

A review of nitrates in drinking water: maternal exposure and adverse reproductive and developmental outcomes

Revisão sobre nitratos na água potável: exposição materna e reações adversas ao desenvolvimento reprodutivo

Deana M. Manassaram¹
Lorraine C. Backer
Deborah M. Moll

Abstract *In this review, we present an update on maternal exposure to nitrates in drinking water in relation to possible adverse reproductive and developmental effects, and discuss nitrates in drinking water in the United States. The current standard for nitrates in drinking water is based on retrospective studies and approximates a level that protects infants from methemoglobinemia, but no safety factor is built into the standard. The current standard applies only to public water systems. Animal studies have found adverse reproductive effects resulting from higher doses of nitrate or nitrite. The epidemiologic evidence of a direct exposure-response relationship between drinking water nitrate level and adverse reproductive effect is still not clear. However, some reports have suggested an association between exposure to nitrates in drinking water and spontaneous abortions, intrauterine growth restriction, and various birth defects. Uncertainties in epidemiologic studies include the lack of individual exposure assessment that would rule out confounding of the exposure with some other cause. We conclude that the current literature does not provide sufficient evidence of a causal relationship between exposure to nitrates in drinking water and adverse reproductive effects*

Key words *Developmental outcomes, Drinking water, Maternal exposure, Nitrate, Nitrite, Reproductive health*

Resumo *Nesta revisão, apresentamos um estudo sobre exposição materna aos nitratos na água potável e possíveis reações adversas ao desenvolvimento reprodutivo, no caso dos Estados Unidos. O padrão de uso de nitratos na água potável é baseado em estudos retrospectivos e leva em conta níveis que protegem os bebês contra a metemoglobinemia. Mas nenhum fator de segurança foi construído a partir desse padrão, sendo que os níveis atuais foram construídos e exigidos apenas para os sistemas públicos de distribuição de água. Estudos com animais encontraram efeitos reprodutivos adversos e advindos das altas doses de nitrate ou nitrito na água. A evidência epidemiológica sobre a relação de exposição-resposta direta entre o nível de nitratos na água potável e efeitos adversos não está clara. No entanto, algumas notificações sugerem associação entre exposição a nitratos na água potável e abortos espontâneos, restrições ao crescimento intrauterino e vários defeitos de nascença. As incertezas nos estudos epidemiológicos incluem a falta de avaliação da exposição individual que excluiria confundir a exposição com outros fatores. Concluímos que a literatura atual não provê suficiente evidência de uma relação causal entre exposição a nitratos na água potável e efeitos reprodutivos adversos.*

Palavras-chave *Desenvolvimento fetal, Água potável, Exposição materna, Nitrate, nitrito, Saúde reprodutiva*

This article was originally published by the journal Environmental Health Perspective 2006; 114:320-327. doi:10.1289/ehp.8407 available via <http://dx.doi.org/> [Online 3 November 2005] and is part of the scientific collaboration between Rev C S Col and EHP
Received 16 June 2005; accepted 3 November 2005.
¹CDC/NCEH/EHHE, Health Studies Branch, 4770 Buford Highway NE, MS F-46, Chamblee, GA 30341 USA. dmanassaram@cdc.gov

The health implications of exposure to nitrates in drinking water were first reported in the scientific literature by Comly in 1945 after observing cyanosis in infants in Iowa, where well water was used in formula preparation¹. Since then, most studies on the health effects of nitrates in drinking water have focused on infants because they are thought to be the most vulnerable to this exposure. More recent evaluations of the health implications of nitrates in drinking water have examined reproductive and developmental effects^{1,2,3,4,5,6,7,8,9,10,11,12}.

Data are insufficient on the incidence of methemoglobinemia among infants in the United States and on the effects that exposure to nitrate levels above the maximum contaminant limit (MCL) may have on other populations such as pregnant women. We do know that people in the United States who get their water from public water systems are occasionally exposed to biologically relevant doses of nitrate in their drinking water¹³. In addition, a considerable number of people (~ 16% of the U.S. population) use private water systems that are usually located in areas considered more vulnerable to nitrate contamination¹⁴.

In this review, we summarize the experimental and epidemiologic studies on nitrates in drinking water with reference to adverse reproductive and developmental effects. Previous reviews by Fan and colleagues^{5,6} focused on nitrates in drinking water, methemoglobinemia, and reproductive toxicity. In this review, we expand on these previous works, with a focus on maternal exposure and reproductive effects. Because a drinking water source may play a crucial role in exposure, we also present a discussion on sources and occurrence of nitrates in drinking water in the United States.

Nitrate and drinking water

Nitrate occurs naturally in soil containing nitrogen-fixing bacteria, decaying plants, septic system effluent, and animal manure. Other sources of nitrate include nitrogenous fertilizers and airborne nitrogen compounds emitted by industry and automobiles¹⁵. Nitrate penetrates through soil and remains in groundwater for decades^{16, 17}. Groundwater is the source for > 50% of drinking water supplies, 96% of private water supplies, and an estimated 39% of public water supplies¹⁸.

Factors affecting nitrate in drinking water. People who get water from shallow wells (< 100

feet below land surface) in areas with well-drained soils and high nitrogen inputs (e.g., close proximity to agricultural areas) have an increased risk of exposure to nitrate-rich groundwater. Agricultural activities are the largest nonpoint sources of nitrate contamination of groundwater because of greater use of nitrogen fertilizer on crops and the trend toward concentrated animal farming^{16,19}. Private wells are usually shallower and closer to sources of nitrate contamination, whereas public supply wells are usually in deeper groundwater aquifers where contamination is less likely¹⁸.

