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Colleen S. Bruning-Fann

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Major professor
John B. Kaneene, DVM MPH PhD

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**THE EFFECT OF NITRATE CONTAINED IN DRINKING WATER
ON THE PRODUCTIVITY AND HEALTH OF FARROWING SWINE**

By

Colleen S. Bruning-Fann

A THESIS

**Submitted to
Michigan State University
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ABSTRACT

THE EFFECT OF NITRATE CONTAINED IN DRINKING WATER ON THE PRODUCTIVITY AND HEALTH OF FARROWING SWINE

By

Colleen S. Bruning-Fann

A cross-sectional study design was utilized to determine the effects of nitrate contained in drinking water on farrowing swine health and productivity. Swine farms (571) in the U.S. were monitored for 3-months. Two water samples collected 3-months apart, were tested for nitrate. Data was gathered through a daily log recorded by the operator and administered questionnaires.

The data were analyzed on a farm basis using stratified analysis, and multiple linear regression or multiple logistic regression. No association was seen between the nitrate concentration of drinking water and average litter size, average percentage stillborn, or the risk of having an above median percentage of mummies. No association was seen between nitrate and the risk of having an above median (>0) percentage of swine ill or dead due to farrowing problems, other reproductive problems, other known health problems, or unknown health problems. The statistical power of this study was .90 (at $\alpha=.05$).

This thesis is dedicated to my
husband John for his unwavering
faith and support and to my children
Lyle and Eric.

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CHAPTER 1

INTRODUCTION

It is estimated that groundwater is the primary source of water for 85% of the rural population and 50% of the entire population of the United States (Council on Agricultural Science and Technology, 1985). Evidence suggests that nitrate levels in groundwater are increasing in much of the world (Vigil et al., 1965; Fraser and Chilvers, 1981; Holländer and Sander, 1987). This is believed to have resulted from human intervention either through agricultural activities, waste disposal, or industrial processes (Bouchard et al., 1992).

Nitrate is associated with a number of adverse health effects both in animal and human health (Bruning-Fann and Kaneene, 1993a; Bruning-Fann and Kaneene, 1993b). The National Academy of Sciences has recommended that concentrations of nitrate in the drinking water of animals not exceed 455 ppm. This recommendation was based on the meager information available at that time (1974). Since then, relatively little additional research has been done concerning the effect of nitrate in drinking water on livestock species.

With groundwater serving as the primary source of drinking water for livestock and the levels of nitrate in this resource increasing, it is timely and important to ascertain the significance of nitrate contamination of groundwater on animal health and productivity. To this end, this study was undertaken to explore the effects of nitrate found in drinking water on farrowing swine health and productivity.

REFERENCES:

Bouchard DC, Williams MK, and Surampalli RY: Nitrate contamination of groundwater: Sources and potential health effects. J Am Water Works Assoc, 85:85-90, 1992.

Bruning-Fann C and Kaneene JB: The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Vet Human Toxicol, 35:237-253, 1993a.

Bruning-Fann C and Kaneene JB: The effects of nitrate, nitrite, and N-nitroso compounds on human health: A review. Vet Human Toxicol, 35:521-538, 1993b.

Council for Agricultural Science and Technology: Agriculture and groundwater quality. Council for Agricultural Science and Technology Report No. 103, p. 62, May, 1985.

Fraser P and Chilvers C: Health aspects of nitrate in drinking water. Sci Total Environ, 18:103-116, 1981.

Holländer R and Sander J: Nitratkonzentrationen von trinkwässern in Südwest-Niedersachsen. Zentralbl Bakter Mikrobiol Hyg, 184:287-296, 1987.

National Academy of Sciences--National Research Council, Subcommittee on nutrient and toxic elements in water: Nutrients and toxic substances in water for livestock and poultry. National Academy of Sciences, Washington, D.C., 1974.

National Academy of Sciences--National Research Council, Assembly of Life Sciences: The health effects of nitrate, nitrite and N-nitroso compounds. National Academy of Sciences, Washington, D.C., 1981.

Vigil J, Warburton S, Haynes WS and Kaiser LR: Nitrates in municipal water supply cause methemoglobinemia in infant. Pub Health Rep, 80:1119-1121, 1965.

CHAPTER 2

OVERALL GOALS AND HYPOTHESES

Goal: To determine the effect of nitrate in drinking water on the productivity and health of farrowing swine.

General

Hypothesis: That nitrate at levels found in the drinking water from wells during the National Swine Survey adversely affects farrowing swine productivity and health.

Specific

Hypotheses: That the nitrate level of drinking water is associated with the farm

1. Average number of pigs born per litter.
2. Average percentage of the litter stillborn.
3. Average percentage of the litter born mummified.
4. Percentage of swine with farrowing problems.
5. Percentage of swine with reproductive problems other than farrowing problems.
6. Percentage of farrowing swine with other health problems.

7. Percentage of farrowing swine with unknown health problems.
8. Mortality rate due to farrowing problems.
9. Mortality rate due to reproductive problems other than farrowing problems.
10. Mortality rate due to other known problems.
11. Mortality rate due to unknown problems.

CHAPTER 3

A REVIEW OF THE EFFECTS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS ON ANIMAL HEALTH

ABSTRACT

The clinical signs of acute nitrate toxicity vary among species. In general, ruminant animals develop methemoglobinemia while monogastric animals exhibit severe gastritis. Nitrate ingestion has also been linked to impaired thyroid function, decreased feed consumption, and interference with the metabolism of vitamin A and E. Hematologic changes observed with chronic high nitrate exposure include both compensatory increases in red blood cells and anemia, along with increased neutrophils and eosinophils.

Unlike nitrate, nitrite is apparently capable of inducing methemoglobinemia in a wide range of species. Methemoglobinemia has been reported in cattle, sheep, swine, dogs, guinea pigs, rats, chickens, and turkeys. In rats, chronic nitrite exposure causes pathologic changes in a variety of tissues, alters motor and brain electrical activity, and affects absorption of nutrients by the gastric mucosa. Nitrite affects the metabolism of sulfonamide drugs in animals such as the pig, guinea pig, and rat.

The N-nitroso compound dimethylnitrosamine causes toxic hepatitis in cattle, sheep, mink, and fox. Nitrosamines have been reported in cows' milk and have been found to pass into the milk of goats under experimental conditions.

INTRODUCTION

Nitrate containing compounds were first implicated in the toxicosis of man in 1793 according to a review by Beck (1909). The earliest recorded case of nitrate poisoning in animals was noted in 1895 by Mayo. It was Comly in 1945, who first associated methemoglobinemia in children with consumption of water high in nitrate and advocated limiting the use of drinking water which exceeded 45 ppm nitrate (10 ppm as nitrate nitrogen).

Since Comly's work, it has become apparent that nitrate is not stable in biological environments but can be converted to nitrite and in the presence of other naturally occurring nitrogen compounds, to N-nitroso compounds. The National Academy of Science after reviewing the literature has recommended that drinking water for humans not exceed 45 ppm nitrate and that of animals not exceed 445 ppm (100 ppm as nitrate nitrogen) (National Academy of Sciences, 1981; National Academy of Sciences, 1974). While the toxicity of nitrates, nitrites, and N-nitroso compounds in humans has been reviewed relatively recently (Hartman, 1982; Green et al., 1982; Magee, 1982; National Academy of Sciences, 1981), it has been 20 years since the effect of nitrate and nitrite on animal health has been reviewed (Singer, 1968; Singer, 1972; Wright and Davison, 1964; Ridder and Oehme, 1974a; National Academy of Sciences, 1974). With the controversy surrounding

the effects of nitrate on health, a wide variety of views on the potential of nitrate to cause adverse health effects has developed. This review was undertaken in an attempt to clarify the situation by considering the effects of nitrate, nitrite and N-nitroso compounds in animals.

Nitrate Measurement:

There are several different ways to report nitrate content including the levels of nitrate ion, nitrate-nitrogen, and potassium or sodium nitrate. Conversion factors may be used to compare the different units of nitrate measurements in various studies (see Table 3.1). Due to differences in molecular weights, 1 ppm potassium or sodium nitrate is equivalent to 0.61 ppm nitrate or 0.14 ppm nitrate-nitrogen. Likewise, conversion from nitrate-nitrogen to nitrate ion can be accomplished by multiplication by a factor of 4.45. Multiplication by 6.1 will convert nitrate-nitrogen to potassium nitrate or sodium nitrate.

Nitrate and Nitrite Metabolism:

When discussing the health effects of nitrate, it is important to consider how nitrate is metabolized by different species. Many of the toxic effects attributed to nitrate are actually the effects of metabolites of nitrate. It is through examining all the effects of the various products that one can

thoroughly understand the importance of nitrate exposure to health.

There are considerable differences in the toxicity of nitrate depending on the animal species involved. The anatomy and microflora of ruminant animals such as cattle and sheep, allows the *in vivo* reduction of nitrate to nitrite to occur rapidly, while the large cecum and colon of the horse provides an area for microbial reduction of nitrate to occur which is intermediate between that of monogastrics and ruminants. In ruminants only a small proportion of nitrate is eliminated in the urine (Kearley et al., 1962; Lewis, 1951a), while in monogastrics such as the dog (Greene, 1954; Greene and Hiatt, 1955), most of the nitrate is eliminated in the urine. In an experiment involving young calves, large quantities of nitrates were excreted in the urine (Prewitt and Merilan, 1958). This finding reflects the fact that calves function initially as monogastrics, becoming ruminants later as their diet changes from milk to roughage.

Nitrate poisoning usually occurs subsequent to reduction to nitrite. Acute nitrate poisoning, though common in ruminants, is rare in monogastric animals. Nitrate poisoning usually occurs subsequent to reduction to nitrite. Nitrite is estimated to be 2.5 times more toxic for ruminants and 10 times more toxic for monogastrics than nitrate (Emerick, 1974). Substantial species differences in the rate of methemoglobin formation (Smith and Beutler, 1966; Spicer,

1950), methemoglobin reduction (Smith and Beutler, 1966), and response to methylene blue are known to occur (Smith and Beutler, 1966). In general, ruminants exhibit the most rapid rates of methemoglobin formation and reduction and respond most rapidly to the administration of methylene blue. In monogastrics, the pig shows the least response to methylene blue and the slowest rate of methemoglobin formation and reduction. Humans tend to be intermediate in methemoglobin formation and reduction but respond exceptionally well to methylene blue.

There are substantial species differences in nitrate and nitrite pharmacokinetics. The elimination half-life of nitrate is 44.681 hours in dogs, 4.233 hours in sheep, and 4.821 hours in ponies, while that of nitrite is 0.499 hours in dogs, 0.475 hours in sheep, and 0.566 hours in ponies (Schneider and Yearly, 1975). Little is known about the long term effects of nitrate exposure in man or animals.

NITRATE, NITRITE, AND N-NITROSAMINES IN RUMINANTS

Methemoglobinemia in Ruminant Animals:

Poisoning in cattle due cornstalks containing high levels of nitrate was first reported by Mayo in 1895. Illness and death in cattle after consuming oat hay was later reported by Newsom et al. (1937). Numerous other reports of "corn stalk" (Case, 1954; Muhrer et al., 1955; Schwarte et al., 1939) and

"oat hay poisoning" soon followed (Bradley et al., 1939; Davidson et al., 1941; Thorp, 1938). Subsequently it was shown that nitrates were the causative agent (Bradley et al., 1939). Since then, methemoglobinemia subsequent to consumption of nitrate containing roughages has been commonly reported in ruminant animals. Nitrate poisoning has resulted from the consumption of green oats (Dodd and Coup, 1957), sugar beet tops (Savage, 1949), variegated thistle (*Silybum marianum*) (Kendrick et al., 1955), turnips (Anon., 1978; Cawley and Collings, 1977), silage (Bjornson et al., 1961; Cawley and Collings, 1977), kale (Cawley and Collings, 1977), Sudan forage (Boermans, 1990; Vermunt and Visser, 1987), Sudan (*Sorghum* species) hay (Brown et al., 1990; Carrigan and Gardner, 1982; Haliburton and Edwards, 1978; Hibbs et al., 1978), ryegrass (Nicholls and Miles, 1980; O'Hara and Fraser, 1975), Italian ryegrass and white clover pasture (Vermunt and Visser, 1987), hay (Brown et al., 1990; Smith and Suleiman, 1991) and pigweed or redroot (*Amaranthus retroflexus*) (Dodd and Coup, 1957).

Toxicity due to well water containing nitrate has also been reported (Bjornson, 1961; Bjornson, 1964; Campbell et al., 1954; Fincher, 1936; Osweiler et al., 1985). An unusual source of nitrate poisoning in cattle involved water consumption from blasted watering holes (dugouts) (Yoong et al., 1990). Water nitrate levels were found to be 4.8 g/L and 7.0 g/L. These extremely high nitrate levels were due to the

use of fertilizer containing nitrate as a portion of the explosive charge.

Acute poisoning can occur when forages exceed 0.5% nitrate (on a dry weight basis) or 500 ppm nitrate in drinking water (Buck, 1970). The finding that some feed samples implicated in toxic episodes contained high levels of nitrite in addition to nitrate suggests that both substances should be measured in feed samples (Smith and Suleiman, 1991).

