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Health Effects of Nitrate Exposure

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For 50 years, physician and public health professionals have known that exposure to high levels of nitrates causes "blue baby syndrome," a condition caused by lack of oxygen in infants. Thousands of cases of this condition have been reported worldwide since its initial diagnosis in 1945, and the current EPA standard has been set in order to protect infants from methemoglobinemia from excessive exposure to nitrate. Unfortunately, in tens of thousands of households, infants continue to drink water contaminated with nitrate at levels deemed unsafe by EPA. And quite likely, the current EPA standard does not adequately protect the public health. The current EPA standard of 10 ppm is based on a 45 year old survey of methemoglobinemia in infants. Since 1950, however, there have been a number of reported cases of methemoglobinemia caused by nitrate at less than 10 ppm in drinking water (Sattelmacher 1964; Simon 1962).

Comparing EPA's nitrate in water standard with other nitrate standards illustrates just how out of step the water standard really is.

- In 1980, the USDA established a zero-tolerance for added nitrate in food destined for infants and children,² and significantly restricted exposure to nitrate in the overall food supply. Unfortunately, this provides no protection for bottle fed infants during the first three to four months of life. This is precisely the time when infants, the most vulnerable population, are at peak susceptibility to the toxic effects of nitrate.
- In Germany and South Africa the drinking water standard for nitrate is 4.4 ppm -- more than twice as strict as the U.S. standard of 10 ppm (Kross, et al. 1995). The European Economic Community has established a nitrate health guideline of 5.6 ppm, and studies of infants in Europe have found that three to four percent of methemoglobinemia cases in infants occurred at doses lower than 10 ppm (Sattelmacher 1964; Simon 1962). Clearly, health authorities in many other countries believe that nitrate poses an unacceptable risk to infants and children below the current EPA standard of 10 ppm.
- Most European countries have banned N-nitrosamines and N-nitroso compounds, along with their nitrate and nitrite precursors from baby bottle nipples due to concern about exposure to these potent carcinogens early in life (Westin 1990). In the U.S., there is no standard, although Mead Johnson, a large producer of infant formula and infant products including baby bottle nipples, reformulated their nipples to remove all N-nitrosamines and precursors.

Unlike virtually all other contaminant standards, the drinking water standard for nitrate contains no margin of safety. Nearly every chemical standard in force today incorporates a ten to 100-fold safety factor to ensure that sensitive members of the population are adequately protected. When there is evidence of possible human carcinogenicity, the EPA adds yet another ten-fold safety factor. The nitrate standard contains no safety factors at all, even though it is targeted towards an especially sensitive population subgroup, infants, and even though nitrate is a precursor compound in the formation of N-Nitroso compounds, many of which are human carcinogens (NRC 1995).

In 1977, the Safe Drinking Water Committee of the National Academy of Sciences, concluded that:

"there appears to be little margin of safety for some infants with the standard at this concentration." (NAS, 1977)

Ten years later, during their review of the proposed 10 ppm standard, the Science Advisory Board of the Reagan Administration EPA had an even harsher assessment, finding that:

"The Agency selects a margin of safety that excludes, for all practical purposes, protection of sensitive members of the population." (Carlson 1987)

The EPA failed to heed this advice and has not added a safety factor to the nitrate standard, arguing that the current standard, which was based on a 45 year-old study of 278 reported cases, is representative of the full range of vulnerability of the infant population of the United States, and that no safety factor is required.

A recent panel of the National Academy of Sciences (NAS 1995) concluded that the 10 ppm standard was adequate to protect public health. This panel, however, ignored the evidence from two international studies (Simon, et al. 1962; Sattelmacher 1964) showing that methemoglobinemia occurred at concentrations below 10 ppm. The bulk of the committee's work then focused on cancer risk. It concluded that:

- While average levels of nitrate exposure in the United States are unlikely to significantly elevate an individuals' cancer risk, tens of thousands of infants are exposed to highly contaminated water with nitrate concentrations far above the national average. Further, the committee's own calculation showed that:
 - Infants being bottle fed with highly nitrate contaminated water would receive a dose of nitrate 80 times higher than the average infant in the population.

The increased risks posed by this exposure were completely sidestepped by the panel even though widely respected animal studies with nitrosamine compounds show that exposure during infancy increases the cancer risk from N-nitroso compounds by a factor of six (Gray et al 1991).

The NAS study acknowledged 27,000 infants drinking water contaminated with nitrate in excess of the federal 10 ppm standard, but then failed to assess whether any increased cancer risk would be associated with such elevated nitrate exposure immediately after birth.

Ultimately the committee undermined its own conclusion that the 10 ppm standard is adequate by recommending that:

"limiting infant exposure to nitrate would be a sensible public health measure. It could be accomplished by minimizing exposure to both foods and water that are high in nitrate..." (NAS 1995 p. 49)

Lastly, the committee recommended further study of possible developmental effects for infants exposed to nitrate. The uncertainties acknowledged by the committees are more than enough to warrant a modest, two-fold safety margin in the current 10 ppm standard.