Drinking water regulations. The U.S. EPA sets the standards for water quality regulations written in the Safe Drinking Water Act (SDWA) and its subsequent amendments²⁰. The SDWA specifies MCL for contaminants, defined as the concentrations above which adverse human health effects may occur²¹. The enforceable MCL applies only to public drinking water systems and government or privately run companies supplying water to at least 25 people or with e" 15 service connections²².

Drinking water standards are usually set at a fraction of the no observed adverse effect levels (NOAELs) because potential health risks are often unknown or hard to predict. The standards are based on data from experimental animal studies and available human health reports. A margin of safety is usually built into the standard to account for issues such as extrapolating from animals to humans. This safety factor (orders of magnitude) is usually higher if potential health effects are more uncertain²³. The MCL for nitrate in drinking water was set at 10 mg/L nitrate–nitrogen (NO₃–N) or 45 mg/L nitrate (NO₃–), on the basis of 214 methemoglobinemia cases reported to the American Public Health Association for which nitrate concentration data were available²⁴. Because the current MCL was nominally based on human exposure data, no uncertainty or modifying factors were used, so there is no safety factor built into the MCL for nitrates in drinking water^{25,24}.

The appropriateness of the current MCL is questioned²⁶. Complicating matters is the existence of methemoglobinemia in infants without exposure to water that contains elevated nitrate levels or any apparent exogenous nitrate or nitrite exposure. These reports suggested that bacterial infection and subsequent overproduction of nitric oxide, or other substances in drinking water, such as copper, cause methemoglobinemia in infants^{26,27,28,29}. However, case reports persist of

methemoglobinemia in infants related to well water containing nitrate levels above the MCL³⁰. Private water systems are not regulated, and methemoglobinemia is not a reportable disease, so there is insufficient data on whether exposure to nitrate levels above the MCL is a problem among users of such systems.

Occurrence of nitrates in drinking water. The National Water Quality Assessment (NAWQA) program of the USGS assessed water quality of aquifer systems that cover the water resources of > 60% of the population in the contiguous United States. On the basis of the NAWQA findings, approximately 15% of shallow groundwater sampled beneath agricultural and urban areas had nitrate levels above the MCL. In comparison, < 10% of samples taken from 100–200 feet deep exceeded the MCL, and no sample was found to exceed the MCL in groundwater that was > 200 feet below the surface¹⁷. Other reports using the NAWQA data showed nitrate levels > 3 mg/L (report assumed levels of e^{-} 3 mg/L because of contamination) in 28% of samples taken from public and private wells. More private wells sampled (11%) exceeded the MCL than did public wells (2%)³¹.

The U.S. EPA National Pesticide Survey³², which sampled private wells in 38 states and public water systems in 50 states, found 1.2% of public water systems and 2.4% of private wells exceeded the MCL for nitrate¹⁶. From this survey, the U.S. EPA estimated that > 4 million people, including some 66,000 infants < 1 year of age, could be served by systems that exceed the MCL for nitrate³². A survey by the Centers for Disease Control and Prevention (CDC) of > 5,500 private wells in nine midwestern states found nitrate levels above the MCL in 13.4% of wells sampled³³. A survey of 3,351 domestic wells found that 9% had nitrate levels exceeding the MCL, compared with 1% of public wells³⁴.

Although varied levels of nitrate in drinking water sources have been reported from state-based and national studies, a relationship between levels of nitrate and source of water is consistent. Specifically, higher levels of nitrate were found more often in groundwater than in surface water, in private wells than in public water supplies, in shallow wells than in deep wells, and in agricultural than in urban areas. The higher levels of nitrate in private water systems are of public health concern because users could be exposed to nitrate levels above the MCL.

Materials and methods

We conducted a Medline³⁵ search to identify publications in English through January 2004. Key words and phrases used to search the database for both animal and human studies included nitrates, nitrites, drinking water, pregnancy, fetus, birth defects, spontaneous abortions, prematurity, stillbirth, health effects, low birth weight, growth, reproductive outcomes, and drinking water contaminants. We also searched for internal publications from the CDC, USGS, and U.S. EPA via the agencies' websites.

Nitrate toxicity

Nitrate toxicity is related primarily to the *in vivo* conversion to nitrite after ingestion^{36,37}. The health hazards from consuming water with nitrate are related to the direct toxicity of nitrite—that is, its ability to directly oxidize hemoglobin, changing it to methemoglobin, which cannot bind oxygen. Accumulation of methemoglobin (methemoglobinemia) occurs if this oxidation process overwhelms the protective reduction capacity of the cells^{38,20}. In addition to drinking water, which contributes an estimated < 3–21% of the average adult intake of nitrate, other sources include vegetables, meat, and meat products preserved with sodium nitrite^{38, 40}. There is also evidence of endogenous nitrate formation from the oxidation of nitric oxide independently of dietary sources⁴¹. A further concern relating to the metabolism of dietary nitrate is the potential *in vivo* formation of N-nitroso compounds from nitrite²⁰. Nitrite is also a substrate in the formation of N-nitroso compounds from nitrosatable drugs. Therefore, higher dietary nitrate intake results in greater amounts being formed if there is concurrent exposure to nitrosatable drugs⁴².

Reproductive toxicity

Animal studies have shown some indication that nitrate, nitrite, and N-nitroso compounds may traverse the placenta and affect the fetus *in utero*^{43, 6,44}. It has been suggested that the placental membrane is effective in separating blood circulation between mother and fetus from the fourth month of pregnancy, thus preventing methemoglobin molecules from crossing⁴⁵. Others have suggested that nitrate or the reduced form (nitrite) may pass to the fetus through a system of

active transport similar to that of iodide, and fetal nitrate plasma levels may exceed that of the mother⁴⁶. Tarburton and Metcalf⁴⁷ found that amyl nitrite caused both adult and cord blood to oxidize from hemoglobin to methemoglobin *in vitro*. Cord blood was oxidized at a 5- to 6-fold greater rate than was adult blood. Whether nitrite may have the same effect *in vivo* and on fetal blood if it traverses the placenta is uncertain⁴⁷.