Nitrate can be absorbed across the ruminant epithelium probably by the $\text{Cl}^-/\text{HCO}_3^-$ exchange mechanism (Würmli et al., 1987). Nitrate can also be microbially reduced in the rumen to ammonia with nitrite as an intermediary step (Lewis, 1951a; Lewis, 1951b; Wang et al., 1961). During this reduction process, nitrite may be absorbed into the bloodstream (Wang et al., 1961) where it reacts with hemoglobin to form methemoglobin (Bodansky, 1951). There is a linear relationship between the amount of nitrate or nitrite administered and the extent of methemoglobin formation within a specific dosage range (Crawford et al., 1966; Lewis, 1951a). At dosages above this level, the reduction of nitrite to ammonia becomes a limiting factor allowing nitrite to accumulate at a much higher concentration in the rumen. At this point, rapid absorption of nitrite into the bloodstream results in a much greater increase in methemoglobin (Crawford et al., 1966; Lewis, 1951a). The administration of 2.5 grams of potassium nitrite intravenously elicited a fatal

methemoglobinemia in sheep within two hours while the intravenous administration of 2.5 grams of potassium nitrate caused no serious problems demonstrating that nitrate must be reduced to nitrite prior to entering the blood stream in order for methemoglobinemia to occur (Pfander et al., 1957).

Clinical Signs of Methemoglobinemia:

Clinical signs of methemoglobinemia become apparent when the percentage of hemoglobin converted to methemoglobin reaches 30 to 40%, and death ensues when the conversion exceeds 80% (Buck, 1969; Buck, 1970). Clinical signs of methemoglobinemia include weakness, ataxia, trembling, hypersensitivity, gasping for breath, and a rapid pulse rate (Bradley et al., 1940a; Davidson et al., 1941; Dodd and Croup, 1957; Fincher, 1936; Kendrick et al., 1955; Mayo, 1895; Newsom et al., 1937; Schwarte et al., 1939; Sloan, 1971; Thorp, 1938). Respiration and urination are frequently increased though temperature remains normal. The most characteristic sign of methemoglobinemia is the chocolate brown appearance of the blood which causes a peculiar "muddy" cyanosis of the mucus membranes and tongue. Symptoms are exacerbated by stress and exertion. Petechial hemorrhages in the epicardium, endocardium, peritoneum, mucous membranes of the respiratory tract and rumen, and the serosal surface of the digestive tract are frequently found on necropsy. Congestion of the

lungs and gastrointestinal tract are often seen, and the odor of nitrogen oxides may be noticeable on necropsy.

Animals poisoned by forages usually die within 2 to 10 hours (Beath et al., 1953). It has been reported that signs typical of nitrite poisoning are more obvious approximately 2 hours after peak blood nitrate and methemoglobin levels (Diven et al., 1962a). The methemoglobin level peaks when the nitrite level of the blood is at maximum (Pirincci and Kelestimur, 1987). Maximum concentrations of rumenal nitrite coincide with peak methemoglobin levels (Wang et al., 1961) indicating the rapidity with which nitrite is absorbed from the gastrointestinal tract and reacts with hemoglobin. In cattle, maximum methemoglobin levels appeared 3 hours after intraruminal dosing (Wang et al., 1961) or drenching (Crawford et al., 1966) with nitrate. When nitrate was fed once daily, the maximum methemoglobinemia occurred approximately 8 hours after feeding, but when fed twice daily, peak levels occurred 4 to 5 hours after feeding (Crawford et al., 1966; Jainudeen et al., 1964).

Blood levels of nitrate and methemoglobin vary directly with the dose of nitrate (Diven et al., 1962a; van 't Klooser, 1982). However, large interanimal variation is seen in the amount of methemoglobin induced in animals of the same species when given the same dose of nitrate (Diven et al., 1962a; Prewitt and Merilan, 1958; Simon et al., 1959a; Winter, 1962; van 't Klooser, 1982). In experimental trials using sublethal

doses in sheep, peak methemoglobin levels were achieved approximately 4 to 8 hours after intraruminal administration of a single 20 gram dose of sodium nitrate in one study (Emerick et al., 1965); another research team found that maximum plasma nitrate and nitrite concentrations occurred 2 hours after drenching and peak methemoglobin formation occurred approximately 4 hours after drenching (Setchell and Williams, 1962). When sheep received a lethal dose of 20 grams of sodium nitrate as a drench, plasma nitrate, nitrite, and methemoglobin levels continued to rise until the animals died at 5 and 6.5 hours after treatment (Setchell and Williams, 1962). These differences are probably related to differing rates of nitrate intraruminal metabolism because when nitrite is administered directly to the blood, the extent of methemoglobin formation is relatively constant (Winter, 1962). Blood levels of nitrate are also related to lactation status with higher nitrate levels seen in dry cows compared to lactating cows (Hambitzer et al., 1987).

Abortion:

Abortion in ruminants has been reported as a sequela following nitrate toxicosis (Bjornson, 1964; Bradley et al., 1940a; Carrigan and Gardner, 1982; Case, 1957a; Case, 1957b; Davidson et al., 1941; Garner, 1958; Haliburton and Edwards, 1978; Hibbs et al., 1978; Jainudeen et al., 1964; Nicholls and Miles, 1980; Ruhr and Osweiler, 1981; Stuart and Oehme, 1982;

Thorp, 1938; Vermunt and Visser, 1987). Abortions occurred 3 to 7 days after the toxicity episode. Case (1957a), reported high levels of methemoglobinemia in stillborn calves born to cows receiving a high nitrate ration. Fetal death and abortion was seen in pregnant cattle given large doses of potassium nitrate (Simon et al., 1959a). Nitrates are the suspected causal agent of "lowland abortion syndrome" in cattle (Simon et al., 1958; Simon et al., 1959b; Sund et al., 1957). Rations containing 3.4% nitrate may have caused abortion in sheep (Davison et al., 1965). In aborted fetuses and ill or dead calves, elevated ocular nitrate concentrations suggest that excessive maternal nitrate ingestion may have been a causative or contributing agent (Johnson et al., 1983). Abortion may be due to methemoglobinemia in the dam, resulting in fetal death from hypoxia or interference with other essential iron containing proteins (Wood, 1980). An experiment on pregnant cows documented low oxygen saturation of umbilical venous blood following nitrite administration to the cow (van 't Klooster et al., 1982) which strongly suggests oxygen transfer to the fetus is decreased after nitrite administration.

Diagnosis:

Methods to ascertain the level of methemoglobin in blood have been described (Evelyn and Malloy, 1938; Hainline, 1965; Schneider and Yeary, 1973). Methemoglobin determination

should be carried out as soon as possible after blood collection because spontaneous methemoglobin reduction (Bodansky, 1951; Eppson et al., 1960; Watts et al., 1969; Wendel, 1939) occurs. The addition of one part blood to 20 parts phosphate buffer (pH 6.6) or a 1:20 dilution with distilled water followed by refrigeration or freezing has been effective in preserving methemoglobin (Ruhr and Osweiler, 1986). Methods to determine serum nitrate and nitrite concentration have been described (Diven et al., 1962b; Schneider and Yeary, 1973). Nitrate levels as measured by ocular fluid offer some advantages over blood determinations. Ocular nitrate values correlate well with serum values and clinical signs while remaining stable for over 24 hours and diagnostically significant for 60 hours after death (Boermans, 1990). However, if death occurs very quickly, ocular nitrate levels will be well below serum values presumably due to a lack of time for equilibration. Urinary nitrite determination may also be a useful diagnostic test (Watts et al., 1969). Recently, utilization of ion-exchange liquid chromatographic determination on nitrate and nitrite in serum, ocular fluid and water has been described (Boermans, 1990; Lippsmeyer et al., 1990).

Factors influencing Toxicity:

The extent of acute toxicity is dependent on a number of factors. Of primary importance is whether nitrate or nitrite

is given, the route and rate of administration, and the total dosage involved. For example, 60% of total hemoglobin was converted to methemoglobin with 25 grams of sodium nitrate intrarumenally, 10 grams of sodium nitrite intrarumenally, or 2 grams sodium nitrite intravenously (Lewis, 1951a).

The rapidity of administration is also very important. Hay that was consumed without problem when fed free-choice, produced intoxication when force fed within 4 hours (Dollahite and Holt, 1969). A single intraruminal dose of 20 grams of potassium nitrate was fatal to sheep while the same dosage incorporated into the feed caused no ill-effects (Sinclair and Jones, 1967). The LD_{50} for cattle when nitrate is given by drenching is estimated to be 330 mg of nitrate ion per kg of body weight (Bradley et al., 1940a) but increases to 990 mg/kg when nitrate is consumed with forage (Crawford et al., 1966). There is no difference between the toxicity of naturally accumulated nitrate and nitrate added to the forage (Davison et al., 1965).

There is evidence that ruminants adapt to the ingestion of nitrate. Several days of dietary nitrate administration were necessary before nitrites appeared in substantial amounts in rumenal fluid (Nakamura et al., 1976; Tillman et al., 1965b) and blood during two studies (Tillman et al., 1965b). Another team of researchers found that rumen flora were capable of nitrate reduction immediately but that methemoglobin did not appear for several days (Wang et al.,

1961). One week after the initiation of daily nitrate treatment, blood nitrate and methemoglobin levels were at their peak (Sinclair and Jones, 1964). Subsequently, blood nitrate decreased and methemoglobin levels returned to normal. In calves given nitrate or nitrite for a period of several weeks, a gradual decline was seen in the induced methemoglobin levels though the dosage of nitrate or nitrite remained the same (McIlwain and Schipper, 1963). Experimental evidence suggests that nitrate intoxication in the ruminant is more likely to occur after a several days of high nitrate ingestion (van 't Klooster et al., 1982). Field reports of toxicity episodes support the premise of nitrate adaptation. During an acute toxicity episode, 4 of the 7 deaths in a group of 90 cattle were from a group of 6 cattle recently introduced into the herd (Slenning et al., 1991).

Under conditions of chronic nitrate ingestion, there are changes in the rumen flora which accounts for adaptation. The ability of rumenal flora to transform nitrate and nitrite is enhanced after nitrate exposure (Cheng et al., 1985; Jamison, 1959; Nakamura et al., 1976). This enhanced ability of rumen microbes to degrade nitrate and nitrite was found to transfer to control animals housed in adjacent pens but not to widely separated control animals (Cheng et al., 1985). Changes in the molecular percentage structure of volatile fatty acids in the rumen is seen in sheep treated with nitrate, nitrite and hydroxylamine suggesting other alterations occur in the

rumenal flora (Jamison, 1958). It is possible that treatment with antibiotics could alter the microbial population in the rumen thereby altering the response to nitrate ingestion (Smith, 1965).

Case, stated that any amount of nitrate over 0.5% of the total ration was a potential cause of trouble (Case, 1957a); however, several studies have shown that under certain conditions, much higher levels of nitrate can be tolerated. Studies have shown that animals on an adequate ration can handle up to 1.2% nitrate without a significant increase in methemoglobinemia (Wallace et al., 1964; Weichenthal et al., 1963). Animals on high quality rations can withstand much higher levels of nitrate than those on deficient rations (Case, 1957a; Case, 1957b; Holtenius, 1957; Pfander et al., 1957; Sapiro et al., 1949; Sokolowski et al., 1960) because of an increased ability to break down nitrite in the rumen (Holtenius, 1957).

Cattle receiving urea in the ration had lower blood urea, nitrate and methemoglobin levels than cattle receiving soybean meal (Clark et al., 1970). Nitrate added to the ration decreased weight gains in sheep fed soybean meal but increased weight gains in sheep fed urea (Hatfield and Smith, 1963). This finding suggests that either soybean meal inhibits the reduction of nitrate, or urea stimulates the reduction of nitrate in the rumen.

The addition of glucose to the ration increases the *in vitro* reduction of nitrate and nitrite (Pfander et al., 1957; Sapiro et al., 1949). With corn or sucrose added to the ration, the average peak methemoglobin level was significantly decreased with intraruminal nitrate administration, but not with intravenous nitrate administration (Emerick et al., 1965). Up to 4% sodium nitrate in the ration, in conjunction with an active ruminal flora and adequate sugar, produced no harmful effects (Clark and Quin, 1951). This evidence shows that carbohydrates act to prevent the accumulation of nitrite in the rumen by increasing the rate of nitrite reduction.

There is evidence that other ration components are important in the utilization of nitrate. The addition of inorganic sulfur to the ration appears to increase the utilization of nitrate in growing lambs (Sokolowski et al., 1961). Adding butylated hydroxy anisole to rations containing up to 2% potassium nitrate increased digestion, absorption, and retention of ration nitrogen but blood nitrate levels were lowered (Ketchum et al., 1957). Digestion and/or absorption of ration nitrogen was also increased when sodium meta bisulfite was added to the ration (Ketchum et al., 1957). Molybdenum is necessary for *in vivo* nitrate reduction while iron and copper may be important (Tillman et al., 1965b). The addition of L-cysteine with administered nitrate suppressed nitrite production and methemoglobin formation (Takahashi and Young, 1991).