The Health Effects of Nitrate

A review of available peer reviewed literature on nitrate toxicity reveals a near systematic failure of the EPA to incorporate current scientific knowledge into drinking water standards. These shortcomings apply to all the toxic effects of nitrate, including:

- **Methemoglobinemia.** Nitrate causes methemoglobinemia in infants and this has been the principle health concern of regulators around the globe. The U.S. standard for nitrate is two times weaker than the standard in Germany and South Africa, and nearly twice as permissive as guidelines set by the European Community.
- **Cancer.** Nitrate is converted to nitrite after ingestion, and this nitrite reacts with both natural and synthetic organic compounds to produce N-Nitroso compounds in the human stomach. Many of these N-Nitroso compounds are carcinogenic in humans (IARC 1978, NAS 1977), and numerous researchers and a substantial body of literature suggest that high nitrate levels in drinking water may increase cancer risks (Mirvish 1983, Mirvish 1991). To date, the EPA has completely ignored the contribution of nitrate in drinking water to the human cancer risk from N-Nitroso compounds.

Infant exposure, particularly when nitrate levels approach the 10 ppm standard, appears to be especially important. Recent animal studies have shown that rats exposed to N-Nitrosodiethylamine during infancy are six times more likely to develop cancer than those exposed after weaning (Gray et al 1991). Human epidemiology studies also have suggested that cancer risks may be higher for those exposed to nitrate contaminated water in the first ten years of life (Cuello 1976).

- **Disruption of thyroid function.** An important study by Danish researchers found that individuals drinking water with a high nitrate content exhibited a dose related increase in hypertrophy, a condition marked by enlargement of the thyroid, the gland responsible for many of the body's endocrine and hormonal functions (Van Maanen, et al. 1994).
- **Birth Defects.** At least five studies have indicated a possible link between exposure to nitrite, nitrate and N-Nitroso compounds and birth defects. The effects of exposure were first observed in animal studies, but have since been observed in human epidemiological studies (Dorsch 1984; Knox 1972; Super 1981).

Given the litany of health effects associated with nitrate exposure and the uncertainty that this volume of evidence brings to the prediction of health risks, sound scientific judgment dictates that the U.S. EPA apply a safety factor of at least two to the current 10 PPM standard for nitrate-nitrogen in drinking water.

The EPA should further establish a Maximum Contaminant Level Goal of 3 ppm nitrate-nitrogen in drinking water, equal to the generally accepted maximum background level of uncontaminated groundwater aquifers used for drinking water in the U.S. This would allow the EPA to at least establish the goal of maintaining ground and surface water resources used for drinking water free from added nitrate contamination.

Nitrate and Methemoglobinemia

Methemoglobinemia, or blue-baby syndrome, is a condition caused by the inability of the blood to deliver enough oxygen to the body. It is the most well-known effect of exposure to elevated levels of nitrate in drinking water.

When nitrate is ingested it is converted to another chemical form, nitrite. Nitrite then reacts with hemoglobin, the proteins responsible for transporting oxygen in the body, converting them to methemoglobin, a form that is incapable of carrying oxygen. As a result, the affected individual suffers from oxygen deprivation.

Infants under four months of age are most susceptible to methemoglobinemia because their stomach is relatively non-acidic, meaning that compared to adults more nitrate is converted to nitrite in the infant stomach, and because infant or fetal hemoglobin reacts to form methemoglobin easier than adult hemoglobin. Diarrhea and other gastric disturbances (often caused by microbial contamination of drinking water) are also thought to play an important role in methemoglobinemia, perhaps because they increase stomach pH and weaken immune systems (NAS 1978). Virtually all reported cases of methemoglobinemia have involved children under the age of six months suffering from gastric disturbances.

Symptoms of methemoglobinemia include anoxic appearance, shortness of breath, nausea, vomiting, diarrhea, lethargy, and in more extreme cases loss of consciousness, and even death. Approximately seven to ten percent of all reported methemoglobinemia cases have resulted in death of the infant, and in recent years at least one death has been reported in the United States (NAS 1977, Johnson et al. 1987). Additional deaths caused by nitrate contaminated drinking water in the United States have almost certainly occurred, but gone unreported (Johnson and Kross 1990).

The Current EPA Standard Has No Safety Factor

The current enforceable drinking water standard (Maximum Contaminant Level or MCL) for nitrate as nitrogen (N) of ten parts per million (ppm) is unique for at least two reasons: (1) unlike most MCL's, which are based on the results of animal studies, the nitrate standard was set based on data from infants reported in a 1951 study of methemoglobinemia occurrence published in the American Journal of Public Health (Walton 1951); and, (2) unlike virtually all other water and food standards based on human data, which use at least a 10 fold safety factor to account for differences in human susceptibility to the toxicant in question, the 10 ppm standard for nitrate has no safety factor at all.

The Walton study analyzed 278 reported cases of methemoglobinemia that occurred in the United States between 1945 and 1950 and found that none of the reported cases occurred at nitrate concentrations below 10 ppm. On the basis of this study, 10 ppm of nitrate as nitrogen (N) was established as the "safe" dose of nitrate in drinking water for infants. In 1962 the American Public Health Service formally recommended a 10 ppm nitrate standard in drinking water, based on the Walton research. This same analysis was relied upon by the EPA during the 1987 MCL standard setting process.