Animal studies. Fan *et al.*⁶ reviewed the experimental data on reproductive toxicity and reported no evidence of teratogenic effects but found indication that nitrates and nitrites may induce abortion in experimental animals^{5,6,48}. These findings include increased levels of nitrite and methemoglobin in rats and subsequently in the fetuses when pregnant rats were given 2.5–50 mg/kg sodium nitrite in drinking water or through intraperitoneal injection. The possibility of increased permeability from placental damage was also tested in this study by giving another group of pregnant rats sodium nitrite after labor had begun. The first newborn had a normal methemoglobin level (1.2%), whereas those born after the chemical was given had higher levels of methemoglobin (10.1%) in their blood⁴⁴.

Globus and Samuel⁴⁹ orally administered 0.5 mg/day sodium nitrite to pregnant mice beginning the first day of gestation and continuing up to the 14th, 16th, or 18th day of gestation. The parameters used to measure embryotoxicity (litter size, gross anatomical defects, weight, number of resorption sites, proportion of fetal deaths) showed no significant difference between this group of mice and the control group given distilled water. The occurrence of skeletal abnormalities was similar in both groups; however, an increase in the production of red blood cells was reported for the offspring of treated mice. The investigators suggested that sodium nitrite may itself traverse the placenta, inducing methemoglobinemia in the fetus that could stimulate erythropoiesis in the hepatic cells⁴⁹.

Inui *et al.*⁵⁰ administered doses of 125, 250, and 500 mg/kg sodium nitrite to hamsters on the 11th or 12th day of pregnancy. Other pregnant hamsters were given similar doses of sodium nitrate or similar doses of dimethylnitrosamine (positive controls). No chromosomal changes occurred in the offspring of animals treated with lower doses of sodium nitrite, but mutation occurred at the highest dose of sodium nitrite (500 mg/kg). The same dose of sodium nitrate did not produce such effects. Morphologic and neoplastic changes were observed in embryonic cells

from hamsters given doses of 250 and 500 mg/kg sodium nitrite. The effects in pregnant hamsters treated with sodium nitrite were similar to those seen in the pregnant hamsters treated with dimethylnitrosamine as a positive control⁵⁰.

Cases of aborted fetuses were observed in pigs pastured in a feedlot of oats and rape. Serologic testing ruled out infectious causes for the abortions but found excessive nitrate. The report indicated that the levels of nitrate found in the rape (5.52%) and oats plants (0.53%) were not safe⁵¹. After observing spontaneous abortions in cattle grazing in weedy pastures, Sund⁵² placed 12 pregnant heifers in pastures with soil known to have high nitrogen levels and high-nitrate weeds and eight pregnant heifers in pastures where prior treatment had killed high-nitrate weeds. Ten of the 12 heifers in the untreated pastures, compared with one of eight in the treated pastures, aborted their fetuses. Blood levels taken at weekly intervals showed low, fluctuating levels of methemoglobin in all the heifers. The study suggested no association between abortion and levels of methemoglobin. However, the report noted that the degenerative changes observed in several organs of the aborted fetuses were indicative of tissue anoxia, symptomatic of methemoglobinemia. Sund concluded that the nitrates in weeds either caused or were related to the incidence of abortion in cattle grazing in pastures with soil high in available nitrogen⁵².

Winter and Hokanson⁵³ indicated that spontaneous abortion may not be a significant effect of chronic nitrate exposure at levels sufficient to induce methemoglobinemia in cattle. Fifteen heifers were given sodium nitrate daily in their feedings from 2 months of pregnancy until they aborted or gave birth. The dosage was adjusted weekly to maintain methemoglobin levels at 20–30% of total hemoglobin. Two heifers aborted (one abortion was caused by vibriosis), and two heifers died from acute nitrate poisoning.

Other adverse reproductive effects, such as mummified fetuses; lesions on the cervix, uterus, and placenta; and maternal death were also reported by earlier experimental studies reviewed by Fan *et al.*⁶. A more recent study examined the effects on embryo growth and viability in 48 cows between 2 and 8 weeks of gestation that were feeding on heavily fertilized grass containing high levels of nitrogen compared with those in control pasture⁵⁴. No evidence was found that embryo survival or growth was affected from 20 days onward in pregnant cows grazing in either pasture.

Two groups of cows, pregnant and nonpregnant, were fed either nitrate rations < 400 ppm nitrate in hay for 5 weeks or a ration of 4,000 ppm nitrate in hay for 8 weeks⁵⁵. Serum progesterone levels were depressed in cows fed the higher nitrate rations. The depressed levels were more evident in nonpregnant cows, less in cows in early pregnancy, and absent in cows in midpregnancy. The investigators concluded that early reproductive toxicity from chronic nitrate exposure may be caused by depression of serum progesterone, but further studies need to be done to confirm this conclusion.

Epidemiologic studies of reproductive effects of nitrate in drinking water

Birth defects. The effects of exposure to nitrates in drinking water on the incidence of birth defects have been evaluated in several epidemiologic studies^{3,56,57, 5,6}. However, the results from epidemiologic studies addressing this topic are equivocal.