Non-Protein Nitrogen:

In low to moderate levels, ruminant animals can utilize nitrate as a source of non-protein nitrogen (Clark and Quin, 1951; Osweiler et al., 1976; Sapiro et al., 1949; Sokolowski et al., 1961; Tollett et al., 1961). The addition of sodium nitrate and a source of sugar to poor quality hay results in accelerated cellulose digestion (Clark and Quin, 1951). At high levels, nitrite can accumulate resulting in methemoglobinemia. The potential for synergy between nitrate and non-protein nitrogen feed supplements has been considered by a number of authors. Non-protein nitrogen has been linked to an episode of acute nitrate toxicity although other factors (use of monensin and improper feeding practices) may have contributed to the deaths (Slenning et al., 1991). In sheep, nitrate decreases the *in vitro* and *in vivo* utilization of urea (Bloomfield et al., 1961a). It appears that nitrate is utilized by microorganisms before urea, thereby decreasing the rate of urea hydrolysis (Carver and Pfander, 1974). Soybean meal, urea or sodium nitrate all appeared to be utilized equally well as sources of N₂ when used to increase the protein content of a ration fed to sheep (Hoar et al., 1968). Carver and Pfander (1973) found urea and nitrate could be fed in a properly supplemented, high concentrate ration as long as an adaptation period was followed. Following an adaptation period, as much as 5.0% potassium nitrate can be given with urea without problems when fed to low producing dairy cows

whether the ration was high or low energy (Sebaugh et al., 1970).

The use of monensin in conjunction with high-nitrate rations may act to precipitate nitrate toxicity in animals. For example, cattle that had been grazing turnips for 6 weeks without incident, developed methemoglobinemia less than 1 day after monensin was introduced. Following recovery, the cattle were once more allowed to graze the turnips. Again, methemoglobinemia developed when monensin was added to the ration (Malone, 1978). The use of monensin has been associated with forage-related nitrate toxicosis in dairy heifers (Slenning et al., 1991). These animals had excessive nitrate exposure as measured by ocular nitrate values but the clinical signs departed somewhat from the classical syndrome as not all animals displayed cyanosis and chocolate-colored blood. One author has attributed the ability of monensin to precipitate nitrate toxicity to a rapid shift in rumen microbial population in favor of the nitrite producers (Malone, 1978).

There are numerous reports of nitrate poisoning occurring after forage was wetted (Davidson et al., 1941; Smith et al., 1959; Thorp, 1938). These accounts suggests the transformation of nitrate to nitrite in the wet forage prior to consumption (Olson and Moxon, 1942). Since nitrite is much more toxic than nitrate, clinical signs would occur at a much lower dose. Conversion of a small amount of nitrate to

nitrite in drinking cups of pigs was noted in one study (Seerley et al., 1965). Also, the conversion of nitrate to nitrite is known to occur in vegetables during storage (Walker, 1975). Alternatively, nitrate concentrations are known to vary considerable even in the same species of plant grown in the same location. Assuming nitrate levels in the feed were extremely variable, clinical signs would become apparent only when the portions of the roughage containing high levels of nitrate were consumed.

Prevention:

There is considerable variation in the nitrate concentration of plants due to differences in the plant species, stage of plant maturity, and growing conditions (Kretschmer, 1958; Ruhr and Osweiler, 1986; Walker, 1975; Wilson, 1943; Wright and Davison, 1964). A wide variety of plants including many of the common forages fed to livestock may accumulate high levels of nitrate (Bradley et al., 1939; Bradley et al., 1940a; Knight, 1985; Ruhr and Osweiler, 1986; Whitehead and Moxson, 1952; Wilson, 1943). The level of nitrate varies considerably even among plants of the same species and is influenced by a number of factors such as available nitrogen (Williams, 1989; Wilson, 1949; Wright and Davison, 1964) and the stage of plant growth (Garner, 1958; Knight, 1985). Also, different parts of the same plant will contain different amounts (Knight, 1985; Whitehead and Moxson,

1952). Growing conditions such as drought have been found to foster nitrate accumulation (Whitehead and Moxson, 1952). The use of herbicides increases both the nitrate content of plants (Ruhr and Osweiller, 1986) and the palatability of some plants thereby increasing the likelihood of a toxic episode. In general, plants in rapid vegetative growth with heavy fertilization, drought, and high or low environmental temperatures are likely to accumulate excessive nitrate.

To prevent excessive nitrate accumulation in plants, care should be exercised in fertilizing crops. Sufficient time must elapse between applications of fertilizer and harvesting. Because drought can cause nitrate accumulation in plants, irrigation during dry periods can prevent excessive nitrate levels in plants. Since stems accumulate more nitrate than leaves, raising the height of the cutter bar to lessen the amount of stem cut will help to lower the nitrate content of the hay (Ruhr and Osweiller, 1986). Ensiling often lowers the nitrate content of forages (Ruhr and Osweiller, 1986).

If high nitrate forages must be used, an adaptation period will allow animals to adapt, thereby preventing problems. Also, dietary supplementation with high quality forages or a source of readily utilized carbohydrate will aid in preventing methemoglobinemia by increasing the rate of nitrite reduction. For each animals ration, the addition of 1 kg grain/200 kg body weight has been suggested (Ruhr and Osweiller, 1986). The addition of L-cysteine to the ration may

be effective in preventing nitrite accumulation and subsequent methemoglobinemia (Takahashi and Young, 1991). Maintaining adequate feed and water is important as several outbreaks of nitrate toxicity occurred after a period of time without feed, probably either due to rapid feed consumption and/or loss of nitrate adaptation (Brown et al., 1990; Slenning et al., 1991; Haliburton et al., 1978).

Treatment:

In mild cases, removal of the source of nitrate and keeping the animal quiet is usually sufficient. More severe cases can be treated with intravenous injections of methylene blue at a rate of 4 to 15 mg/kg of body weight or more (Burrows, 1980). In cattle, 8 mg/kg of body weight is usually sufficient (Beath et al., 1953; Bradley et al., 1940a; Bradley et al., 1940b). Although ascorbic acid reduces methemoglobin, the mode of action is much slower (Pirinççi et al., 1988).

Chronic Toxicity:

Hematologic Effects:

Studies in which animals developed significant methemoglobinemia for an extended period of time as a result of the ingestion of high levels of nitrate, suggest that compensatory increases in hemoglobin concentration, packed red cell volume, and blood volume occur in both cattle and sheep

(Davison et al., 1965; Jainudeen et al., 1964; Winter and Hokanson, 1964). One study found that methemoglobin and total hemoglobin increased proportionally and therefore the percentage of methemoglobin was unchanged (Crawford et al., 1966). It has been reported that sodium nitrate administered intrarumenally in sheep for 5 weeks at 0.2 g/kg increased the erythrocyte count whereas at 0.3 g/kg, a hypochromic anemia was seen (Keleştimur et al., 1988). Other blood changes such as higher leukocyte counts, increased neutrophil and eosinophil percentages, and lower lymphocyte percentages were seen in treated sheep whether 0.2 or 0.3 g/kg of sodium nitrate was given, compared to controls (Keleştimur et al., 1988).

Transplacental Transfer:

The finding of nitrate in amniotic fluid of cows given sodium nitrate orally suggests nitrates can pass the bovine placental barrier (Slanina et al., 1990). Pregnant heifers given nitrate in the ration at levels sufficient to maintain a daily peak methemoglobin level at 40 to 50% of total hemoglobin, had calves with elevated red blood cell and hemoglobin concentrations (Winter and Hokanson, 1964). This transplacental effect may be due to fetal anoxia subsequent to maternal methemoglobinemia or from the passage of nitrate or nitrite across the placenta.

Transmammary Transfer:

The composition of milk from animals receiving high levels of nitrate has been studied by several researchers. In one study, nitrate but not nitrite was found in the milk of ewes and a cow given sodium nitrate orally (Slanina et al., 1990). The methemoglobin levels of lambs increased after consumption of this high nitrate milk (Slanina et al., 1990). However, other studies found either a small increase in the nitrate content of milk from cows consuming high nitrate feed (Davison et al., 1964) or no significant difference in milk nitrate levels (Kammerer et al., 1992; Sebaugh et al., 1970). The differences between the studies are likely due to the large dosage in the first experiment relative to the other studies.

It has been demonstrated experimentally that ingested potentially carcinogenic nitrosamines could appear in the blood and milk of lactating animals (Juszkiewicz and Kowalski, 1974). The formation of nitrosamines in the rumen occurred only under acidic conditions, which do not normally exist in the healthy animal. However, nitrosamines have been found in some plants and fish meals which may be used in animal feeds (Juszkiewicz and Kowalski, 1974).

Thyroid:

The goitrogenic effect of nitrate discovered in rats by Wyngaarden et al. (1952), stimulated the study of this effect

in ruminants. The concentration of serum I^{131} as protein-bound iodine in sheep receiving nitrate in the diet was significantly less six days after injection of I^{131} (Bloomfield et al., 1961b; Bloomfield et al., 1962) but higher at 27 days compared to controls (Bloomfield et al., 1962). A tendency for dietary nitrate administration to decrease thyroid activity as measured by triiodothyronine I^{131} uptake was noted initially (Carver and Pfander, 1973) but further study found no significant effect (Carver and Pfander, 1974). No effect from the ingestion of nitrate was seen on thyroid weights or thyroid acinar heights in dairy cattle (Jainudeen et al., 1965). These data do not support a goitrogenic effect of nitrate in ruminant animals except perhaps a transitory effect prior to nitrate adaptation.

Pituitary:

The only study to monitor the pituitary gland reported pituitaries from cattle fed up to 660 mg/kg body weight were heavier than those from cattle not receiving nitrate (Jainudeen et al., 1965). It is unknown if pituitary function was affected.

Vitamin A:

Vitamin A deficiency in cattle was first attributed to dietary nitrate by Case (1957a). Since then, several theories have been examined concerning the effect of nitrate and

nitrite on vitamin A status. One theory proposes that nitrite oxidizes iron-containing enzymes, such as dioxygenase enzymes, thereby decreasing the conversion of beta-carotene to vitamin A (Wood, 1980).

Another theory states that vitamin A is readily destroyed by oxidation and since nitrates are oxidizing agents, nitrates could destroy vitamin A (Mitchell et al., 1967). *In vivo* experiments indicate nitrite is capable of destroying carotene (Keating et al., 1964; Olson et al., 1963; Pugh et al., 1962) and vitamin A (Keating et al., 1964; Pugh et al., 1962; Roberts and Sell, 1963). However, nitrate seems to affect carotene and vitamin A in some circumstances but not in others. The conversion of carotene to vitamin A *in vitro* was reduced by nitrate (Reddy and Thomas, 1962) but *in vitro* carotene destruction was not increased (Davison and Sen, 1963). The addition of high levels of nitrate to the rumen liquor of steers *in vitro* increased the destruction of vitamin A only in steers fed high concentrate diets (Keating et al., 1964). Experiments in which nitrate was added to the ration have failed to find any effect on the *in vitro* (Keating et al., 1964) or *in vivo* destruction of vitamin A (Mitchell et al., 1965; Mitchell et al., 1967) or *in vitro* destruction of carotene (Davison and Sen, 1963). Likewise, feeding 2% potassium nitrate to sheep had no effect on vitamin A concentrations in abomasum fluids, indicating little destruction of vitamin A *in vivo* (Roberts and Sell, 1963).

Dietary administration of nitrite decreased liver vitamin A levels in sheep (Holst et al., 1961). These results indicate that only with relatively large doses of nitrate and nitrite (greater than is seen with the dietary addition of nitrate) are carotene and vitamin A metabolism noticeably affected.

The thyroid is important in the conversion of carotene to vitamin A in rats (Johnson and Baumann, 1947). Research indicates that this is also true in ruminants. Treatment of steers with the thyroid hormone triiodothyronine resulted in increased plasma carotene and liver vitamin A in those supplemented with carotene or vitamin A and increased plasma carotene and vitamin A in steers without supplementation (Jordan et al., 1963). In addition, the *in vitro* conversion of carotene to vitamin A was reduced with hypothyroidism and also by the presence of nitrate *in vitro* (Reddy and Thomas, 1962). Since the thyroid is important in transforming carotene to vitamin A, anything that alters thyroid activity should also affect vitamin A status. Though many studies have been undertaken with the assumption that nitrate affects thyroid function, that premise has not been proven.

The last proposed mechanism of vitamin A impairment involves hydroxylamine. Hydroxylamine has been shown to be an intermediate product in the reduction of nitrate to ammonia *in vitro* (Lindsey and Rhines, 1932). It has been proposed that hydroxylamine is also formed in the ruminant and that it is hydroxylamine which adversely affects vitamin A status

(Tillman et al., 1965a). Though administration of hydroxylamine did decrease plasma vitamin A levels in sheep, there was no effect on liver vitamin A levels (Tillman et al., 1965a). The authors of that article suggest that not enough time had elapsed during the experiment to allow changes in liver vitamin A to become apparent. However, since only very low levels of serum hydroxylamine have been found in cattle receiving nitrate (Winter, 1962), it is unlikely that this mode of action is biologically important.

Regardless of the hypothesized mode of nitrate action, a number of studies have measured serum carotene, serum vitamin A, liver carotene, and liver vitamin A in relation to various levels of nitrate or nitrite. Although there are significant relationships between serum carotene, serum vitamin A, liver carotene, liver vitamin A, these items are not directly correlated in cattle (Diven et al., 1960).