Two retrospective German studies found that 3-4 percent of the reported methemoglobinemia cases in that country occurred at nitrate concentrations of 11 ppm or less (Sattelmacher 1962; Simon 1964). Infants suffering from gastric disturbances, respiratory illness, or diarrhea are particularly sensitive to methemoglobinemia and virtually all reported cases have involved this sensitive subpopulation.

In addition to infants, other populations may be more susceptible to the effects of elevated nitrate. These include African Americans, Alaskan Eskimos, and Native Americans, who lack a hereditary enzyme that helps reduce methemoglobin levels in the blood (NAS 1978; Aldrich 1980) as well as individuals suffering from stomach conditions such as gastric ulcers, pernicious anemia, adrenal insufficiency, gastritis, or gastric carcinoma, all of which reduce stomach acidity and cause more nitrate to be converted to hazardous nitrite (NAS, 1978). Pregnant women, with naturally high levels of methemoglobin in the latter period of their pregnancy, may also be sensitive to the effects of nitrate (Kross 1994, NAS 1977). A final group that is particularly sensitive includes dialysis patients, who are uniquely susceptible to methemoglobinemia. For water used for dialysis, a standard of 2 ppm has been recommended because of this sensitivity (Carlsen and Shapiro 1970, Fan et al. 1987).

There have been no estimates of the total size of the nitrate sensitive subpopulation, although it is quite large -- studies have indicated that enzyme deficiencies affect approximately 7 percent of African American males (Aldrich, 1980) -- a population of over one million, and over 140,000 people receive dialysis treatment each year (USRDS 1994).

In addition to the known sensitive populations, a recent reported case of methemoglobinemia in Wisconsin also provides cause for concern (MMWR 1993). In this case, an infant was hospitalized due to acute methemoglobinemia. When testing of the tap water was conducted, the nitrate concentration of the well water was found to be 9.9 ppm -- below the current EPA standard. The well was also found to be contaminated with copper, and the case was thought to have been caused by a synergistic effect of copper and the nitrate acting together.

A final cause for concern is that some baby foods contain naturally occurring nitrate. A recent study found that a number of baby foods, including bananas, carrots, garden vegetables, spinach, green beans, and beets, contain high concentrations of nitrate (Dusdieker, et al 1994). A young infant drinking contaminated water and eating commercial baby food that is also high in nitrate can easily exceed acceptable intake levels and become ill. The current EPA standard contains no safety standard to account for this simultaneous consumption of nitrate in food and drinking water.

The No Safety Approach Is Invalid

In theory, drinking water standards with no safety factors could be set based on human data, if the sample sizes for the study were large enough and statistically designed to capture all of the variability in the exposed human population.

The nitrate standard is based on a single 45 year-old study of 278 reported cases of methemoglobinemia in infants. It is inappropriate for two reasons. First, the sample size is far too small to account for the variable sensitivity of the entire U.S. population. And second, the standard fails to account for a number of confounding factors, including the fact that infants are also exposed to nitrate in food, and that exposure to nitrate may cause additional long-term health problems in addition to methemoglobinemia.

EPA justifies the absence of a safety factor by arguing that the Walton study is in fact a study of sensitive infants, because the condition of methemoglobinemia only occurs in sensitive infants. Therefore, according to the EPA, the fact that methemoglobinemia was not reported at contamination levels under 10 ppm by Walton, proves that nitrate is not likely to afflict any infant anywhere at levels under 10 ppm, because the sensitive infants in this study accurately represent the sensitivity to nitrate of all infants in the United States.

Several facts contradict this assertion. First, the study is only of reported cases, which, according to most medical experts, underrepresents the total number of cases. Methemoglobinemia is underreported because reporting is often not required, and because the symptoms mimic other conditions including congenital heart conditions, and even sudden infant death syndrome (Johnson and Kross 1990). As the National Academy of Sciences found in their recent study, "...the absence of reported cases [of methemoglobinemia below 11.3 ppm] might in part be due to the lack of requirements for reporting cases..." (NAS 1995)

And second, scientific data document methemoglobinemia at doses below 10 ppm (Sattelmacher 1964; Simon 1962). At a minimum, in order to fully justify not adopting a safety factor for nitrate, the EPA must lower the MCL to the lowest dose at which methemoglobinemia occurred in the infants in these more recent studies.

Methemoglobinemia Occurrence is Vastly Underreported

The only two surveys conducted in the last 15 years indicate that only a small percentage of methemoglobinemia cases are reported or publicized. In 1974, a Nebraska researcher noted that there had been no cases reported in the literature, and set out to determine if it was due to lack of occurrence (Grant 1981). A survey was sent to 910 physicians in a 72 county area, 442 of whom included infants in their practice. This survey asked all physicians if they had seen a case of nitrate induced methemoglobinemia, and reported that 33, or seven percent, had seen a case in the period 1973-1978. During that time not a single case had been reported in the medical literature.