In a case-control study of Mexican-American women, Brender *et al.*⁴² examined nitrosatable drug exposure and the occurrence of neural tube defects (NTDs) in relation to dietary nitrites and nitrates. They examined 184 cases of NTD-affected pregnancies from the Texas Neural Tube Defects Projects and 225 women with normal live births. All participants were interviewed to obtain detailed dietary information, periconceptional medication use, and drinking water source. The water sources of 110 women (43 cases and 67 controls) were also tested for nitrate. Nitrosatable drug use was reported as a risk factor for having an NTD-affected pregnancy [odds ratio (OR) = 2.7; 95% confidence interval (CI), 1.4–5.3]. Among those who had their water tested for nitrate, drinking water nitrate level \geq 3.5 mg/L nitrate-N was associated with having an NTD-affected pregnancy (OR = 1.9; 95% CI, 0.8–4.9). The risk estimate increased drastically (OR = 14; 95% CI, 1.7–660) for women who took nitrosatable drugs and had nitrate levels \geq 3.5 mg/L nitrate-N in their drinking water source. The authors concluded that because the level of nitrate in the water sampled was relatively low, and women were not asked about frequency and amount of water consumed, the amount of nitrate in the water directly contributed to the increased risk observed among women who used nitrosatable drugs⁴².

A study of 71,978 infants born from 1982 through 1996 was conducted in a Swedish county served by 80 municipal water systems⁵⁶. The study assessed the possible association between mothers' preconception or early pregnancy exposure to chlorination by-products and nitrate in public drinking water and incidence of congenital cardiac defects. The study population was identified through the Swedish Birth Registry and was limited to infants whose mothers used the municipal water system and had addresses for the preconception or early pregnancy period that could be geocoded. The Registry of Congenital Malformations provided information on cardiac defects. Additional data on the pregnancy, delivery, and newborn health were obtained from medical records and the hospital discharge registry. Exposure assessment was ascertained by using a geographic information system to link the study subjects to specific water supplies. Groundwater as a source of drinking water was reported as a potential risk factor for cardiac defects (adjusted OR = 1.31; 95% CI, 1.09–1.57). A very small and not statistically significant excess risk for cardiac defects was found to be associated with levels \geq 2 mg/L nitrate-N compared with those < 2 mg/L nitrate-N (adjusted OR = 1.18; 95% CI, 0.97–1.44)⁵⁶.

A case-control study in California investigated the potential association between maternal exposure to nitrates in drinking water and diet before pregnancy and the risk of NTDs in the mothers' infants⁵⁷. Case infants (538) with NTDs (both live and stillborn singleton births) born from 1989 through 1991 were selected from California's birth defects program. Control infants (539) were live births with no malformations selected from each area birth hospital for the same time period. Exposure assessment was done through interviews with the mothers, which included a detailed beverage and dietary questionnaire, and the state's community water systems data for sources serving their preconception addresses. The authors found an increased risk for NTDs among babies born to mothers living in areas where the drinking water nitrate level was above the MCL compared with those in areas below the MCL (OR = 2.7; 95% CI, 0.76–9.3). This association did not change after adjusting for other dietary nitrate intake (OR = 1.9; 95% CI, 0.73–4.7). Increased risk with increasing levels of nitrates was observed. Risk estimates were higher among groundwater users; however, other risk factors (e.g., Hispanic ethnicity, young age, low socio-

economic status, and no vitamin use) for NTDs were also more common among groundwater users.

In this study, the authors examined risk separately for anencephaly and spina bifida⁵⁷. Increased risk for anencephaly in babies was associated with their mother living in an area where the nitrate in drinking water was above the MCL (OR = 4.0; 95% CI, 1.0–15.4). This association was not substantially altered when adjusted for dietary intake of nitrate. A doubling in risk for anencephaly in babies whose mothers lived in areas where the nitrate level in groundwater was ≥ 5 mg/L compared with < 5 mg/L was observed. No increased risk was observed in users of mixed water (i.e., a combination of surface water and groundwater). There was no increased risk for spina bifida with any level of nitrate exposure or source of drinking water⁵⁷.

Fan and Steinberg⁵ summarized the studies conducted on maternal exposure to nitrates in drinking water and birth defects by Scragg *et al.*⁸, Dorsch *et al.*⁴ in South Australia, Arbuckle *et al.*³ in New Brunswick, Canada, and Bove *et al.*⁵⁸ in New Jersey. The study by Dorsch *et al.*⁴ suggested an increased risk of bearing a child with a congenital malformation among women whose homes were served by water with a nitrate concentration > 5 ppm. Although the effects of nitrate cannot be discounted, the finding that the risks associated with multiple defects were increased suggests possible multiple risk factors. The study by Arbuckle *et al.*³ reported a protective relationship for users of public and spring water sources compared with private sources, but the ORs were not statistically significant. Five water samples exceeded the Canadian MCL (44 ppm) for nitrate. Bove *et al.*⁵⁸ reported a positive association with water contamination and NTDs but cautioned that the study did not provide sufficient evidence of causation for any of the contaminants in question.

These studies assessed exposure to nitrates in drinking water on the basis of the source of the water. The lack of individual exposure assessment—whether the women actually drank the water—is a limitation in some investigations. The presence of other substances, such as pesticides, toxic metals, and chemicals (e.g., chlorinated solvents and chlorinated disinfection by-products), in private and public water systems may be correlated with the presence of nitrate. Some of these constituents are reported as risk factors for congenital malformations^{58,59}.

Spontaneous abortions. Aschengrau *et al.*⁶⁰

investigated the quality of community drinking water and the occurrence of spontaneous abortions among a group of women in the Boston, Massachusetts, area. The population, selected from 1976 through 1978, consisted of women who lived in Massachusetts during their pregnancy, lived in a town with a public water supply, and were admitted to a specific community hospital. Cases were 286 women who had a miscarriage during their first 27 weeks of pregnancy, and controls were 1,391 women who had live births. The women were interviewed to obtain demographic and behavioral information, and water quality data were obtained from public records of routine analyses of public tap water. All levels of nitrate were below the MCL. A negative association was reported for any detectable level of nitrate and the occurrence of spontaneous abortions. The report noted that risk estimates may have been diluted by the measurement, recording, and classification of exposure, because this information was obtained indirectly from public water supply records of the communities where the women lived at the time the spontaneous abortions occurred⁶⁰.