Most studies in ruminants, found no significant effect due to dietary nitrate on either plasma carotene (Davison et al., 1965; Goodrich et al., 1964; Miller et al., 1965; Wallace et al., 1964; Weichenthal et al., 1963), plasma vitamin A (Davison et al., 1965; Miller et al., 1965; Smith et al., 1962; Wallace et al., 1964; Weichenthal et al., 1963), liver carotene (Wallace et al., 1964; Weichenthal et al., 1963), or liver vitamin A (Cline et al., 1963; Davison et al., 1965; Hale et al., 1961; Smith et al., 1962; Wallace et al., 1964; Weichenthal et al., 1963). Although a few studies found

nitrate to decrease plasma vitamin A (Hatfield et al., 1961) and liver vitamin A (Goodrich et al., 1962; Goodrich et al., 1964; Hatfield et al., 1961) and some studies suggest nitrate may inhibit the conversion of carotene to vitamin A (McIlwain and Schipper, 1963) or increase the depletion of liver vitamin A (Jordan et al., 1963). The weight of evidence supports the view that nitrate has relatively little effect on *in vivo* vitamin A status in ruminants.

Vitamin E:

In sheep receiving dietary nitrite, no evidence of vitamin E deficiency (as measured by hemolysis time) was noted (Holst et al., 1961). The addition of vitamin E to sheep receiving nitrate did not have any beneficial effect on vitamin A storage or the rate of gain (Cline et al., 1963). Apparently, nitrate has no effect on vitamin E in the ruminant.

Weight Gain and Feed Efficiency:

In most studies, no significant difference was seen in weight gain in cattle (Davison et al., 1963; Smith et al., 1962; Wallace et al., 1964) or sheep (Cline et al., 1963; Davison et al., 1965; Eppson et al., 1960) receiving nitrate in the ration. Either no difference in weight gain was seen in steers fed high-nitrate silage as compared to those fed low-nitrate corn silage (Jordan et al., 1961; Jordan et al.,

1963) or average daily weight gains were increased for the higher-nitrate silage group (Zimmerman et al., 1962). These results are consistent with the finding that nitrate can be utilized as a source of non-protein nitrogen in ruminants (Clark and Quin, 1951; Osweiler et al., 1976; Sapiro et al., 1949; Sokolowski et al., 1961; Tollett et al., 1961).

Nitrate in the ration at 1% or more can decrease feed intake in cattle (Hale et al., 1961, Hale et al., 1962; Jones et al., 1966; Weichenthal et al., 1963) while the same result occurs in sheep at 3% or greater (Carver and Pfander, 1974; Davison et al., 1965; Goodrich et al., 1964). The addition of vitamin A lessened the decrease in feed consumption seen with sheep (Goodrich et al., 1964) but not with cattle (Weichenthal et al., 1963). When the amount of feed was limited, adding 2.5% nitrate (34 grams/head daily) did not affect rate of weight gain, feed efficiency or carcass grade (Goodrich et al., 1964). This evidence strongly supports the premise that nitrate does not decrease feed efficiency. Rather, any decrease in weight gain is brought about by decreased feed consumption possibly related to poor palatability.

Reduction in daily weight gain in steers was seen when 1% nitrate was fed with a ration low in total digestible nutrients (low-concentrate). But when the steers were fed a ration high in total digestible nutrients (high-concentrate), an increase in weight gain was seen (Hale et al., 1961, Hale et al., 1962). These findings are consistent with evidence

that high nitrate levels reduce feed consumption and carbohydrates in a high-concentrate ration increase the utilization of nitrate as a source of non-protein nitrogen.

Reproduction:

Research in sheep and cattle has shown nitrate to have little effect on reproduction. No detrimental effect on the maintenance of pregnancy was observed in heifers fed nitrate at levels sufficient to maintain a daily peak methemoglobin level of up to 50% of total hemoglobin (Winter and Hokanson, 1964), nor were significant differences found in length of gestation, birth weight of calves, average length of estrous cycle or the number of services per conception (Davison et al., 1963). In sheep, no adverse effects were seen from nitrate ingestion on the pregnancy (Eppson et al., 1960), the birth weights of the lambs born (Setchell and Williams, 1962; Sinclair and Jones, 1964 ; Davison et al., 1965), or growth of the lambs (Eppson et al., 1960).

The only effect seen from nitrate administration relates to progesterone levels. While serum progesterone concentrations were not statistically different in early pregnancy of mid-pregnancy cows fed the same high and low nitrate rations (Page et al., 1990), serum progesterone concentrations were decreased in open, luteal phase cows fed a high nitrate ration (1,600 ppm nitrate) compared to open,

luteal phase cows fed a low nitrate ration (<400 ppm nitrate). The mechanism of action is unknown.

Milk Production:

Since Case first reported lower milk production in dairy cows receiving high nitrate rations, several studies have explored this area (Case, 1957a). A study utilizing only two cows also reported declines in milk production (Muhrer et al., 1956) as did an epidemiological study involving nitrate in the drinking water (Olson et al., 1972). However, the small sample size in the first study and the uncertainty that confounding factors (such as feed, housing, and management factors) were controlled in the second study make these results uninterpretable. Most studies have found no significant difference in the rate of milk production of cows receiving high levels of nitrate compared to cows receiving low levels of nitrate (Davison et al., 1963; Jones et al., 1966; Kahler et al., 1975; Morris et al., 1958; Murdock and Hodgson, 1972; Page et al., 1990; Sebaugh et al., 1970). A decrease in milk production was reported in cattle given nitrate at levels that produced 24 to 35.5% methemoglobinemia (Stewart and Merilan, 1958). High producing cows and those cows not previously treated with nitrate, were the most affected by nitrate administration. Clearly, nitrate has no effect on milk production unless given at doses sufficient to cause significant methemoglobinemia.

N-Nitroso Compounds In Ruminants

Outbreaks of toxic hepatitis occurred in Norway in 1961 and 1962 affecting a substantial number of cattle and sheep (Hansen, 1964; Koppang, 1964). Ruminants of all ages and both sexes were stricken although pregnant ewes and dairy cows were most susceptible. Initially inappetence, decreased milk production, decreased rumenal movements, and lethargy were seen. As the disease progressed, apathy, anorexia, abdominal pain, and ataxia of the hind legs occurred. The animals became unresponsive to stimuli and exhibited an abnormal posture of the head and extremities. A peculiar characteristic odor was often perceptible from the breath, skin, and milk of these cattle. Morbidity and mortality varied considerable from herd to herd. The most striking post mortem findings were grossly enlarged livers with variations in coloring such as light yellowish-brown areas alternating with darker, hyperemic areas. The causative agent was a toxic substance contained in nitrite preserved herring meal fed to these animals as a protein supplement (Koppang, 1964). It was determined that sodium nitrite used to preserve the herring had reacted with amines present in stale fish to produce highly toxic dimethylnitrosamine (Sakshaug et al., 1965).

Nitrosamines (N-nitroso compounds) have been found in certain plants and fish meals used as animal feeds (Juszkiewicz and Kowalski, 1976) and it has been demonstrated experimentally that ingested volatile nitrosamines can pass

into the milk of goats (Juszkiewicz and Kowalski, 1974). The possibility that toxic N-nitrosamines may pass into the milk of cattle is suggested by the unusual odor associated with the milk of cattle poisoned with dimethylnitrosamine (Hansen, 1964). N-nitrosodimethylamine has been reported in milk from cows which the authors attribute to nitrate ingestion (Rubenchik et al., 1988). The importance of these findings to humans remains to be determined.

NITRATE, NITRITE, AND N-NITROSAMINES IN MONOGASTRICS

Acute Nitrate Toxicity:

Acute nitrate intoxication has been experimentally induced in pigs by the administration of large doses of potassium nitrate. On necropsy, the pigs which died within 24 hours of dosing with nitrate had a severe gastritis with blood. The submucosa of the stomach was swollen and edematous. No changes were seen in blood color, heart, mesenteric lymph nodes, spleen, kidneys, bladder, respiratory tract, and large or small intestines. Necropsy lesions in a pig which received multiple sublethal doses of sodium nitrate consisted of hyperemic liver, kidneys, and stomach, and edematous stomach walls (Wanntorp and Swahn, 1953). A pig that lived 9 days after nitrate administration exhibited similar pathology (Gwatkin and Plummer, 1946).

Histopathologic examination revealed extensive liver and kidney lesions. The severe gastritis noted in the acute cases of nitrate toxicosis resembles that seen with salt poisoning (Ellis, 1942; Blood et al., 1983).

There are only a few cases of acute nitrate toxicity reported in swine subsequent to consumption of high-nitrate feed (Case, 1957c; Smith et al., 1959) or water (Bjornson et al., 1961). Smith et al., (1959) noted illness and death in pigs which had consumed wet oat and wheat straw. Clinical signs included labored breathing, inappetence, and increased water consumption. One animal had a convulsive seizure prior to death. Findings at postmortem examination were mild congestion of the lungs and acute gastritis. Samples of the wet straw were positive for both nitrate and nitrite, though the specific levels were not mentioned. It is not known if the nitrate, nitrite, or both compounds were the causal agent, however the necropsy lesions more closely resembled those seen with nitrate intoxication as described by Gwatkin and Plummer (1946). Serum nitrate levels increased in pigs as the amount of nitrate in the ration increased. In pigs fed 2% potassium nitrate in the ration, serum nitrate was 16 mg 100 ml compared to control pigs at 1-3 mg 100 ml (Garner et al., 1958).

Acute nitrate toxicosis has been reported in a dog receiving large daily doses of potassium nitrate from the owner (Whitehead, 1953). The dog exhibited behavioral changes, vomiting, diarrhea, polyuria, polydipsia, depression,

inappetence, and dehydration. It recovered completely following cessation of nitrate administration. A rabbit given 5 g. of potassium nitrate orally developed a rapid, weak pulse, muscular trembling and died within one hour. The necropsy revealed congestion of the lungs, the cardiac portion of the stomach and a portion of the small intestines (Mayo, 1895).

Water containing nitrate has been reported to cause toxicity in chinchillas (Bjornson, 1964) and turkeys (Adams et al., 1969). Clinical signs of nitrate toxicity in turkeys include slow growth, excessive salivation, incoordination, and anorexia. Nitrate at levels of 3,990 ppm and above caused increased mortality. Necropsy lesions were suggestive of salt toxicity (Adams et al., 1969).

Experiments using rats have shown that the LD₅₀ to be 5 g of sodium nitrate per kg of body weight (Wanntorp and Swahn, 1953). Clinical signs appeared soon after administration of sodium nitrate by intragastric tube. Initially, listlessness was noted, followed by apathy. In 2-3 hours, coma developed, lasting until death occurred at 6-24 hours after administration. Experiments with Japanese quail (*Coturnix coturnix japonica*) show mortality is significantly increased when the birds are given drinking water containing nitrate at 3960 ppm or more (Adams, 1974).

Since pigs, dogs, rabbits, chinchillas, turkeys, quail, rats, and man (Phillips, 1968b; Carlson and Shapiro, 1970;

Christian, 1928; Eusterman and Keith, 1929) can all develop acute nitrate toxicosis, it seems likely that most if not all mammalian species are susceptible to nitrate given a sufficiently large dose.

Methemoglobinemia:

Methemoglobinemia has been induced in horses by dosing with 50 g of nitrate per 100 lb of body weight. Clinical signs appeared 11 hours after administration in one animal and 21 hours in the other. The animals became weak, ataxic, restless, and perspired profusely. The administration of nitrate at 100 grams/cwt. resulted in death with 70% of the hemoglobin converted to methemoglobin (Bradley et al., 1940a).

Experimentally, sodium nitrate has caused methemoglobinemia in rabbits, with maximum methemoglobin readings seen between 4 and 7 hours after ingestion (Kilgore et al., 1959). Furthermore, methemoglobin levels increased as the level of nitrate in the diet increased whether the source of nitrate was naturally contained in the feed or added to the diet as sodium nitrate (Kilgore et al., 1959). The results of balance studies show that some conversion of nitrate to nitrite does occur *in vivo* in the rabbit (Kilgore et al., 1959).

Cyanosis was seen in a rat (2.49 g methemoglobin per 100 ml of blood) which had consumed nitrate at a rate of 10 g/kg of body weight (Kilgore et al., 1959). This suggests that in

some monogastrics given large doses of nitrate, some conversion to nitrite may occur.

Toxicity in turkeys grazing on plants high in nitrate has been reported (Riggs, 1945). Though the turkeys had been grazing this field for some time without problems, toxicity was seen after a rain. The turkeys developed anorexia, cyanosis, and diarrhea. Pathologic changes included dark blood, dehydration, mucoid enteritis, hyperemia of the intestinal mucosa, and petechial hemorrhages in the epicardium. Experimentally, the author was able to reproduce the syndrome in a turkey with potassium nitrite but not with sodium nitrate. In this case, the weight of evidence points to the reduction of nitrate to nitrite in the plants prior to consumption by the turkeys.

In order for methemoglobin to result from nitrate ingestion, the reduction of nitrate to nitrite must occur *in vivo*. Microbial reduction is known to occur in human infants and ruminant animals where conditions suitable for nitrate reducing bacteria exist. That nitrate ingestion can result in methemoglobinemia in the horse and rabbit suggests the enlarged cecum of these species may provide a suitable environment for nitrate reduction.