A similar survey was carried out after a nitrate contaminated well caused the death of an infant by methemoglobinemia in South Dakota (Johnson, et al. 1987). The author sent questionnaires about methemoglobinemia to all doctors in the 12 county area of the Big Sioux aquifer. The survey found that many cases of methemoglobinemia went unreported (Meyer 1994). Although this was only the second reported case in South Dakota history, doctors reported that they had treated at least 80 cases in the period 1950-1980 -- in a region that comprises only 0.28% of the U.S. population. The study concluded that "the common perception that methemoglobinemia occurs rarely may be wrong..."

A 1994 study noted succinctly that "signs of methemoglobinemia...may be difficult to detect in an infant by a caretaker or an unsuspecting physician" (Dusdieker 1994) and in a 1990 review, researchers in Iowa and South Dakota concluded that many cases of preventable nitrate poisoning were still occurring. They reported that:

"the contamination of ground and rural drinking water supplies by nitrates...continues to be a serious potential hazard throughout the world. Nitrate poisoning certainly contributes to national infant death rate statistics." (Johnson and Kross 1990)

Other Consequences of Nitrate Exposure

Although methemoglobinemia is the most immediate life threatening effect of nitrate exposure, there are a number of equally serious longer-term, chronic impacts. In numerous studies, exposure to high nitrate drinking water has been linked to a variety of effects ranging from hypertrophy (enlargement) of the thyroid, to 15 different types of cancer to two kinds of birth defects and even to hypertension. All of these effects have been observed in human epidemiology studies, and are often supported by further human physiology or animal studies. These results indicate that the current MCL does not provide adequate protection against chronic effects of exposure to elevated concentrations of nitrate.

In 1978, food regulations, set by the USDA, were strengthened in order to reduce the risk from nitrite and nitrate present in cured meats. Since 1978, only 120 ppm nitrite has been allowed in nitrite preserved products, and these products also must contain either ascorbate or erythorbate, two compounds that prevent the formation of hazardous N-Nitroso compounds. This has reduced exposure to hazardous nitrites by a factor of five or more (Mirvish 1991). And most importantly, current USDA regulations specifically ban the use of added nitrate or nitrite in "baby, junior, and toddler foods" (CFR 9 at 318.7).

Nitrate and Hypertrophy of the Thyroid

A recent study by Danish researchers revealed a strong correlation between prolonged exposure to nitrate contaminated water causes hypertrophy of the thyroid, the gland responsible for many of the body's endocrine and hormonal functions (van Maanen, et al. 1994). The study compared three dose groups -- low nitrate (0 ppm nitrate in drinking water), medium nitrate (4-10 ppm) and high nitrate (> 10 ppm) -- and found that "a dose-dependent difference in the volume of the thyroid was observed between low and medium vs. high nitrate exposure groups, showing development of hypertrophy at nitrate levels exceeding [11.3 ppm]". Adding further weight to the human epidemiological evidence, the researchers also noted that similar effects had been observed in laboratory animals, where nitrate interfered with the uptake of iodine by the thyroid.

Nitrate, Nitrite, Nitrosamines and Cancer

For many years, public health professionals have known that nitrate has the potential to form carcinogenic compounds when it reacts with naturally occurring materials (NAS 1977, 1978; Mirvish, 1991, 1983). Historically, however, there has been uncertainty over the risk posed by nitrate in drinking water, in large part because of questions over the relative importance of the dose of nitrate in water compared to larger amounts consumed via food, and a lack of understanding of the full series of reactions in the human body that lead to the formation of N-Nitroso compounds. Nonetheless, over the last 20 years, numerous human epidemiology and physiology studies (Table 6 [0]) as well as animal studies have shown a potential link between nitrate intake in water and increased risk of cancer.

Evidence from animal and human studies suggests that exposure to elevated levels of N-Nitroso compounds during infancy can significantly increase lifetime cancer risks (Cuello, et al 1976; Gray, et al. 1991). While questions do remain over the link between nitrate in drinking water and cancer, prudent public health principles and the evidence of potential carcinogenic effects provide strong support for an additional safety factor in the nitrate standard.

Nitrate itself is not carcinogenic, but instead acts as a "procarcinogen", meaning that it reacts with other chemicals to form carcinogenic compounds via a multiple step process. First, nitrate is converted into nitrite after consumption. Second, 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 which are carcinogens. In animal or human studies, this class of 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 -- a wider range of tumors than any other group of carcinogens (Mirvish 1991). 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).

There is strong evidence that many of these compounds are carcinogenic in humans. In 1978, the International Agency for Research on Cancer reviewed 11 N-Nitroso compounds for which adequate data was available, and concluded that all 11 "should be regarded for practical purposes as if [they] were carcinogenic in man" (IARC 1978). Citing several human epidemiological studies, the National Academy of Sciences noted that "there is no reason to suppose that man is not susceptible." (NAS 1977). In humans, the organs thought to be most at risk from cancers caused by nitrosamine formation are the stomach, esophagus, nasopharyngeal cancer, and cancer of the bladder.