Gelperin *et al.*⁷ evaluated data from 1959 through 1966 on infant and fetal deaths in 16 Illinois communities, nine of which had nitrate levels ranging from 43 ppm to 123 ppm (NO₃) in their water supply. Communities were grouped into three categories, consistently having high nitrate (above the MCL), having high nitrate in spring only, or not having high nitrate levels. The community water supply data provided nitrate levels. No significant increase in fetal deaths was found in areas consistently reporting nitrate levels above the MCL in their water compared with other areas.

Skrivan⁶¹ measured methemoglobin levels in the blood of pregnant women over a 2-year period to evaluate whether the observed mean values of methemoglobin would be similar in women who experienced spontaneous abortions and in those who experienced term delivery. The mean values in women before spontaneous abortion did not differ significantly from the mean values among women with term deliveries⁶¹.

An earlier evaluation of methemoglobin levels in pregnancy reported a relationship between methemoglobinemia and miscarriages in humans⁶². The study tested methemoglobin levels in 25 women in their first trimester of pregnancy. Higher levels were observed in women who spontaneously aborted or who threatened abortion in the first trimester. The report noted ni-

trates and nitrites as the most common methemoglobin inducers and concluded that high maternal methemoglobin levels are possibly related to miscarriages.

Case reports. A report on a cluster of spontaneous abortions in LaGrange, Indiana, cited nitrate-contaminated water from private wells as the possible cause⁶³. The cases included a 35-year-old woman who experienced four consecutive miscarriages and a 37-year-old and a 20-year-old who each experienced one miscarriage. All three women lived within 1 mile of each other and were in the first trimester of pregnancy at the time of the miscarriages. Testing of the wells serving the homes of the women found nitrate to be the only elevated contaminant. The wells had nitrate levels over the MCL, with reported levels of 19.0 mg/L, 26 mg/L, and 19.2 mg/L nitrate-N for the three women, respectively. Although these incidents of spontaneous abortion may have been related to the ingestion of nitrate contaminated drinking water, other possible explanations such as genetic defects in the fetuses and cluster by chance could not be ruled out⁶³.

Other reproductive effects. Besides birth defects and prenatal mortality, reproductive toxicity includes less readily observed effects that may be influenced by chronic low-level exposure to a toxic substance. These effects include sterility, intrauterine growth restriction, premature birth, and complications of pregnancy. Several of these outcomes have been addressed in epidemiologic studies of the potential effects of nitrate exposure on reproductive health. Hypothetically, the oxidation of hemoglobin to methemoglobin, which limits the oxygen-carrying capacity of the blood, may interfere with the course and outcome of pregnancy^{48, 10, 11, 12}.

Bukowski *et al.*⁶⁴ conducted a population-based case-control study on singleton births that occurred from 1991 through 1994 to mothers who resided in Prince Edward Island, Canada, and who used municipal and private water systems. The study examined the potential impact of groundwater nitrate exposure on prematurity and intrauterine growth restriction (IUGR). The study included 210 cases of IUGR, 336 cases of premature births, and 4,098 controls that were identified through a Reproductive Care Program (RCP) database. The authors developed a nitrate level exposure map using data on public and private wells collected from 1990 through 1993. Premature birth was defined as birth at < 37 weeks of gestation, IUGR was defined as birth weight < 2,500 g at term birth, and controls were de-

defined as "normal" live births with no other diagnoses. The nitrate data were subdivided into six exposure categories on the basis of increasing nitrate-N levels, with the highest exposure having levels ≥ 5 mg/L. The study subject was assigned to an exposure group on the basis of the mother's residential address at the time of delivery. The RCP database provided additional data including demographics, lifestyle, health, and reproductive history⁶⁴.

Bukowski *et al.*⁶⁴ found a significant relationship between IUGR and higher nitrate levels. Using the lowest exposure category (median level of ≤ 1.3 mg/L nitrate-N) as the comparison group, an adjusted OR showed an excess risk for higher exposure categories with median levels 3.1 mg/L (OR = 2.31; 95% CI, 1.47-3.64) and 4.3 mg/L (OR = 2.56; 95% CI, 1.44-4.45). An increased but not significant risk for the highest exposure group of 5.5 mg/L (OR = 1.34; 95% CI, 0.31-3.99) was observed, as well as a significant dose-response association between nitrate exposure and prematurity. The authors reported excess risk estimates for the exposure categories with median levels of 3.1 mg/L (OR = 1.82; 95% CI, 1.23-2.69), 4.3 mg/L (OR = 2.33; 95% CI, 1.46-3.68), and 5.5 mg/L (OR = 2.37; 95% CI, 1.07-4.80). The dose-response relationship is fairly consistent and suggestive; however, assessing exposure to nitrate based on ecologic classification makes it difficult to interpret the findings exclusively in terms of water nitrate exposure⁶⁴.

In a study in Bulgaria, Tabacova *et al.*¹¹ investigated the association between maternal exposure to environmental nitrogen sources and subsequent complications of pregnancy. The study was done in an area known to have nitrate levels in drinking water ranging from 8 to 54 mg/L nitrate-N (depending on the season and the water supply source), as well as in the food supply and oxides of nitrogen in ambient air. The study, which included pregnant women at ≥ 24 weeks' gestation, analyzed blood methemoglobin levels as markers of exposure to nitrogen compounds. Personal interviews with the women furnished medical and lifestyle histories. Only 16% of the study group experienced a normal pregnancy. The others experienced complications, including anemia (67%), threatened spontaneous abortion/premature labor (33%), and toxemia (23%), with some women having more than one of these diagnoses. Among women with pregnancy pathology, methemoglobin levels were 0.1-11.2% of total hemoglobin, compared with 0.4-2.8% in

women with normal pregnancy. The mean methemoglobin levels for all categories of pregnancy complications were above the physiologic normal level of 2%, with the highest mean level occurring in the toxemia group. The report suggests an increased risk for pregnancy complications from exposure to nitrogen compounds. However, the lack of individual exposure assessment and the presence of several potential sources of exposure prevent implicating drinking water as a single source¹¹.