Studies in other monogastrics indicate that exposure to nitrite but only rarely nitrate, can result in methemoglobinemia. One researcher observed methemoglobinemia in dogs after oral administration of high levels of sodium

nitrate (Hiatt, 1940). In a later experiment, this researcher was unable to experimentally induce methemoglobinemia in dogs with nitrate even after nitrate reducing bacteria were introduced into the gut (Greene and Hiatt, 1954). Other studies on nitrate metabolism provide no evidence that nitrate is reduced to nitrite in the dog, nor that methemoglobinemia may be a sequela to nitrate administration (Greene and Hiatt, 1954; Greene and Hiatt, 1955; Keith et al., 1930). These studies indicate that for nitrate to induce methemoglobinemia in the dog and probably most other monogastrics, nitrate must be converted to nitrite prior to ingestion.

There are several reports of methemoglobinemia and elevated methemoglobin levels in pigs due to nitrite (London et al., 1967; Sleight et al., 1972; Wood et al., 1967; Emerick et al., 1965) in the literature. Unusual cases include poisoning from the consumption of whey containing nitrite (Wanntorp and Swahn, 1953) and a case where nitrate contained in well water was converted to nitrite during the cooking of soup (Winks et al., 1950). Elevated methemoglobin levels have been reported in pigs given sodium nitrite in the ration at a rate of 0.5% (Koch et al., 1963). One study reported the methemoglobin levels in pigs increased as the amount of nitrate increased in drinking water (Anderson and Stothers, 1978) while another study found no significant effect (Woods et al., 1967). The difference in the study results may be due to the high levels of sulfate in the water in one study which

by itself also seemed to increase the methemoglobin levels. Experimentally, pigs given up to 100 ppm nitrite in the drinking water had small but significant increases in methemoglobin levels compared to control groups (Seerley et al., 1965).

Oral administration of sodium nitrite to pigs resulted in methemoglobinemia with clinical signs appearing 10-15 minutes after intake. Fasted pigs reached maximum methemoglobin levels at 1.5 to 2 hours after oral dosing, with normal methemoglobin levels seen in 8-10 hours (Wanntorp and Swahn, 1953). Sows given subcutaneous injections of sodium nitrite developed maximum methemoglobin levels approximately 2 hours after administration (Sleight et al., 1972). The severity of clinical signs varied directly with the dose of nitrite as did the extent of methemoglobinemia (London et al., 1967; Sleight et al., 1972).

Clinical signs are apparent when 20% of the total hemoglobin is present as methemoglobin, and consist of restlessness, frequent urination, and detectable dyspnea (London et al., 1967). As methemoglobinemia worsens, ataxia, weakness, dyspnea, and cyanosis develop (Wanntorp and Swahn, 1953; Winks et al., 1950; London et al., 1967; Sleight et al., 1972). Finally, the most severely affected animals are unable to rise, vomiting occurs along with severe dyspnea and cyanosis. Death results when more than 75% of the total hemoglobin is present as methemoglobin and is often preceded

by severe convulsions. No lesions are present on necropsy though the blood is the characteristic dark brown (chocolate) color characteristic of methemoglobinemia.

Sodium nitrite given intravenously to dogs at a rate of 30 mg/kg of body caused methemoglobinemia (Wendel, 1939). Methemoglobin reached maximum concentrations of 60 to 70 mg 100 ml in 60 to 100 minutes. Reconversion to hemoglobin occurred at a rate of 0.03 mg 100 ml per minute with no methemoglobin present after 8 to 9 hours. Another study reported nitrite levels peaked in the blood, saliva, and bile of dogs in approximately 30 minutes following intravenous administration of sodium nitrite (Fritsch et al., 1985). The time differences between the studies may be due to the different doses of sodium nitrite administered, or there may be a lag period between nitrite entering the blood and the development of methemoglobin.

Experimentally, the methemoglobin level of rats given sodium nitrite peaked at 1-1.5 hours after oral administration (Wanntorp and Swahn, 1953). Methemoglobin levels are reduced by 50% every 90 minutes (Shuval and Gruener, 1972) until no methemoglobin remains after 4-6 hours (Wanntorp and Swahn, 1953). Increases in methemoglobin accompany increases in the level of nitrate in the diet of rats whether the source of nitrate is naturally contained in the feed or added to the diet as sodium nitrate (Kilgore et al., 1959).

Chicks fed a ration containing 0.4% nitrite had greater mortality than controls. Chicks receiving nitrite exhibited anorexia, mild ataxia, dyspnea, paleness, and tired quickly when excited (Sell and Roberts, 1963). Similar clinical signs along with poor growth, muscle tremors and frothing around the mouth were reported for nitrate and nitrite toxicity in turkeys and chickens (Marrett and Sunde, 1968). With nitrite at 400 ppm in the ration, turkeys exhibit reduced growth, a lack of balance, rapid breathing, frothing at the mouth, dehydration and high mortality (Sunde, 1964).

Factors Influencing Methemoglobinemia:

The presence of food in the gastrointestinal tract apparently has a protective effect towards nitrite toxicity. Fatal methemoglobinemia was seen in pigs (20 to 45 lbs) given 5 grams of potassium nitrite when fasted, but when given the same dosage on a full stomach, mild methemoglobinemia developed (Gwatkin and Plummer, 1946). It is likely the food slowed the rate of absorption of nitrite to a level the pigs could tolerate. Pigs given sodium nitrite intravenously developed less methemoglobinemia when treated at 1 week of age than when treated at 3 months or 5 1/2 months of age (Emerick et al., 1965). This may be related to the slower rate of *in vitro* methemoglobin reduction seen for older pigs compared to those 1 week of age (Emerick et al., 1965).

One study found that ascorbic acid deficient guinea pigs developed significantly higher levels of methemoglobin from nitrite administration than ascorbic acid supplemented controls (Kociba and Sleight, 1970). However, another study reported ascorbic acid had no effect on the susceptibility of guinea pigs to nitrite toxicity (Kilgore et al., 1964). Large interanimal variation in response to nitrite is often seen. It is likely that the small sample size (4 guinea pigs) in the latter study contributed to the study's failure to detect a difference in nitrite response due to ascorbic acid administration. Diets containing 1% ascorbic acid slightly reduced nitrite-induced methemoglobin blood levels in guinea pigs compared to diets with 0.02% ascorbic acid. The finding that ascorbic acid protects against methemoglobinemia agrees with human studies (Armstrong et al., 1958; Bodansky, 1951; Deeny et al., 1943; Shuval and Gruener, 1972; Super et al., 1981) and what is known about the mode of action of ascorbic acid (Bodansky and Gutmann, 1947; Bodansky, 1951).

The addition of 1% methionine to the guinea pig ration in conjunction with 1% or 2% ascorbic acid resulted in a 50% reduction in methemoglobin levels (Stoewsand et al., 1973). High nitrate-containing beets or high-nitrate diets enhanced nitrite induced methemoglobinemia in guinea pigs (Stoewsand et al., 1973). The mechanism of action may be related to the ability of guinea pigs to reduce nitrate to nitrite in the oral cavity (Woolley and Sigel, 1982). The sulfhydryl

compounds glutathione and ergothioneine are protective against nitrite induced methemoglobinemia in rats though the mode of action is unknown (Mortensen, 1953).

Experiments showed that pregnant rats had higher methemoglobin levels than non-pregnant rats receiving the same doses of nitrite (Gruener and Shuval, 1973; Shuval and Gruener, 1972). This finding agrees with previous work showing the increased sensitivity of pregnant rats to nitrite (Metcalf, 1962). Starvation was found to increase the sensitivity of rats to nitrite while riboflavine increased the resistance of hemoglobin to oxidation with nitrites (Metcalf, 1939).

Young turkeys were more sensitive to the effects of nitrate and nitrite than young chickens (Sunde, 1964). The addition of vitamin A to the ration mitigated the toxicity of nitrite in studies involving chickens and turkeys (Marrett and Sunde, 1968; Sell and Roberts, 1963; Sunde, 1964). These studies point to interference with vitamin A utilization as a mode of action for nitrites in chickens and turkeys.

Abortion and Placental Transfer:

Case (1957c) first reported abortion 2 weeks prior to full term along with stillbirths and deformities in pigs which had been pastured on oats and rape containing high levels of nitrate. Testing showed a low level of plasma vitamin A and excessive nitrate in the pigs. Since then, several

researchers have explored the effect of nitrate and nitrite on the fetus.

Elevations in serum nitrate were found in dead pigs born to sows fed potassium nitrate compared to control pigs, with the increases in serum nitrate corresponding to the levels of nitrate fed in the dams' ration (Garner et al., 1958). Though litter size was unchanged, liveability and the number of strong pigs appeared to decrease with higher levels of nitrate (Garner et al., 1958). These results suggest nitrate ingestion by sows may have some affect on their fetuses.

In a group of sows given sodium nitrite at high doses, only 1 of the 8 sows which survived the nitrite treatment, had fetal deaths associated with the toxic episode (Sleight et al., 1972). This sow was given nitrite for 2 consecutive days and farrowed 4 days after the second dose. At farrowing, 2 of the 9 pigs appeared to have died during the period of nitrite toxicosis. This study found a narrow range between the dose that killed sows and the dose that allowed sows to survive, suggesting that toxicity episodes severe enough to cause death of the fetuses without causing maternal death would be rare.

No methemoglobin was found in the fetuses of sows which died within 2 hours of nitrite administration (Sleight et al., 1972). However, oxygen saturation of fetal blood decreased significantly ($P < 0.001$) after nitrite was administered to the dam (Sleight et al., 1972). The oxygen saturation of fetal blood declined as time elapsed between administration of

nitrite to the dam and blood collection from the fetus, though the correlation was not linear. These findings suggest that fetal death may be due to hypoxia in the fetuses as a result of maternal methemoglobinemia. Sleight et al. suggest that little of the nitrite crossed the placental barrier because the methemoglobin levels of the fetuses did not appear to be affected by nitrite administration. However, the study did not measure nitrite levels in fetal blood. It is also possible that not enough time had elapsed between dosing the sow and testing the fetuses to allow for a full transfer of nitrite and methemoglobinemia to occur.

Abortions as a sequelae to nitrite toxicosis, occurred in a high percentage of ascorbic acid deficient guinea pigs while no abortions occurred in the control group (Kociba and Sleight, 1970). However, fetal methemoglobin levels were similar in both ascorbic acid deficient and control guinea pigs following nitrite administration (Kociba and Sleight, 1970). This result suggests that the nitrite-induced abortions are not related to methemoglobinemia in the fetuses.

In a series of experiments with rats, Gruener et al. (1973), demonstrated the transfer of nitrite through the placenta and production of methemoglobinemia in the fetuses. The transplacental transfer effect was discovered to have a threshold dose of 2.5 mg/kg of sodium nitrite. Nitrite levels rose in fetal blood approximately 20 minutes after the dam's level rose and the increase in fetal nitrite was followed by

a rise in fetal methemoglobin. These studies indicate that nitrite can cross the placenta, at least in one species.

Rat fetuses were found to have approximately ten times more methemoglobin reductase than adult pregnant rats (Gruener et al., 1973). This suggests that rat fetuses are less sensitive to the oxidation properties of nitrites than adults, in contrast to humans where fetuses have less methemoglobin reductase than adults.

Maternal Milk:

Dogs given intravenous nitrate had increased milk nitrate but the levels remained at or below plasma levels (Green et al., 1982). In rats, although the dams showed high methemoglobin levels due to ingestion of nitrites in the drinking water (after giving birth), no rise in methemoglobin levels were seen in suckling rats (Shuval and Gruener, 1972). These findings suggest that the passage of nitrate and nitrite into maternal milk may be species specific.

Chronic Nitrate and Nitrite Exposure:

Tissue pathology:

In rats given massive doses of sodium nitrite for up to 18 weeks, degenerative vascular and parenchymatous lesions were found in the heart, lung, brain, kidney and testes (Hueper and Landsberg, 1940). Pathological examination of

tissues from rats receiving drinking water that contained up to 3000 mg of nitrite per liter for 24 months showed no changes in the pancreas, adrenal, or brain though necropsy did reveal lesions in the lungs and heart, especially with higher doses of nitrite (Shuval and Gruener, 1972). After 24 months of study, most control rats had some degree of thickening of the intramural coronary arteries, many with marked hypertrophy and narrowing. However, the rats receiving water containing nitrate (especially at 3000 mg/l) had coronary arteries that were thin and dilated (Shuval and Gruener, 1972). No explanation is known for these changes.

Elevated serum urea, creatinine, and glutamic-pyruvic transaminase concentrations provide evidence of kidney and liver damage in chickens fed nitrate and nitrite in the ration (Atef et al., 1991). Lower bursa of Fabricius weights, decreased hemagglutination response, and reduced delayed hypersensitivity reaction in chickens fed nitrate and nitrite (Atef et al., 1991) suggests interference with the immune system, although further work is required to confirm this inference.

Central Nervous System:

Motor activity was significantly decreased in rats given nitrites in the drinking water at doses which produced up to 15% methemoglobinemia (Shuval and Gruener, 1972). Vitamin C given concurrently with drinking water containing 2000 mg/l of

nitrites prevented methemoglobinemia, but motor activity remained decreased (Shuval and Gruener, 1972). Three-month-old rats exposed to nitrite in the drinking water for 2 months had irreversible changes in brain electrical activity even at levels of 100 mg/l (Shuval and Gruener, 1972). It appears nitrite exerts an effect on the central nervous system other than that due to methemoglobinemia.