Human Evidence that Nitrate in Drinking Water Can Cause Cancer

Epidemiology. Since 1976, at least 8 different human epidemiology studies conducted in 11 countries show a relationship between increasing rates of stomach cancer and increasing nitrate intake (Hartmann, 1983; Mirvish 1983).³ The National Academy of Sciences, in their recent study on Nitrate and Nitrite in Drinking Water, ignored the vast majority of these studies, arguing that for most adults, nitrogen intake via water was relatively small. Those conclusions do not hold true for infants and others with highly contaminated tap water.

Links between nitrate and cancer have been found in at least three separate European countries. A 1984 study in the Piemonte Region of Italy compared the incidence of gastric cancer in regions with high and low nitrate in drinking water. Researchers found a positive relationship between communities having a significantly elevated cancer risk and a high nitrate content (>4.5 ppm as nitrate) in their drinking water (Gilli 1984). High nitrate communities were 13 times more likely to have higher than average stomach cancer rates than low-nitrate communities. Although this study did not adjust for many social or medical risk factors, for historical changes in nitrate concentrations or for a variety of other factors, the data do provide statistically significant evidence across a broad geographical area consisting of more than 150 communities of a potential link between nitrate contaminated drinking water and stomach cancer.

In Britain, a 1985 study of 253 urban areas found a negative relationship between water nitrate levels and stomach cancer rates (Beresford 1985). However, EPA and others have criticized this analysis because nitrate concentration data and cancer occurrence data are not from the appropriate time period -- that is, that the study ignores the long latent period between nitrate exposure and cancer occurrence (ECETOC 1988; USEPA 1990).

An English study which overcame this problem did in fact find a positive relationship between nitrate content and stomach cancer in males, although no relationship was observed for females (Clough 1983). This study analyzed 43 districts and boroughs in the county of Kent with water nitrate concentrations ranging from 0 to 11 ppm nitrate as N, and reported that, for both males and females, an increase in nitrate concentrations was linked with an increase in stomach cancer occurrence. For both groups, a dose response relationship was observed for nitrate concentrations up to 7 ppm. For women, risks increased at higher concentrations, while for men, no further increase in risk was observed at higher concentrations. Women exposed at greater than 7 ppm had gastric cancer risks approximately 10 percent higher than women exposed at concentrations between 0 and 2.5 ppm. Men exposed at greater than 7 ppm had gastric cancer occurrence rates approximately 17 percent higher than women exposed at concentrations between 0 and 2.5 ppm. Notably, increases in cancer occurrence were found at concentrations well below the current MCL.

A 1982 study in Denmark also found a link between nitrate exposure and occurrence of stomach cancer. This study compared stomach cancer rates in Aalborg, a town with approximately 5-7 ppm nitrate in drinking water, with surrounding communities that had lower nitrate concentrations. Stomach cancer rates in the high nitrate region were approximately 25% higher than in the low nitrate region. Although the authors only compared cancer rates in two communities, and did not control for many outside factors, this study did support a possible link between nitrate exposure and stomach cancer. The author concluded that, while the results did not provide proof of a link between water nitrate and cancer, they did "support the hypothesis of a possible role for nitrate in the etiology of stomach cancer" (Jensen 1982).

Three epidemiology studies in South America have shown an association between high rates of gastric cancer and nitrogen fertilizer use, nitrate intake, or nitrate contaminated water on cancer rates (Armijo 1975,1981; Cuello 1976). Perhaps the most compelling was a study of the incidence of stomach cancer rates in Narino, Colombia -- the first to link nitrate exposure via water and stomach cancer -- published in the Journal of the National Cancer Institute in 1976. This research compared nitrate content of well water with gastric cancer risk, finding a significant correlation between the two. Even more disturbing were data in the study showing that cancer occurrence rates were higher for members of the population using wells (which generally had higher nitrate content) at a young age, between 0 and 10 years old. The authors noted in their abstract that the "data could be construed as presumptive epidemiologic evidence for the role of nitrate availability in the etiology of stomach cancer" (Cuello 1976).

A 1992 study conducted in China showed a link between nitrate exposure in drinking water and gastric cancer (Xu, et al. 1992). This study, which was based upon determinations of exposure and disease for individuals rather than groups, found that individuals

at higher risk for gastric cancer had an average exposure to nitrate in drinking water that was more than twice as high as individuals at lower risks. These findings led the authors to conclude that "the results suggest that nitrate in drinking water probably plays an important role in gastric carcinogenesis."

Finally, a recent 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). As noted previously, N-Nitroso compounds have been found to cause cell lymphomas in animal studies. The study, which was designed to suggest possible factors in the increased incidence of non-Hodgkins lymphoma in many regions of Nebraska, reported that NHL incidence was twice as high in counties where more than 20% of the wells were contaminated by nitrate or in the 33% of counties with highest fertilizer use. Previous studies also found relationships between pesticide use and NHL, leading the authors to conclude that "these findings suggest that NHL in eastern Nebraska may be related to the use of pesticides and nitrogen fertilizers" (Weisenburger 1990).

