, stridor, tachypnea (respiratory rate ≥ 60 respirations/min in an infant younger than 2 months of age, ≥ 50 respirations/min in an infant 2–12 months of age, and ≥ 40 respirations/min in a child 1–8 years of age), chest indrawing, the presence of wheezes and/or rales, and the presence of bronchial breathing (with or without the presence of the signs of URI such as cough, the presence of nasal secretions, and nasopharyngeal congestion). Recovery from RTI was shown by the absence of these complaints and the disappearance of the signs and symptoms for not < 30 days (*20,21*).

We drew blood samples to estimate methemoglobin [represented as percent hemoglobin (Hb)] in accordance with the modified method of Evelyn and Malloy, as described by Davidson and Henry (*22*). We determined cytochrome $b_5$ reductase activity (diphospho pyridine nucleotide reduced-dependent diaphorase activity—represented as international units per gram Hb) by the methemoglobin ferricyanide method (*23*).

***Statistical analysis.*** We completed multivariate statistical analysis (correlation and regression analysis) using Microsoft Excel software (Microsoft Corp., Redmond, WA) on a personal computer. We also calculated *p*-value for significance (*p*-value < 0.05 was considered significant).

## Observations

In all, we examined 88 children in village units with 26, 45, 100, 222, and 449 mg nitrate ion/L in drinking water. Table 1 shows the number of cases examined, the methemoglobin levels (met-Hb), the average cytochrome $b_5$ reductase activity, and the number and percent of RRTI cases for each nitrate concentration.

We noted RRTI in 40–82% of cases. A correlation analysis indicated high positive correlation ($r$ = 0.731) between met-Hb and RRTI. The correlation of RRTI was poor with nitrate in drinking water ($r$ = 0.565) and cytochrome $b_5$ reductase activity ($r$ = -0.120).

Regression analysis between percent RRTI and met-Hb indicated a high correlation coefficient ($R^2$ = 0.803) at 95% significance level with a *p*-value of 0.04. This high coefficient of regression indicates high goodness-of-fit, explaining approximately 80% of the variability in RRTI with met-Hb concentration.

## Discussion

We observed a high percentage (40–82%) of RRTI at various nitrate concentrations in drinking water in children up to 8 years of age. This percentage is much higher than that reported in the literature (*1–4*). The wide variations observed (represented as SD) in met-Hb and cytochrome $b_5$ reductase activity are due to the fact that the health risk from exposure to nitrates is an age-dependent phenomenon (*9,19,24*). The health risk is therefore related not only to the nitrate concentration in drinking water and food but also to conditions conducive to their reduction to nitrites and to the protective autoreduction capacity of red blood corpuscles (RBCs) through cytochrome $b_5$ reductase, an intracellular enzyme of RBCs. To avoid bias, we used age matching as the criterion for the selection of children in all five village units.

A significant positive coefficient of correlation (0.731), a high coefficient of regression ($R^2$ = 0.803), and $p$ < 0.05 indicate a probable cause–effect relationship between high met-Hb concentration and RRTI. In fact, these high methemoglobin levels are primarily caused by high nitrate concentrations in drinking water, and there is ample evidence (*10,24,25*) to indicate that higher nitrate concentration in drinking water causes methemoglobinemia.

Considering the significant cause–effect relationship suggested by observations of humans and the observations of animal studies (*6–8*), the following possible pathophysiology of a causal relationship of recurrent respiratory infection and high nitrate ingestion in drinking water has been proposed.

Ingested inorganic or organic nitrates are converted to nitrite by microflora in the oral cavity (*13*) and in the gastrointestinal tract by intestinal microflora (*26,27*). This conversion will result in increased oxidation of hemoglobin to methemoglobin and increased production of nitric oxide (*26–28*) (Figure 1). The conversion of nitrite to nitric oxide is nonenzymatic (*15,16,29,30*). The oxidation of hemoglobin to methemoglobin results in the formation of the superoxide radical by the transfer of a single electron. The enzyme superoxide dismutase, present in the erythrocytes, catalyzes the conversion of superoxide radical ($O^{\bullet}$) to $H_2O_2$ and $O_2$. The $H_2O_2$ is then decomposed by glutathione peroxidase or catalase, both of which are also present in erythrocytes (*14,31*). 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 (*24,32*), there is increased production of reactive $NO^{\bullet}$ and $O^{\bullet}$ (*14*).

***The fate of NO•.*** Hemoglobin scavenges nitric oxide through the high-affinity ferrous sites on heme to form *S*-nitrosothiol (with an affinity to nitric oxide 8,000 times that of their affinity for oxygen) (*33*) by binding at b 93 cysteine residue on the globin chain. As hemoglobin binds oxygen in the lungs, its binding affinity to *S*-nitrosothiol increases. As hemoglobin releases oxygen at the