Hematological Effects:

Sows given 2% nitrate in the ration had serum nitrate levels up to 16 mg 100 ml compared to 1-3 mg 100 ml for controls (Garner et al., 1958). These figures indicate that some of the absorbed nitrate circulates in the bloodstream. No difference in hemoglobin or methemoglobin levels were seen in pigs given drinking water that contained nitrate at levels up to 500 ppm nitrate for 79 days (Bouwkamp and Counotte, 1988). However, when pigs were given sodium nitrite at levels sufficient to cause a prolonged increase in methemoglobin, hematologic changes included increased hemoglobin, packed cell volume, and lymphocytosis (London et al., 1967). Nitrate administered in the drinking water of turkey poults at levels of 675 ppm (Arends et al., 1967) or up to 1,485 ppm (Adams et al., 1969) for 24 weeks had no effect on blood hematocrit values. However, sodium nitrate (4.2 g/kg of diet) and sodium nitrite (1.7 g/kg of diet) added to the ration for 4 weeks

resulted in increased methemoglobin levels from 1.1% of total pigment to 8% and 25.6%, respectively (Atef et al., 1991).

Pregnant rats given 2,000 mg/l of sodium nitrite in the drinking water for an extended period developed anemia while non-pregnant rats exposed to the same treatment did not (Gruener et al., 1973). In chickens fed nitrate and nitrite, erythrocyte counts increased initially (at 7 days into the trial), but then decreased in comparison to control birds (Atef et al., 1991). It appears compensatory increases in hematocrit are seen at low nitrite levels but with prolonged or high-dose administration, anemia results. The cause of the anemia is unknown.

Immunosuppression:

Rats given 400 ppm sodium nitrite or 400 ppm sodium nitrate in the drinking water for 6 months developed much lower antibody titers than control rats when injected with Newcastle virus (224). This suggests nitrate and nitrite exert an immunosuppressive effect in rats.

Thyroid Gland:

The goitrogenic action of nitrate and possibly nitrite was originally discovered in rats by Wyngaarden et al. (1952). After seventeen days of nitrate treatment, the thyroid glands showed minimal hypertrophy, hyperplasia and a slight reduction in iodine concentrations. Since then a number of researchers

have repeated these findings in rats. Dietary nitrate was found to significantly increase the weight (Bloomfield et al., 1961b; Welsch et al., 1961) and cause hyperplasia and hypertrophy (Welsch et al., 1962) of thyroid glands in rats. The mode of action of nitrate on the rat thyroid is through inhibition of both the collection (Bloomfield et al., 1961b; Wyngaarden et al., 1952) and retention of the iodide ion in the thyroid gland (Wyngaarden et al., 1952).

A goitrogenic effect due to nitrite has also been seen in chickens. Nitrite in the feed of chicks at a rate of 0.4% increased thyroid gland weights. Massive doses of vitamin A partially overcame the thyroid hypertrophy caused by nitrite (Sell and Roberts, 1963). No effect was seen from the administration of 675 ppm nitrate in the drinking water of turkeys on thyroid activity or the size of the thyroid, spleen, or adrenal gland (Arends et al., 1967). It is not known if the differences between the results seen with turkeys and chickens reflect species differences or differences in study protocol.

Dogs given sodium nitrate at levels up to 1,000 ppm in drinking water for 1 year showed no evidence of thyroid dysfunction (Kelley et al., 1974). The finding that nitrate ingestion interferes with rat and chicken thyroid function but has no effect on dogs indicates substantial species differences in the effect of nitrate on this organ.

Vitamin A:

Several researchers have explored the effect of nitrate and nitrite on vitamin A, with conflicting results. In pigs, one study found no difference due to nitrate in the vitamin A levels of the liver (Anderson and Stothers, 1978). While several authors found 0.3% nitrate decreased liver vitamin A levels even when beta-carotene 3,520 IU/kg or vitamin A 1,172 IU/kg were provided (Garrison et al., 1966; Wood et al., 1967). Conflicting findings were also seen with nitrite in pigs as one study noted nitrite at levels up to 100 ppm in the drinking water for 105 days had no effect on liver vitamin A values (Seerley et al., 1965). Other researchers reported liver vitamin A levels decreased with 0.04% nitrite even though beta-carotene 3,520 IU/kg or vitamin A 1,172 IU/kg were provided (Garrison et al., 1966; Wood et al., 1967). The only study to measure serum vitamin A levels in pigs found that in general serum vitamin A varied inversely with the level of nitrite (London et al., 1967). With such disparity in results, no conclusions can be drawn as to the effect of nitrate or nitrite on vitamin A metabolism in the pig.

Nitrate was found to decrease vitamin A stores in the liver (Yadav et al., 1962) and to be associated with more rapid depletion of vitamin A stores in the liver of rats (Garner et al., 1958). In rats with adequate vitamin A stores fed a high-nitrate silage diet, liver stores of vitamin A were maintained by the addition of vitamin A but declined when carotene was given (Smith et al., 1961). One study found

liver storage of vitamin A was not effected in rats fed nitrate when vitamin A was administered regardless of whether vitamin A was given orally as an oil solution, orally as a water solution, or injected in an oil solution (Emerick and Olson, 1962). However, nitrate did significantly reduce liver storage of vitamin A with intragastric administration of carotene (Emerick and Olson, 1962). These results indicate nitrate acts to either destroy carotene, inhibit the conversion of carotene to vitamin A, or to prevent the absorption of vitamin A in the rat. It appears that vitamin A supplementation may overcome this effect of nitrate. A possible mechanism of action may be through the goitrogenic effect nitrate has on the thyroid in rats. The thyroid is known to be important in the conversion of carotene to vitamin A (Johnson and Baumann, 1947) and rats given adequate iodine stored more vitamin A than rats on a thyroid deficient diet (Yadav et al., 1962). Nitrite administered to vitamin A deficient rats caused a more rapid depletion of liver vitamin A (O'Dell et al., 1960). Also, nitrite given to vitamin A depleted rats reduced the liver storage of vitamin A whether given orally in an oil-based solution, orally as a water based solution, or with intragastric administration of carotene (Emerick and Olson, 1962). However, when an oil-based vitamin A solution was injected in vitamin A deficient rats receiving nitrite, storage was increased. The reason for this increase in storage is unexplained. Apparently, nitrite interferes

with liver storage of vitamin A either through inhibited storage, accelerated depletion, or both mechanisms.

The addition of nitrite at 200 ppm to the drinking water, reduced vitamin A levels in the liver of chickens and both the vitamin A and beta-carotene levels in the liver of turkeys (Adams et al., 1966). Chickens receiving 0.4% nitrite in the feed had significantly reduced liver vitamin A levels compared to control chickens except when high levels of vitamin A were injected. Adding vitamin A or carotene to the ration did not increase liver stores to the same extent (Sell and Roberts, 1963). This finding suggests that nitrite acts to decrease to absorption of vitamin A and carotene, to increase the destruction of carotene and vitamin A, or both. Chickens receiving 0.74% potassium nitrite had lower concentrations of vitamin A than control birds in both the ventriculus and intestinal ingesta suggesting that nitrite acts to destroy vitamin A in these organs (Roberts and Sell, 1963).

Vitamin E:

Relatively little research has been done on the effect of nitrite on vitamin E. Vitamin E levels varied inversely with the level of nitrite administered to pigs though signs of vitamin E deficiency did not develop (London et al., 1967). The addition of 0.3% nitrite to a ration considered to be adequate in vitamin E, precipitated a vitamin E deficiency in rats on that diet (O'Dell et al., 1960). Research thus far

indicates nitrite has a detrimental effect on vitamin E though the mode of action remains unknown.

Reproduction:

No teratogenic effects were noted in pig fetuses 10 days after treatment, when sows were given doses of sodium nitrite at 30 mg/kg of body weight on days 15, 31, 32 and 33 of gestation (Sleight et al., 1972). A review of other animal studies found no evidence for teratogenic effects attributable to nitrate or nitrite (Fan et al., 1987). Although human studies have attempted to link nitrate consumption to congenital malformations (Scragg et al., 1982; Dorsch et al., 1984), the weight of evidence does not support this conclusion.

In pigs, no effect on corpora lutea numbers (Tollett et al., 1960) or percent implantation (Tollett et al., 1960) was seen from dietary nitrate. Litter size was found to be unaffected by the addition of up to 2% nitrate in the ration of pigs though liveability and number of strong pigs appeared to decrease at higher nitrate levels (Garner et al., 1958). Birth weight, litter size at weaning, and daily weight gain in pigs whose dams were consuming drinking water containing nitrate at 300 ppm were found to be unaffected (Seerley et al., 1965). There is very little evidence that nitrate exerts a detrimental effect on reproductive performance in pigs.

Administration of nitrate in the drinking water of rabbits at concentrations of up to 500 ppm for 2 successive gestations had no detrimental effects on conception, litter size, or the weights of the offspring at birth or weaning (Kammerer and Siliart, 1993). Nor were the plasma levels of estradiol or progesterone affected.

Reproduction in guinea pigs was not affected by potassium nitrate in the drinking water at levels up to 10,000 ppm (Sleight and Atallah, 1968). But at 30,000 ppm, reproductive performance was 8% of the untreated control group (Sleight and Atallah, 1968). Nitrite caused massive fetal death at much lower dosages than nitrate. Fetal losses in pregnant guinea pigs given drinking water containing nitrite at 5,000 or 10,000 ppm were 100% (Sleight and Atallah, 1968). Nitrite administered at a rate of 60 mg/kg subcutaneously to guinea pigs during the last 15 days of pregnancy caused fetal death approximately 1 hour after administration (Sinha and Sleight, 1971). Nitrite was found in the fetuses, though at lower levels than the dams, indicating at least partial passage of the nitrite ion across the placenta (Sinha and Sleight, 1971). A relatively narrow range was found in the doses of nitrite which either had no effect on reproduction, caused fetal death, or caused maternal death (Sinha and Sleight, 1971). Methemoglobin concentrations were significantly lower in fetuses than guinea pig sows, and no changes occurred in the placenta prior to fetal death (Sinha and Sleight, 1971).

These data suggest that fetal death occurred due to hypoxia subsequent to maternal methemoglobinemia. Conception appeared unaffected by nitrate or nitrite.

Drinking water with nitrite levels of 2,000 mg/l and 3,000 mg/l given to pregnant rats had a pronounced effect on offspring mortality, particularly in the 3-week period prior to weaning (Shuval and Gruener, 1972). Though birth weights were unaffected, growth rates were slower in the groups receiving high-nitrite water. Dullness and thinning of the fur was observed in pups whose groups received high-nitrite water (Shuval and Gruener, 1972). The mechanism of action is unknown.

In chickens, no consistent relationship was seen in the rate of egg production when consuming nitrate at levels up to 300 ppm or nitrite at levels up to 200 ppm nor were egg weight or shell thickness affected (Adams et al., 1966). Chickens consuming drinking water with nitrate at 300 ppm exhibited a lowered peak of early egg production though later egg production (during lower environmental temperatures) approached that of control groups (Bentley et al., 1965). These results may indicate that more water was consumed during the warmer periods resulting in higher nitrate dosages in the chickens, or that early production is more sensitive to the effect of nitrates than later production, or that the effect of nitrite is greater during periods of warmer environmental temperatures.

Diarrhea:

Laying hens consuming 150 or 300 ppm nitrate in the drinking water had fluid feces for the first three weeks of the trial, after which the hens apparently adjusted and no further problems were noted (Adams et al., 1966).

Growth:

Feed efficiency in swine was not adversely affected by nitrate (Koch et al., 1963; Seerley et al., 1965) or nitrite (Koch et al., 1963). One study found slightly better feed efficiency with high nitrite administration which the investigators attribute to decreased activity levels because of dyspnea caused by nitrite-induced methemoglobinemia (London et al., 1967).

No significant differences in average daily weight gain (Anderson and Stothers, 1978; Seerley et al., 1965; Wood et al., 1967), feed consumption (Anderson and Stothers, 1978), or feed-to-gain ratio (Anderson and Stothers, 1978) were seen in pigs consuming up to 300 ppm nitrate in the drinking water. Nitrate at levels of 200 ppm in weaned pigs and 500 ppm in finishing pigs had no effect on rate of weight gain, feed efficiency, or feed consumption (Bouwkamp and Counotte, 1988). Tollett et al. (1960) reported significantly depressed weight gains in pigs fed over 1.84% nitrate, though the amount of feed consumed was not monitored. Studies with ruminant animals show decreased weight gains to be strongly associated

with decreased feed consumption. The evidence points to nitrate having no effect on feed efficiency or weight gain, except perhaps in regards to decreased feed consumption.

Giving guinea pigs drinking water that contained nitrate at levels of up to 10,000 ppm nitrate or nitrite at up to 5,000 ppm had no effect on weight gain, or the consumption of feed or water (Sleight and Atallah, 1968). Vitamin A depleted rats fed 3% nitrate or 0.5% nitrite weighed significantly less than controls at the end of the 6-day trials (Emerick and Olson, 1962). No significant differences were seen in growth or development of 3-month-old rats (non-vitamin A depleted) given up to 3000 mg/l of nitrite in the drinking water (Shuval and Gruener, 1972). This points to interference with vitamin A metabolism as the cause of decreased weight gain in rats.