Physiology. Although the evidence from the database of human epidemiology studies suggests a link between nitrate contamination and cancer, three critical human physiology studies add significant weight because they indicate that nitrate in water may contribute more than nitrate in food to total body burden of nitrite, and because they prove that the entire series of reactions necessary to convert nitrate into carcinogenic N-Nitroso compounds can occur in the human body.

In a 1982 study, researchers compared two groups, one drinking nitrate contaminated water (at 20 ppm) and another drinking water with lower levels of nitrate. The study found that salivary nitrate concentrations more than doubled and salivary nitrite concentrations quadrupled after drinking nitrate contaminated water, and noted that "ingestion of nitrate rich water can provide a steady and prolonged supply of nitrite to the gastric system" (Weisenberg 1982). A second noteworthy finding of the study was that the threshold for water affecting salivary nitrate was lower than the threshold for food. In previous findings, researchers had indicated a food threshold intake of approximately 13 milligrams nitrate before any increase in salivary nitrate was noted. However, Weisenberg, et al. (1982) reported that "the dose of nitrate ingested from water needed to increase the salivary nitrite seems to be much lower." This may be particularly important because salivary nitrite generally accounts for 75% of all exposure to hazardous nitrite or N-nitroso compounds (NAS 1977).

In a second study published in 1992, researchers at the University of Nebraska Medical Center were able to provide convincing evidence that nitrate consumed via drinking water could produce nitrosamines in the human body (Mirvish 1991). In the study, 44 Nebraska men were divided into two groups, one drinking well water high in nitrates (>18 ppm) and one group drinking well water lower in nitrate (<18 ppm). Each of these men was also given a small dose of proline, a compound which reacts with nitrite to form nitrosoproline (NPRO), a nitrosamine. During the study the researchers measured NPRO concentration in the urine and found that the high nitrate group had higher levels of NPRO, proving that drinking water high in nitrate can lead to increased production of carcinogenic nitrosamines in the human body. Similar results were observed in a Danish study of populations drinking water high in nitrate, leading those researchers to note that "it would seem well advised to reduce the intake of nitrate from drinking water." (Moller 1989).

These studies provide strong physiological evidence supporting the association between nitrate contamination of drinking water and increased cancer rates observed in the human epidemiology studies. They clearly indicate that nitrate ingested via water can undergo the full set of reactions needed to ultimately produce carcinogenic N-Nitroso compounds in the human body, and they further suggest that given an equal amount of nitrate in food and water, that nitrate in water may account for a proportionately greater amount of actual exposure to both nitrate and nitrite.

Nitrate, Infants, and Children: A High Cancer Risk? Just as children are more sensitive to methemoglobinemia than adults, there is strong experimental and epidemiological evidence to support the conclusion that infants, children, and the fetus, when exposed to nitrate, may face notably higher cancer risks later in life ([Table 7](#) [0]).

There are several possible reasons for this higher sensitivity or risk in the young. Infants have a lower stomach acidity than adults, meaning that when nitrate is consumed in water more of it will be converted into the hazardous nitrite form. In addition, children drink more water per unit of body weight than adults -- bottle-fed infants subsist on a diet composed entirely of formula and water, and relative to body weight, the average infant drinks approximately seven times more tap water than the average adult.

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 conducted in an area of high nitrate exposure in Colombia focused on the effect of nitrate exposure early in life. The study found that increases in stomach cancer rates were associated with consumption of well water high in nitrate and that individuals exposed during the first ten years of life formed a high risk group (Cuello et al. 1976).

Studies with N-Nitroso compounds on both fetal and infant equivalent animals support this finding. Research on rats has shown that nitrite is transported through the placental barrier and delivered to the fetus, and that a high dose of nitrate to the pregnant dam can cause subacute methemoglobinemia in fetal rats (Shuval and Gruner 1972). A second study showed the potential significance of fetal exposure to N-Nitroso compounds. In this case a single dose of a nitrosamide (ethylnitrosourea) given to the mother on the 15th day of the pregnancy was sufficient to cause rare malignant ocular tumors in the offspring. Significantly, these cancers occurred relatively early in the life of the offspring, indicating even greater potency for the compound than is suggested by the otherwise compelling fact that a single dose during gestation was sufficient to cause cancer in 100 percent of the surviving fetuses (Druckrey 1976).

Further reason for concern about infant exposure to nitrate comes from a massive study of 1,040 rats fed an N-Nitroso compound, N-Nitrosodiethylamine (NDEA).

This study was designed to determine a threshold for exposure below which no adverse effects occurred, but instead the authors found "no indication of any 'threshold'" for tumor formation (Peto et al., 1991). The investigation found that the N-Nitrosamines caused tumors of the liver and esophagus, with a strong dose-response relationship between the concentration of NDEA and the tumor formation rate.

In conjunction with this study, the authors examined the effects of age of initial exposure on cancer incidence (Gray et al. 1991). In this part of the study, the rats were broken into three groups with treatment starting at either three, six, or 20 weeks of age.