Tabacova *et al*¹² also evaluated mother–infant pairs in an area of Bulgaria with chronic exposure to pollution by nitrogen compounds, including drinking water nitrate levels of 8–54 mg/L nitrate–N in municipal water system, 13–400 mg/L nitrate–N in wells, and nitrogen oxides in ambient air. They examined methemoglobin levels as markers for exposure to nitrogen compounds, measures of oxidative stress, and the health status of infants at birth. Fifty-one mother–infant pairs were recruited from a local hospital; interviews were conducted with the mothers, and clinical records were reviewed for information on demographics, lifestyle, and medical factors. Maternal and cord blood were tested for methemoglobin and markers of oxidative stress. More than half of maternal blood and almost half of cord blood had methemoglobin levels > 2%. The study indicated a strong association between maternal methemoglobin and cord blood methemoglobin. Maternal and cord blood methemoglobin levels were higher in cases of abnormal birth outcomes (preterm birth, fetal distress, and low birth weight) than in cases of normal birth outcomes. The authors also reported an association between methemoglobin levels in cord blood and adverse birth outcome. Overall, the results show that nitrate exposure may have a role in adverse reproductive effects. However, the small sample size, multiple sources of exposure, and the lack of individual exposure assessment make the findings difficult to interpret in terms of nitrate exposure from drinking water per se. Other pollutants potentially associated with abnormal birth outcomes were not discussed¹².

Fan *et al*^{5,6} discussed the study by Super *et al*⁹ that evaluated infant morbidity in a rural area of southwest Africa known to have wells with high levels of nitrates. Super *et al*⁹ found no association between the incidence of premature birth or the size of infant at birth and living in an area with nitrate levels > 20 mg/L nitrate–N compared with areas with levels ≤ 20 mg/L. No association was found between birth weight, current or previous

premature births, stillbirths, and spontaneous abortions and nitrate levels > 20 mg/L nitrate–N. However, increased deaths among infants previously born to mothers in regions with nitrate levels > 20 mg/L nitrate–N were reported.

Summary and discussion

The contamination of groundwater by nitrates is ubiquitous, but the magnitude of the risk posed to human health is still unclear. That the potential toxicity of nitrate in drinking water is largely based on studies using high concentrations of nitrite is a complication. Another complication is that human studies have provided little evidence that adverse health effects result from chronic exposure to low levels of nitrate in drinking water. Although the current MCL for nitrates is also in debate, the public drinking water supply rarely exceeds this limit. The lack of data on unregulated systems, however, is an important issue. The available data on the occurrences of nitrates in drinking water indicate that users of private water systems are most at risk for exposure to nitrate levels above the MCL. However, a lack of studies focusing on users of private water systems means that the extent of the problem is unknown.

Experimental animal studies on nitrate or nitrite (in the form of sodium or potassium nitrate/nitrite) and adverse reproductive and developmental outcomes provide moderate evidence for an association between exposure to nitrate and fetal loss, neonatal mortality, maternal toxicity, and decrease in number of litters and live births. Epidemiologic evidence for increased risk for adverse reproductive and developmental outcomes in humans from exposure to nitrate in drinking water is sparse and suggestive at best. Nevertheless, the findings of excess birth defects in some studies suggest the need for further studies.

In most studies presented in this review, exposure to nitrates in drinking water was assessed primarily through water quality data for water systems serving women's addresses during pregnancy or at time of delivery. Although this form of exposure assessment provides information in a timely and cost-efficient way, community-based water quality data provide only a rough estimate of individual exposure. This does not account for other issues in exposure assessment such as other sources of nitrate exposure, individuals not drinking tap water, or use of private water sys-

tems. The recent study by Brender *et al.*⁴² is an example of the extent of exposure assessment that should be considered when evaluating nitrate exposure and reproductive effects.

Drinking water contaminants other than nitrates have been reported to be associated with increased risk of adverse pregnancy outcomes^{58,59}. Identifying which contaminant in a community water system is associated with a particular adverse reproductive outcome when multiple contaminants are present is difficult. Future studies should conduct individual exposure assessments such as maternal interviews concerning water consumption habits in and outside the home and other risk factors such as occupational exposures or smoking.

Many studies on birth defects are also limited by the time between the end of pregnancy and the maternal interview. This is difficult for individual studies to overcome because birth defects are rare outcomes and prospective cohort studies may not be feasible to conduct. Prospective cohort studies on end points such as spontaneous abortions are more feasible and

would provide knowledge about the potential effects of nitrates in drinking water on this outcome. Spontaneous abortion may be a more sensitive indicator of adverse reproductive effects from relatively low levels of drinking water contamination.

States with large numbers of private wells where groundwater is vulnerable to contamination should be encouraged to increase monitoring or surveillance of such systems. Future research could include long-term monitoring or surveillance of water systems vulnerable to contamination. This could provide valuable exposure assessment information to conduct studies on drinking water contaminants such as nitrates. A discussion on the appropriateness of the current MCL is beyond the scope of this review. However, future studies with improved exposure assessment (including other dietary sources and medications), adequate sample size, and evaluating endogenous nitrate exposure could help to determine whether nitrates in drinking water at the current MCL increase the risk for reproductive and developmental effects.

Collaborations

We thank K Bradley for conducting the literature searches, J Peters for drawing the forest plots, and H Pall, D Ray, and A Spurgeon for reviewing the original manuscript.