A reduction in growth was seen in chicks given drinking water containing 200 ppm nitrite (Adams et al., 1966). Decreased weight gain was also noted in Balady chickens but because of this breed's foraging habits, feed consumption could not accurately be measured (Atef et al., 1991). Chicks consuming 0.4% sodium nitrite had significantly decreased feed consumption and rate of weight gain (Sell and Roberts, 1963). When daily feed consumption was equalized between chicks receiving nitrite and controls, no difference in weight gain was seen if chicks were supplemented with vitamin A. However, chicks on a nitrite-containing, vitamin-A-free ration gained far less than all other treatment groups while consuming, on

average the same amount of feed (Sell and Roberts, 1963). Clearly nitrate acts to decrease feed consumption in chickens. It appears that decreased feed efficiency when seen, is related to interference with vitamin A metabolism.

Nitrate administered at levels up to 1,485 ppm continuously in the drinking water of turkey poults from 1 day of age to 24 weeks had no significant effect on growth rate or feed conversion (Adams et al., 1969). One study reported water containing 675 ppm nitrate given to turkeys for the first 4 weeks of life, resulted in turkeys which were heavier than controls at 16 weeks of age (Kienholz et al., 1965). Subsequent work mentioned that males were more affected than females, and that testes weights were far smaller in the nitrate treated groups (Arends et al., 1967) thus suggesting that nitrate has a "caponizing" effect. Nitrate in the feed at 1000 ppm for the first 4 weeks did not promote growth at later ages (Arends et al., 1967). The finding that nitrate in the feed did not have a similar effect and the disagreement with the study done by Adams et al. (1969) casts doubt on the existence of the "caponizing" effect of nitrate. Feed consumption and growth decreased in turkeys as the amount of nitrite (50 to 200 ppm) in the drinking water increased (Adams et al., 1966). It is likely that the decrease in growth is related to a decrease in food consumption.

Weight gain and feed consumption in Japanese quail (*Coturnix coturnix japonica*) decrease with nitrate in the

drinking water at 1,320 ppm and 3,960 ppm but not at 2,640 ppm, suggesting that the decreased feed consumption and weight gain may have been due to some other factor rather than nitrate (Adams, 1974).

Sulfonamide Drug Metabolism:

Nitrites have been shown to affect the metabolism of sulfonamide drugs in animals. The deamination of sulfathiazole, sulfamethazine, and sulfadiazine in the rat (Nelson et al., 1987; Paulson, 1986; Paulson, 1987; Woolley and Sigel, 1982), sulfadiazine in the guinea pig and rat (Woolley and Sigel, 1982), and sulfamethazine in swine (Paulson and Feil, 1987; Struble and Paulson, 1988) is accelerated by high dietary nitrite.

Since nitrite is required for the deamination reaction, dietary nitrate must be reduced to nitrite *in vivo* (e.g. in the oral cavity or stomach) if nitrate is to affect sulfonamide drug metabolism. Studies have shown that rats and most calves do not reduce nitrate, while guinea pigs can readily convert nitrate to nitrite, and swine are intermediate in their ability (Paulson, 1987; Wooley and Sigel, 1982). The concentrations of nitrite in the oral cavity of swine increased when fed 500 and 1000 ppm nitrate though the amount of nitrate reduced was small (Paulson and Aschbacher, 1990). Bacterial reduction of nitrate is suspected as nitrate was converted to nitrite in regular pigs but was not converted in

germ-free pigs (Struble and Paulson, 1988). Likewise in guinea pigs, orally administered nitrate was reduced to nitrite but not when administered intramuscularly or intraperitoneally (Wooley and Sigel, 1982). These results are in agreement with human studies which show nitrate to be reduced to nitrite in the oral cavity by bacterial activity (Tannenbaum et al., 1974; Tannenbaum et al., 1976).

Experimental evidence in swine shows that at 1000 ppm nitrate in the ration the amount of desaminosulfamethazine increased from 1.1 ppm to 6.3 ppm (Paulson and Aschbacher, 1990). Because production of this metabolite of sulfamethazine is enhanced by nitrite, this finding indicates the deamination of sulfamethazine can occur in swine subsequent to the reduction of nitrate to nitrite *in vivo*. The effect of nitrite on sulfonamide drug metabolism has been thoroughly reviewed elsewhere (Paulson, 1987). In general, high levels of ingested nitrite act to increase excretion of drug-related compounds in the feces and decrease concentrations of the parent drug in the blood and other tissues (Paulson, 1987). Animals that receive high levels of nitrite or that reduce ingested nitrate to nitrite in the oral cavity or gastrointestinal tract, may require a different dose of the sulfonamide drug to provide the same level of prophylactic or therapeutic action (Paulson, 1987). Ascorbic acid counteracts the effect of nitrite ingestion on

sulfamethazine disposition in rats (Paulson, 1987) and may exert a similar effect in other animals and humans.

Intestinal Absorption:

In sedentary rats, orally administered potassium nitrate whether given acutely (one dose at 800 mg/kg of body weight) or chronically (100 mg/kg of body weight for 90 days), did not affect intestinal absorption nor alter the metabolic parameters of the small intestinal mucosa (Grudzinski and Szymanski, 1991b and 1991a). However when the rats were exercised, potassium nitrate caused reduced gastric mucosal absorption as measured by D-xylose absorption in both the acutely and chronically dosed rats (Grudzinski and Szymanski, 1991c and 1991d). In the chronically dosed rats, decreased alkaline phosphatase and Na^+/K^+ -ATPase activity, and atrophy of intestinal villi was seen with exercise (Grudzinski and Szymanski, 1991c).

Sodium nitrite given orally to rats at a dose of 80 mg/kg of body weight, increased gastric mucosal absorption as measured by D-xylose absorption and increased the uptake of oxygen in the small intestine (Grudzinski and Szymanski, 1991b). However with chronic administration of sodium nitrite at 5 and 10 mg/kg of body weight for 90 days, the absorption of D-xylose was decreased by the rat gastric mucosa while oxygen uptake was unchanged (Grudzinski and Szymanski, 1991a). Alkaline phosphatase and Na^+/K^+ -ATPase were unaffected by

sodium nitrite at a dose of 80 mg/kg of body weight but were significantly reduced with daily administration of doses of 10 mg/kg (Grudzinski and Szymanski, 1991a). Exercise exacerbated the effects of both acute and chronic nitrite administration (Grudzinski and Szymanski, 1991c and 1991d). Further study revealed that while nitrite reduces the activity of alkaline phosphatase and Na^+/K^+ -ATPase in intestinal mucosa, it does not increase Na^+/K^+ -ATPase inhibition produced by ouabain and inhibition of alkaline phosphatase produced by L-phenylalanine (Grudzinski and Szymanski, 1991e).

On histopathologic examination, the rats that received sodium nitrite at a dose of 80 mg/kg body weight had a reduction in the amount of mucus on the gastric surface, small erosions, slight swelling of the villi, and a decrease in lactate dehydrogenase activity in the superficial layer of glands (Grudzinski and Szymanski, 1991b). Chronic daily dosing with sodium nitrite at 10 mg/kg of body weight caused considerable swelling and atrophy of villi within 21 days (Grudzinski and Szymanski, 1991a). Further research is needed to explain the mechanism and importance of these findings.

N-Nitroso Compounds:

Outbreaks of toxic hepatitis occurred in Norway in 1961 and 1962 affecting a substantial number of cattle and sheep (Hansen, 1964; Koppang, 1964). The toxic substance was found

to have been contained in nitrite-preserved herring meal fed to the animals as a protein supplement (Koppang et al., 1964). It was determined that sodium nitrite used to preserve the herring had reacted with amines present in stale fish to produce highly toxic dimethylnitrosamine (Sakshug et al., 1965). As research progressed, toxic herring meal was found to be the cause of hepatosis seen in mink and foxes since 1957 (Koppang, 1966; Koppang and Helgebostad, 1966). Further research has shown mink to be exceptionally susceptible to the effects of dimethylnitrosamine (Carter et al., 1969). In fur animals, this disease is a slowly progressive disease with restlessness, fur biting and dry, dull, fur observed initially. As the disease advances, inappetence develops along with ascites, anemia, and thin tarry feces. The mortality rate can be quite high. On necropsy, the liver is markedly affected. Both the gross and histologic appearance of the liver varies with the acuteness of the disease (Koppang, 1966; Koppang and Helgebostad, 1964).

DISCUSSION

Methemoglobinemia in ruminant animals subsequent to consumption of high-nitrate forages will continue to be a problem especially with erratic rainfall patterns. Monitoring of forage nitrate levels and judicious use of fertilizers, herbicides, and irrigation are useful in limiting nitrate

accumulation in plant material. Methemoglobinemia in animals due to nitrate-contaminated water is rarely reported, though a human fatality was described in the United States as recently as 1986 (Johnson et al., 1987). In regard to nitrate toxicity in ruminants, there remain a number of unanswered questions. Many substances such as soybean meal, urea, antibiotics, and monensin affect nitrate metabolism. It would be beneficial to explore the nature and extent of these interactions in order to better understand and prevent toxicity episodes.

Large doses of nitrate are toxic to monogastric animals without the occurrence of methemoglobinemia. Methemoglobinemia has been reported in monogastrics from the consumption of high-nitrate forage under conditions suitable for nitrate reduction to nitrite in the forage prior to consumption.

Ingested nitrites or nitrates in species with nitrate reductase activity, may be of regulatory interest as deaminated sulfonamide drugs in swine and calves have much longer half-lives but are not detected by the Bratton Marshall test (commonly used to measure sulfonamide drug residues in animal tissues) (Paulson, 1986). In this case, sulfonamide drug residues would be increased in food animals while the efficacy of the analysis for the total tissue residue burden was decreased.

A variety of other effects have been attributed to nitrate. A goitrogenic effect on thyroid function by nitrate has been demonstrated to occur in some species but not in others. This aspect of nitrate may be a species specific response, though the data remain incomplete. Little work has been done on pigs, an animal that may serve as an appropriate model for the response of nitrate in humans. Hematological changes noted in cattle, sheep, pigs, rats and chickens suggest that humans may respond in a similar manner to chronic nitrate exposure. The finding of nitrite-induced tissue pathology and irreversible changes in brain electrical activity of rats is of considerable concern. The question of whether such devastating effects also occur in humans remains unanswered.

Vitamin A deficiency has been associated as a risk factor for cancer both epidemiologically and experimentally (Rose, 1983). The work done in animals such as the rat, chicken and turkey showed nitrate to have a detrimental affect on vitamin A metabolism in some species. This suggests the possibility that nitrate may exert an effect on cancer through this mechanism.

A hypothesis for the role of nitrate, nitrite and N-nitroso compounds in the etiology of gastric and other human cancers has been proposed (Correa, 1987; Correa et al., 1975). A number of researchers have examined the effect of these compounds on the rate of gastric cancer (Cuello et al., 1976;

Buitti et al., 1990; Gilli et al., 1984; Takács, 1987; Zemla, 1980). Epidemiologic studies using humans have been burdened by a variety of difficulties, and the importance of nitrate, nitrite and N-nitroso compounds in the etiology of gastric cancer remains uncertain. To determine the nature and extent of exposure to these compounds, one must determine the sources, amount, and time period involved, all of which are extremely difficult with human subjects. Because of the difficulties encountered when using humans in studies, a monogastric animal might serve as a suitable research subject.

Experiments in cattle, guinea pigs and rats suggest the ability of nitrate to cross the placenta. Animal studies suggest nitrite may also traverse the placenta (Gruener et al., 1973; Globus and Samuel, 1978) or exert a transplacental effect (Inui et al., 1979; Inui et al., 1979). Investigations of nitrate poisoning indicates abortion may occur in ruminants subsequent to acute toxicity. The possibility of nitrate traversing the human placenta (Gelperin et al., 1971) or having a role in infant death or malformation has been suggested, but remains unproven (Scragg et al., 1982; Dorsch et al., 1984; Schmitz, 1961). A review of animal data found no evidence for teratogenic effects attributable to nitrate or nitrite ingestion (Fan, 1987). The finding of nitrates in the milk from cattle and dogs and N-nitroso compounds in the milk from goats is worrisome. At this time, it is not known whether nitrate, nitrite or N-nitroso compounds cross the

placenta or can pass to children through their mothers' milk. Until further research clarifies the situation, pregnant women and nursing mothers would be wise to avoid ingestion of nitrate, nitrite, and N-nitroso compounds in food or water.

Currently, little is known about the long term effects of nitrate, nitrite and N-nitroso compounds on animal health. With the number of adverse health effects known or suspected to be associated with nitrate, nitrite, and N-nitroso compounds in human health, further animal research is needed to answer the remaining questions.