The results of this research showed that the initial age of exposure had a significant effect on liver tumor rates, and that young animals were particularly sensitive to the cancer causing effects of the chemical. After the same duration of exposure, rats starting treatment at three weeks of age had a cancer rate three times higher than rats starting treatment at six weeks, and six times higher than rats starting treatment at 20 weeks of age.

The authors reported that, although they could not identify the exact cause, their result:

"indicates a profound influence of nitrosamine treatment of the liver during the first few weeks of life on subsequent tumor onset rates, due to some temporary factors that greatly enhance the sensitivity of the organ." (Gray, et al 1991)

The National Academy of Sciences Conclusions on Nitrate and Cancer

In its recent report on nitrate in drinking water, a subcommittee of the National Research Council concluded that cancer risk from nitrate in drinking water is negligible. For most of the U.S. population, where nitrate contamination in drinking water is low, this assessment is likely accurate.

For this average person in the U.S. population, the subcommittee estimated nitrate consumption via drinking water at 2 mg per day, or three percent of daily exposure. Food exposure was estimated at 73 mg per day, or 97 percent of daily exposure.

In areas with high nitrate contamination, described as "nitrate-rich water⁴" by the subcommittee, nitrate consumption via drinking water was estimated at 160 mg per day, or 69 percent of daily exposure. This exposure level is many times greater than the average exposure via drinking water, and contributes to a total daily exposure more than triple the average exposure.

The flaw in the committee's analysis is the failure to assess the increased cancer risk associated with high consumption of nitrate during the first years of life.

For newborns whose sole food source is infant formula reconstituted with nitrate-rich tap water, the exposure during the first six months of life would be 80 times greater than the average bottle fed infant. The committee made no assessment of the effect of elevated nitrate exposure during infancy on cancer risk other than to note that the epidemiological literature provides contradictory evidence of an association between high nitrate consumption in the general population and cancer risk. None of these studies cited by the committee examined the potential increase in risk associated with high nitrate consumption during infancy.

According to the subcommittee, five percent of nitrate ingested is converted to carcinogenic N-Nitroso compounds in the digestive tract. And there is reason to believe that exposure to N-Nitroso compounds during the first year of life present significantly higher cancer risks than exposure during other periods of life (see previous).

The question that the committee failed to address is: What is the increase in cancer risk for the infants subsisting on water based formula that is contaminated with nitrate at levels much higher than those deemed to present negligible risk over a lifetime. Reliable data indicate that early life exposure to N-Nitroso compounds significantly increases cancer incidence in rats. It follows that highly elevated early life exposure in humans might have the same effect.

Infants also lack vitamins in their diet that inhibit the formation of N-Nitroso compounds. Vitamin C and E found in many fruits and vegetables are known to inhibit the formation of carcinogenic N-Nitroso compounds. Consuming ample vitamin C and E can mitigate much of the potential cancer risk associated with a diet high in nitrate. Newborns drinking nitrate contaminated water have no such dietary defenses. This lack of risk mitigating components that are present in the adult diet may contribute to increased cancer risks from heavy nitrate exposure early in life.

Other Chronic Effects: Birth Defects and Hypertension

A number of other human or animal studies support two other potential adverse, chronic effects of nitrate exposure: birth defects and hypertension.

At least five studies have indicated a possible link between exposure to nitrite, nitrate and N-Nitroso compounds and birth defects. The effects were first observed in animal studies, but have since been observed in human epidemiological studies ([Table 8 \[0\]](#)).

Studies in rats and hamsters have indicated that N-Nitroso compounds are potent teratogens (Druckery 1966; Givelber 1969). In both of these animals, 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.

Other studies have indicated that nitrite can be transferred from the mother to the fetus and could affect behavioral development at sublethal doses (Shuval and Gruener 1972). When pregnant rats were exposed to high nitrite concentrations in water, nitrite was transferred through the placenta, and resulted in high nitrite levels and subacute methemoglobinemia in the fetuses. The same study indicated that in pregnant rats exposed to sodium nitrite in their drinking water "a pronounced effect on mortality" of the infants in the high dose group (Shuval and Gruener 1972). This effect -- an increase in infant death rates -- has also been observed in human epidemiology studies. A study of African mothers exposed to elevated nitrate levels in their drinking water found that an increase in infant deaths was associated with increasing exposure of pregnant mothers and infants to nitrate (Super, et al. 1981). This may have been either due to undetected toxic methemoglobinemia or to malformations and weaknesses in the infant caused by fetal nitrate exposure.

After these animal studies indicated the teratogenic effects of N-Nitroso compounds, a number of human epidemiology studies were conducted. The first, in 1972, attempted to compare rates of anencephaly (a birth defect causing severe brain malformation) with dietary intakes of a number of food products (Knox 1972). The clearest link was found between anencephaly rates and intake of cured meat containing high levels of nitrite. Over a ten year period, 1960-1970, anencephaly rates were closely linked to seasonal and yearly variations in national intake of cured meat products. This study provided the first suggestive evidence in humans that nitrite consumption in food could have adverse impacts on the fetus.