**Table 1.** RRTI cases for different levels of nitrate in water.

| Total cases | Nitrate (mg/L) | RRTI +ve | Cases (%) | Average age (mean ± SD) | Methemoglobin (% Hb) (mean ± SD) | Cyto (IU/g Hb) (mean ± SD) |
|---|---|---|---|---|---|---|
| 15 | 26 | 6 | 40 | 2.53 ± 2.15 | 10.44 ± 6.44 | 10.64 ± 10.60 |
| 24 | 45 | 17 | 71 | 2.87 ± 2.43 | 16.07 ± 9.56 | 9.39 ± 15.50 |
| 11 | 95 | 8 | 73 | 2.58 ± 2.11 | 19.44 ± 9.36 | 14.70 ± 14.31 |
| 27 | 222 | 15 | 56 | 2.99 ± 1.86 | 7.88 ± 3.09 | 8.01 ± 12.65 |
| 11 | 459 | 9 | 82 | 2.85 ± 1.82 | 15.44 ± 8.1 | 6.46 ± 2.9 |

Abbreviations: cyto, cytochrome $b_5$ reductase activity; +ve, cases with a history of RRTI.

periphery, its affinity for *S*-nitrosothiol is reduced and nitric oxide is released in the tissues (*33*). 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 nitrosohemoglobin in hypoxic tissue in turn reduces regional vascular resistance.

Nitric oxide is a biogenic messenger, an EDRF (*15,16*), and it activates the guanylyl cyclase system (*17*) [it converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP)], raising the cGMP pool and therefore inducing, among other things, vasodilatation (*17*) by lowering the intracellular calcium ion (*16*).

***The fate of O•.*** In a normal cell, $O_2^-$ will be scavenged by the enzyme superoxide dismutase, and $H_2O_2$, which is a product of this reaction, by glutathione peroxidase and catalase (*24,31*). 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 amounts (e.g., excessive nitrate ingestion), or if any of the protective mechanisms are defective (*24,31*).

Apart from this mechanism, there is an increased formation of methemoglobin, which creates a condition of hypoxia, causing vasoconstriction of the pulmonary arterioles.

The vasoactivity of the pulmonary arterioles depends on the balance between constricting and dilating stimuli (*17*). Once the pulmonary arteriolar dilatation predominates, it will lead to congestion, the presence of inflammatory cells, and the breakdown of alveoli. This situation will be worsened by increased production of free oxide radicals, possibly causing irreversible cell damage. These changes in pulmonary circulation and alveoli lead to mismatching of ventilation and perfusion, and therefore provide high-risk conditions suitable for recurrent respiratory infections.

The suggested pathophysiology also explains the changes observed in an animal study (*8*).

## Conclusions

There is a strong interdependence between methemoglobin concentration and RRTI in children up to 8 years of age. Methemoglobinemia, primarily caused by high nitrate concentrations in drinking water, is the most likely cause for RRTI. These findings are important because of the high mortality in children due to ARI (*1–5*). Because the children under normal circumstances can consume such high nitrate in drinking water, the high levels may lead to methemoglobinemia, hypoxia, excessive production of free radicals of nitric oxide and oxygen, and may cause changes in pulmonary circulation and alveoli. These changes may lead to mismatching of ventilation and perfusion, making the respiratory system more susceptible to recurrent infections, as we observed in this study.

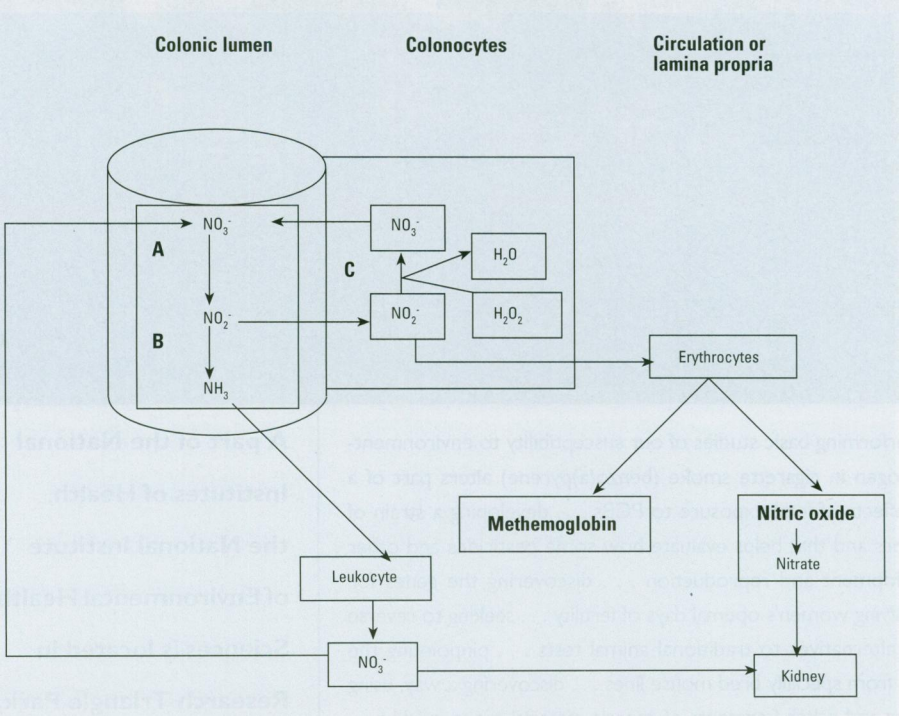


**Figure 1.** Nitrate metabolism. *A*, Bacterial nitrate reductase; *B*, bacterial nitrite reductase; *C*, catalase.

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