This study was funded by the Department for Environment, Food, and Rural Affairs. The authors declare they have no competing financial interests.

References

- Comly HH. Landmark article Sept 8, 1945: cyanosis in infants caused by nitrates in well-water. *JAMA* 1987; 257:2788–2792.
- Arbuckle TE, Hewitt D, Sherman GJ. Re: “Congenital malformations and maternal drinking water supply in rural South Australia: a case-control study” [Letter]. *Am J Epidemiol* 1986;124:344.
- Arbuckle TE, Sherman GJ, Corey PN, Walters D, Lo B. Water nitrates and CNS birth defects: a population-based case-control study. *Arch Environ Health* 1988; 43:162–167.
- Dorsch MM, Scragg RK, McMichael AJ, Baghurst PA, Dyer KF. Congenital malformations and maternal drinking water supply in rural South Australia: a case-control study. *Am J Epidemiol* 1984; 119:473–486.
- Fan AM, Steinberg VE. Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regul Toxicol Pharmacol* 1996; 23:35–43.
- Fan AM, Willhite CC, Book SA. Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Regul Toxicol Pharmacol* 1987; 7:135–148.
- Gelperin A, Moses VK, Bridger C. Relationship of high nitrate community water supply to infant and fetal mortality. *Ill Med J* 1975; 147:155–157, 186.
- Scragg RK, Dorsch MM, McMichael AJ, Baghurst PA. Birth defects and household water supply. Epidemiological studies in the Mount Gambier region of South Australia. *Med J Aust* 1982; 2:577–579.
- Super M, Heese H, MacKenzie D, Dempster W. An epidemiological study of well water nitrates in a group of south west African/Namibian infants. *Water Res* 1981; 15:1265–1270.
- Tabacova S, Balabaeva L. Environmental pollutants in relation to complications of pregnancy. *Environ Health Perspect* 1993; 101(suppl 2):27–31.
- Tabacova S, Balabaeva L, Little RE. Maternal exposure to exogenous nitrogen compounds and complications of pregnancy. *Arch Environ Health* 1997; 52:341–347.
- Tabacova S, Baird DD, Balabaeva L. Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. *Arch Environ Health* 1998; 53:214–221.
- U.S. EPA. *Estimated National Occurrence and Exposure to Nitrate/Nitrite in Public Drinking Water Supplies* Washington, DC: U.S. Environmental Protection Agency 1990; 2–32.
- U.S. EPA. *Drinking Water from Household Wells* EPA 816-K-02-003. Washington, DC: U.S. Environmental Protection Agency; 2002a.
- USGS. *Nutrients in the Nation's Waters: Too Much of a Good Thing?* USGS Cir 1136. Denver, CO: U.S. Geological Survey; 1996c.
- Spalding R, Exner M. Occurrence of nitrate in ground-water. *J Environ Qual* 1993; 22:392–402.
- USGS. *The Quality of Our Nation's Waters: Nutrients and Pesticides* Circular 1225. Reston, VA:U.S. Geological Survey; 1999.
- USGS. *Nitrate in Ground Waters of the US—Assessing the Risk* FS-092-96. Reston, VA: U.S. Geological Survey; 1996a.
- USGS. *Nutrients in the Nation's Waters: Identifying Problems and Progress* FS-218-96. Reston, VA: National Water Quality Assessment Program, U.S. Geological Survey; 1996b.
- Safe Drinking Water Act of 1974. Public Law 42 U.S.C. s/s 300f. [accessed 2005 Jan 5]. Available from: <http://www.epa.gov/region5/defs/html/sdwa.htm>
- U.S. EPA. *2002 Edition of the Drinking Water Standards and Health Advisories* EPA-822-R-02-038. Washington, DC:U.S. Environmental Protection Agency; 2002b.
- U.S. EPA. *Factoids: Drinking Water and Ground Water Statistics for 2002* EPA 816-K-03-001. Washington, DC:U.S. Environmental Protection Agency; 2003. p.1–10.
- RAIS. Toxicity Summary for Nitrates. Oak Ridge, TN:Risk Assessment Information System. 1995. [accessed 2005 Feb 17]. Available from: http://risk.lsd.ornl.gov/tox/profiles/nitrates_f_V1.shtml#te
- Walton G. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. *Am J Public Health* 1951; 41:986–996.
- Johnson CJ, Kross BC. Continuing importance of nitrate contamination of groundwater and wells in rural areas. *Am J Ind Med* 1990;18:449–456.
- Avery AA. Infantile methemoglobinemia: reexamining the role of drinking water nitrates. *Environ Health Perspect* 1999; 107:583–586.
- Felsot AS. Re-examining the link between nitrates and “blue baby” syndrome: a necessary first step for managing ground water quality to protect public health. *Agric Environ News* 1998; 150:1–14.
- Hanukoglu A, Danon PN. Endogenous methemoglobinemia associated with diarrheal disease in infancy. *J Pediatr Gastroenterol Nutr* 1996; 23:1–7.
- Hegesh E, Shiloah J. Blood nitrates and infantile methemoglobinemia. *Clin Chim Acta* 1982; 125:107–115.
- Knobeloch L, Salna B, Hogan A, Postle J, Anderson H. Blue babies and nitrate-contaminated well water. *Environ Health Perspect* 2000; 108:675–678.
- Squillace PJ, Scott JC, Moran MJ, Nolan BT, Kolpin DW. VOCs, pesticides, nitrate, and their mixtures in ground-water used for drinking water in the United States. *Environ Sci Technol* 2002; 36:1923–1930.
- U.S. EPA. *National Pesticide Survey Update and Summary of Phase II Results*. 570991021. Washington, DC: Office of Water, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency; 1992.