Later studies indicated that birth defects may also be caused by nitrate in water. A 1984 study of ground and surface water drinkers in South Australia analyzed birth defects in relation to nitrate contamination in drinking water and found statistically significant dose response relationships between birth defects of the central nervous system and musculoskeletal system and increasing nitrate concentration of drinking water (Dorsch, et al. 1984).

This study broke mothers into three groups, those consuming 0-1.1 ppm nitrate, those consuming 1.1-3.5 ppm of nitrate, and those consuming over 3.5 ppm of nitrate in their drinking water. Women in the 1.1-3.5 ppm category experienced a threefold increase in risk of a birth defect, while women in the highest exposure category faced a fourfold increased risk. Interestingly, this study also found a seasonal gradient in risk, with greater risks occurring for conception during the spring and summer. The authors attributed this to the possibility of increased water consumption in the warmer months.

While the authors (and other critics of the work) have noted that, because of the design of the study, they could not exclude other causes besides nitrate, they discussed two factors that strengthened the association:

- The fact that a true dose-response relationship was observed, and
- Other studies that indicate, in humans or animals exposure to nitrate, nitrite, or N-Nitroso compounds is associated with birth defects.

Based on these findings, the authors concluded that

...the internal cohesion of our findings and their consistency with our earlier study and experimental evidence, lend weight to the possibility of a real association between groundwater nitrate consumption and malformations. (Dorsch, et al. 1984)

Based on the results of the Australian study, a similar follow-up study was performed attempting to link defects of the central nervous system with nitrate exposure via drinking water in New Brunswick, Canada (Arbuckle, et al. 1988). This study, although it did not contradict the results of the Australian study, found the evidence for an association between nitrate and birth defects to be weaker. In the study, no statistically significant risk relationship was found between CNS birth defects and nitrate exposure. The authors discuss a number of reasons for the variability in study results, including differences in sensitivity of the populations, biases in exposure estimates in the Australian study, and differences in levels of exposure between the Australian and Canadian groups.

Although both studies analyzed risks at exposure below the current MCL of 10 ppm, the majority of the Canadian population was not exposed above 3 ppm. Notably, when the relationship between nitrate exposure and birth defects was analyzed for two higher exposure subgroups of the population (well water drinkers and those in a high occurrence region, both of which were exposed at relatively high nitrate levels, although still below the MCL), moderate but not statistically significant increases in CNS birth defect rates were observed with increasing nitrate exposure. The authors of this study concluded that these results warranted further study, and that future studies must include a larger population exposed to higher nitrate levels.

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. As noted previously, studies have indicated that N-Nitroso compounds are transported through the placenta to the fetus (Shuval and Gruener 1972) and that fetal exposure can cause cancer later in life (Druckrey 1966). The same author reported that a single dose of a nitrosamide given to pregnant rats on day 15 of the pregnancy can cause birth defects in the offspring.

Human epidemiology studies have also indicated a link between nitrate intake and hypertension. A 1971 study published in the American Journal of Public Health analyzed the risk of hypertension and hypertension mortality and found a positive relationship between hypertension and increased nitrate exposure through drinking water (Morton 1971). The study was designed with the goal of identifying the cause of a localized area of increased hypertension risk in eastern Colorado, and analyzed a number of drinking water parameters (hardness, sodium, nitrate and chlorine) in relation to this risk. Among the six watersheds that were analyzed, the strongest relationship was between elevated nitrate levels and increased hypertension. In the study, Republican River watershed residents, with the highest mean nitrate concentration at 3.1 ppm (3 times lower than the current MCL) had hypertension mortality rates that were more than twice the rates of the next highest region.

A second study, conducted seven years later, reported on eighteen communities in Weld County, Colorado (Mallberg 1977). This study separated community water supplies into a high-nitrate group and a low-nitrate group, and did not report any epidemiological evidence of increased hypertension with increased nitrate consumption. However, the study did provide some evidence supporting the initial findings. Among residents in the exposed communities, there was an earlier onset of hypertension, with the exposed group exhibiting a peak among the 50-59 age group that was not evident in the non-exposed communities.

Supporting these epidemiological findings, complementary evidence from workers exposed to high levels of organic nitrates appeared to strengthen the link. Among explosives industry employees exposed to high levels of nitrate in the workplace, a variety of adverse cardiovascular effects are observed, including elevated blood pressure, increased risk of angina pectoris, and sudden coronary death (Morton 1971).

Notes

1. Nitrate concentrations are generally reported in one of two ways: either measured as nitrogen, or measured as nitrate. The EPA standard of 10 ppm is based on nitrate measured as nitrogen. For conversions, this is equivalent to 45 ppm nitrate measured as nitrate. In this text, when referring to nitrate concentrations we are referring to nitrate measured as nitrogen. This is the format generally used by American scientists.
2. Although for infants eating baby food there may still be exposure from nitrate that occurs naturally in vegetables.
3. These studies were ecologic studies, meaning that groups of individuals, not the individuals themselves, were the subjects of the study.
4. Water that contains concentrations of nitrate-nitrogen approaching the current EPA standard of 10 ppm.