33. CDC. *A Survey of the Quality of Water Drawn from Domestic Wells in Nine Midwest States: 1998*. National Center for Environmental Health. NCEH 97-0265. Atlanta, GA: Centers for Disease Control and Prevention; 1998.
34. USGS. *Nutrients in Ground Water and Surface Water of the United States—an Analysis of Data through 1992*. Report 95-4031. Denver, CO: U.S. Geological Survey; 1995.
35. PubMed. Homepage. Bethesda, MD: National Library of Medicine. 2005. [accessed 2005 Feb 17]. Available from: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>
36. NAS. *Drinking Water and Health: Inorganic Solutes*. Washington, DC: National Academy of Sciences; 1977. p.411-439.
37. Swann PF. The toxicology of nitrate, nitrite and N-nitroso compounds. *J Sci Fd Agric* 1975; 26:1761-1770.
38. Jaffe ER. Methemoglobin pathophysiology. In: The Function of Red Blood Cells: Erythrocyte Pathobiology. *Proceedings of an International Symposium on Erythrocyte Pathobiology*. New York: Alan R. Liss; 1981. p.133-151.
39. ATSDR. *Case Studies in Environmental Medicine: Nitrate/Nitrite Toxicity*. Atlanta, GA: Agency for Toxic Substances and Disease Registry; 2001.
40. Wogan GN, Generoso W, Koller LD, Smith RP. *Nitrate and Nitrite in Drinking Water*. Washington, DC: National Academic Press; 1995.
41. Walker R. The metabolism of dietary nitrites and nitrates. *Biochem Soc Trans* 1996; 24:780-785.
42. Brender JD, Olive JM, Felkner M, Suarez L, Marckwardt W, Hendricks KA. Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. *Epidemiology* 2004; 15:330-336.
43. Bruning-Fann CS, Kaneene JB. The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. *Vet Hum Toxicol* 1993; 35:521-538.
44. Gruener N, Shuval HI, Behroozi K, Cohen S, Shechter H. Methemoglobinemia induced by transplacental passage of nitrites in rats. *Bull Environ Contam Toxicol* 1973; 9:44-48.
45. L'hirondel J, L'hirondel JL. *Nitrate and Man: Toxic, Harmless or Beneficial?* New York: CABI Publishing; 2002.
46. Hartman PE. Nitrates and Nitrites: Ingestion, pharmacodynamics, and toxicology. In: de Serres FJ, Hollaender A, eds. *Chemical Mutagens: Principles and Methods for Their Detection*. New York: Plenum Press; 1982. p.211-294.
47. Tarburton JP, Metcalf WK. Kinetics of amyl nitrite-induced hemoglobin oxidation in cord and adult blood. *Toxicology* 1985; 36:15-21.
48. NAS. *The Health Effects of Nitrates, Nitrites, and N-Nitroso Compounds*. Washington, DC: National Academy of Sciences; 1981.
49. Globus M, Samuel D. Effect of maternally administered sodium nitrite on hepatic erythropoiesis in fetal CD-1 mice. *Teratology* 1978; 18:367-378.
50. Inui N, Nishi Y, Taketomi M, Mori M. Transplacental action of sodium nitrite on embryonic cells of Syrian golden hamster. *Mutat Res* 1979; 66:149-158.
51. Case AA. Abortion in swine due to excessive nitrate. *J Am Vet Med Assoc* 1957; 131:226.
52. Sund JM. Weeds containing nitrates cause abortion in cattle. *Agron J* 1957; 49:278-279.
53. Winter AJ, Hokanson JF. Effects of long-term feeding of nitrate, nitrite, or hydroxylamine on pregnant dairy heifers. *Am J Vet Res* 1964; 25:353-361.
54. Laven RA, Biggadike HJ, Allison RD. The effect of pasture nitrate concentration and concentrate intake after turnout on embryo growth and viability in the lactating dairy cow. *Reprod Domest Anim* 2002; 37:111-115.
55. Page RD, Gilson WD, Guthrie LD, Mertens DR, Hatch RC. Serum progesterone and milk production and composition in dairy cows fed two concentrations of nitrate. *Vet Hum Toxicol* 1990; 32:27-31.
56. Cedergren MI, Selbing AJ, Lofman O, Kallen BA. Chlorination byproducts and nitrate in drinking water and risk for congenital cardiac defects. *Environ Res* 2002; 89:124-130.
57. Croen LA, Todoroff K, Shaw GM. Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. *Am J Epidemiol* 2001; 153:325-331.
58. Bove FJ, Fulcomer MC, Klotz JB. *Public Drinking Water Contamination and Birth Weight, Fetal Deaths, and Birth Defects. A Crosssectional Study. Report on Phase IV-A*. Trenton, NJ: New Jersey Department of Health; 1992.
59. Bove F, Shim Y, Zeitz P. Drinking water contaminants and adverse pregnancy outcomes: a review. *Environ Health Perspect* 2002; 110(suppl 1):61-74.
60. Aschengrau A, Zierler S, Cohen A. Quality of community drinking water and the occurrence of spontaneous abortion. *Arch Environ Health* 1989; 44:283-290.
61. Skrivan J. Methemoglobinemia in pregnancy (clinical and experimental study). *Acta Univ Carol [Med] (Praha)* 1971; 17:123-161.
62. Schmitz JT. Methemoglobinemia - a cause of abortions? Preliminary report. *Obstet Gynecol* 1961; 17:413-415.
63. CDC (Centers for Disease Control and Prevention). Spontaneous abortions possibly related to ingestion of nitrate-contaminated well water—LaGrange County, Indiana, 1991-1994. *MMWR Morb Mortal Wkly Rep* 1996; 45:569-572.
64. Bukowski J, Somers G, Bryanton J. Agricultural contamination of groundwater as a possible risk factor for growth restriction or prematurity. *J Occup Environ Med* 2001; 43:377-383.