Table 3.1: Conversion Factors

Nitrate in PPM = Nitrate in mg/kg = Nitrate in mg/L

PPM X 0.0001 = % Nitrate

% Nitrate X 10,000 = PPM

Potassium or sodium nitrate X 0.61 = Nitrate

Potassium or sodium nitrate X 0.14 = Nitrate-nitrogen

Nitrate-nitrogen (NO⁻-N) X 4.45 = Nitrate

Nitrate-nitrogen (NO⁻-N) X 3.29 = Nitrite

Nitrate-nitrogen (NO⁻-N) X 6.1 = Potassium or Sodium Nitrate

REFERENCES

- Adams AW: Effects of nitrate in drinking water of Japanese quail: Poultry Sci, 53:832-834, 1974.
- Adams AW, Emerick RJ, and Carlson CW: Effects of nitrate and nitrite in the drinking water on chicks, poults and laying hens. Poultry Sci, 45:1215-1222, 1966.
- Adams AW, West JL and Kahrs AJ: Some effects on turkeys of nitrate in the drinking water. Poultry Sci, 48:1222-1229, 1969.
- Anderson DM and Stothers SC: Effects of saline water high in sulfates, chlorides and nitrates on the performance of young weanling pigs. J Anim Sci, 47:900-907, 1978.
- Anonymous: MAFF Investigations Service Report--Nitrate poisoning. Vet Rec, 102:183, 1978.
- Arends LG, Kienholz EW, and Nockels CF: Growth stimulation effects of sodium nitrate for bronze turkeys. Poultry Sci, 46:1228 (abstract), 1967.
- Armstrong JG, McKendry JBJ, and Slemon KW: Methaemoglobinaemia in infancy. Can Med Assoc, 79:392-395, 1958.
- Asbury AC and Rhode EA: Nitrite intoxication in cattle: The effects of lethal doses of nitrite on blood pressure. Am J Vet Res, 25:1010-1013, 1964.
- Atef M, Abo-Norage MAM, Hanafy MSM, and Agag AE: Pharmacotoxicological aspects of nitrate and nitrite in domestic fowls. Br Poultry Sci, 32:399-404, 1991.
- Beath OA, Gilbert CS, Eppson HF and Rosenfeld I: Oat-hay and oat-straw poisoning. In Wyoming Agricultural Experiment Station bulletin 324, 46-48, 1953.
- Beck EG: Toxic effects from bismuth subnitrate with reports of cases to date. J Am Med Assoc, 52:14-18, 1909.
- Bentley AB, Kinder QB, Garner GB, and Savage JE: Effect of nitrate in water on performance of laying hens. Poultry Sci, 44:1351 (abstract), 1965.
- Bjornson CB, McIlwain P, Eveleth DF, and Bolin FM: Sources of nitrate intoxication. Vet Med, 56:198-200, 1961.
- Bjornson CB: Sources of nitrate intoxications in North Dakota. North Dakota Farm Research Bimonthly Bulletin, 23:16-20, 1964.

Blood DC, Radostits OM, and Henderson JA: *Vet Med, Sixth Edition*. Bailliere Tindall, Philadelphia, 1983.

Bloomfield RA, Garner BB, Welsch C, and Muhrer ME: Effect of dietary nitrate on urea utilization *in vitro* and *in vivo*. *J Anim Sci*, 20:925-926 (Abstract), 1961.

Bloomfield RA, Welsch CW, Garner GB, and Muhrer ME: Effect of dietary nitrate on thyroid function. *Science*, 134:1690, 1961.

Bloomfield RA, Welsch CW, Garner GB and Muhrer ME: Thyroidal I^{131} metabolism in nitrate fed sheep. *J of Anim Sci*, 21:988 (Abstract), 1962.

Bodansky O and Gutmann H: Treatment of methemoglobinemia. *J Pharmacol and Exp Therap*, 90:46-55, 1947.

Bodansky O: Methemoglobinemia and methemoglobin-producing compounds. *Pharm Rev*, 3:144-196, 1951.

Boermans HJ: Diagnosis of nitrate toxicosis in cattle, using biological fluids and a rapid ion chromatographic method. *Am J Vet Res*, 51:491-495, 1990.

Bouwkamp FT, and Counotte GHM: Verslag van een onderzoek naar de gevolgen van extra nitraattoevoeging aan drinkwater voor mestvarkens en gespeende biggen. *Tijdschr Diergeneeskd*, 113:737-747, 1988.

Bradley WB, Eppson HF and Beath OA: Nitrate as the cause of oat hay poisoning. *J Am Vet Med Assoc*, 94:541-542, 1939.

Bradley WB, Beath OA and Eppson HF: Oat hay poisoning. *Science*, 89:365, 1939.

Bradley WB, Eppson HF and Beath OA: Livestock poisoning by oat hay and other plants containing nitrate. In *University of Wyoming Agricultural Experiment Station bulletin* 241, 3-20, 1940.

Bradley WB, Eppson HF and Beath OA: Methylene blue as an antidote for poisoning by oat hay and other plants containing nitrates. *J Am Vet Med Assoc*, 96:41-42, 1940.

Brown CM, Burrows GE, and Edwards WC: Nitrate Intoxication. *Vet Human Toxicol*, 32:481-482, 1990.

Buck WB: Laboratory toxicologic tests and their interpretation. *J Am Vet Med Assoc*, 155:1928-1941, 1969.

Buck WB: Diagnosis of feed-related toxicoses. J Am Vet Med Assoc, 156:1434-1443, 1970.

Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, Bonaguri C, Cipriani F, Cocco P, Giacosa A, Marubini E, Minacci C, Puntoni R, Russo A, Vindigni C, Faumeni JF Jr, Blot WJ: A case-control study of gastric cancer and diet in Italy: II. Association with nutrients. Int J Cancer 45: 896-901, 1990.

Burrows GE: Nitrate intoxication. J Am Vet Med Assoc, 177:82-83, 1980.

Campbell JB, Davis AN and Myhr PJ: Methaemoglobinaemia of livestock caused by high nitrate contents of well water. Can J Comp Med, 18:93-101, 1954.

Carlson DJ and Shapiro FL: Methemoglobinemia from well water nitrates: A complication of home dialysis. Ann Int Med, 73:757-759, 1970.

Carrigan MJ, and Gardner IA: Nitrate poisoning in cattle fed sudax (*sorghum* sp. hybrid) hay. Aust Vet J, 59:155-157, 1982.

Carter RL, Percival WH and Roe FJC: Exceptional sensitivity of mink to the hepatotoxic effects of dimethylnitrosamine. J Pathol, 97:79-88, 1969.

Carver LA and Pfander WH: Some metabolic aspects of urea and/or potassium nitrate utilization by sheep. J Anim Sci, 38:410-416, 1974.

Carver LA and Pfander WH: Urea utilization by sheep in the presence of potassium nitrate. J Anim Sci, 36:581-587, 1973.

Case AA: Abortion in swine due to excessive nitrate. J Am Vet Med Assoc, 131:226, 1957.

Case AA: Some aspects of nitrate intoxication in livestock. J Am Vet Med Assoc, 130:323-329, 1957.

Case AA: Nitrate poisoning from plants. N Am Vet, 38:46A, 1957.

Case AA: Corn stalk intoxication. Sheep Breeder, 74:14-15, 1954.

Cawley GD, Collings DF, and Dyson DA: Nitrate poisoning. Vet Rec, 101:305-306, 1977.

Cheng KJ, Phillippe RC, Kozub GC, Majak W, and Costerton JW: Induction of nitrate and nitrite metabolism in bovine rumen fluid and the transfer of this capacity to untreated animals. *Can J Anim Sci*, 65:647-652, 1985.

Christian HA: Diuretic action of nitrates and their use in the treatment of dropsy. *J Am Med Assoc*, 91:121 (Society Proc), 1928.

Clark R, and Quin JI: Studies on the alimentary tract of the Merino sheep in South Africa. XXIII.--The effect of supplementing poor quality grass hay with molasses and nitrogenous salts. *Onderstepoort J Vet Res*, 25:93-103, 1951.

Clark JL, Pfander WH, Bloomfield RA, Krause GF and Thompson GB: Nitrate containing rations for cattle supplemented with either urea or soybean meal. *J Anim Sci*, 31:961-966, 1970.

Comly HH: Cyanosis in infants caused by nitrates in well water. *J Am Med Assoc*, 129:112-116, 1945.

Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M: A model for gastric cancer epidemiology. *Lancet* 12: 58-60, 1975.

Correa P: Modulation of gastric carcinogenesis: Updated model based on intragastric nitrosation. In Bartsch et al, eds: *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms*. IARC Scientific Publications No. 84, IARC, Lyons, : 485-491, 1987.

Crawford RF, Kennedy WK and Davison KL: Factors influencing the toxicity of forages that contain nitrate when fed to cattle. *Cornell Vet*, 56:3-17, 1966.

Cuello C, Correa P, Haenszel W, Gordillo G, Brown C, Archer M, Tannenbaum S: Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. *J Natl Cancer Inst* 57: 1015-1020, 1976.

Davidson WB, Doughty JL and Bolton JL: Nitrate poisoning of livestock. *Can J Comp Med*, 5:303-313, 1941.

Thorp F Jr: Further observations on oat hay poisoning. *J Am Vet Med Assoc*, 92:159-170, 1938.

Davison KL, Hansel W, Krook L, McEntee K and Wright MJ: Nitrate toxicity in dairy heifers. I. Effects on reproduction, growth, lactation, and vitamin A nutrition. *J Dairy Sci*, 47:1065-1073, 1964.

Davison KL, Hansel W, McEntee K, and Wright MJ: Reproduction, growth and lactation of heifers fed nitrate. *J Anim Sci*, 22:835 (Abstract), 1963.

Davison KL, McEntee K and Wright MJ: Responses in pregnant ewes fed forages containing various levels of nitrate. *J Dairy Sci*, 48:968-977, 1965.

Davison KL and Seo J: Influence of nitrate upon carotene destruction during in vitro fermentation with rumen liquor. *J Dairy Sci*, 46:862-864, 1963.

Deeney J, Murdock ET, and Rogan JJ: Familial idiopathic methaemoglobinaemia. *Br Med J*, 1:721-723, 1943.

Diven RH, Reed RE, Trautman RJ, Pistor WJ and Watts RE: Experimentally induced nitrite poisoning in sheep. *Am J Vet Res*, 23:494-496, 1962.

Diven RH, Pistor WJ, Reed RE, Trautman RJ and Watts RE: The determination of serum or plasma nitrate and nitrite. *Am J Vet Res*, 23:497-499, 1962.

Diven RH, Pahnish OF, Roubicek CB, Erwin ES, and Page HM: Vitamin A and carotenoid inter-relationships in bovine plasma and liver. *J Dairy Sci*, 43:1632-1638, 1960.

Dodd DC and Coup MR: Poisoning of cattle by certain nitrate-containing plants. *NZ Vet J*, 5:51-54, 1957.

Dollahite JW and Holt EC: Nitrate Poisoning. *Southwest Vet*, 23:23-28, 1969.

Dorsch MM, Scragg RKR, McMichael AJ, Baghurst PA and Dyer KF: Congenital malformations and maternal drinking water supply in rural south Australia: A case-control study. *J Epidemiol*, 119:473-486, 1984.

Ellis NR: Salt tolerance and salt poisoning of swine. In *Yearbook of Agriculture*. United States Government Printing Office, Washington, D.C.: 803-809, 1942.

Emerick RJ, Embry LB and Seerley RW: Rate of formation and reduction of nitrite-induced methemoglobin in vitro and in vivo as influenced by diet of sheep and age of swine. *J Anim Sci*, 24:221-230, 1965.

Emerick RJ and Olson OE: Effect of nitrate and nitrite on vitamin A storage in the rat. *J Nutr*, 78:73-77, 1962.

Emerick RJ: Consequences of high nitrate levels in feed and water supplies. *Fed Proc*, 33:1183-1187, 1974.

Eppson HF, Glenn MW, Ellis WW and Gilbert CS: Nitrate in the diet of pregnant ewes. *J Am Vet Med Assoc*, 137:611-614, 1960.

Eusterman BB and Keith NM: Transient methemoglobinemia following administration of ammonium nitrate. *Med Clin N Am*, 12:1489-1496, 1929.

Evelyn KA and Malloy HT: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. *J Biol Chem*, 126:655-662, 1938.

Fan AM, Willhite CC, and Book SA: Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Reg Toxicol Pharmacol*, 7:135-148, 1987.

Fincher MG: Sodium nitrate poisoning; a report of cases. *Cornell Vet*, 26:271-273, 1936.

Fritsch P and De Saint Blanquat G: Excretion of nitrates and nitrites in saliva and bile in the dog. *Food Chem Toxicol*, 23:655-659, 1985.

Garner GB: Learn to live with nitrates. *University of Missouri Agricultural Experiment Station Bulletin* 708, 1958.

Garner GB, O'Dell BL, Radar P, and Muhrer ME: Further studies on the effects of nitrate upon reproduction and vitamin A storage with rats and swine. *J Anim Sci*, 17:1213 (Abstract), 1958.

Garrison GW, Wood RD, Chaney CH, and Waddill DG: Effects of nitrate and nitrites in drinking water on the utilization of carotene in swine. *Kentucky Agricultural Experimental Station Progress Report* 164, pages 85-87, 1966.

Gelperin A, Jacobs EE, and Kletke LS: The development of methemoglobin in mothers and newborn infants from nitrate in water supplies. *Illinois Med J*, 140:42-44, 82, 1971.

Gilli G, Corrao G, Favilli S: Concentrations of nitrates in drinking water and incidence of gastric carcinomas: First descriptive study of the Piemonte region, Italy. *Sci Total Environ*. 34: 35-48, 1984.

Globus M and Samuel D: Effect of maternally administered sodium nitrite on hepatic erythropoiesis in fetal CD-1 mice. *Teratology*, 18:367-378, 1978.

Goodrich RD, Emerick RJ, and Embry LB: Effects of sodium nitrate on vitamin A status of sheep. *J Anim Sci*, 21:997 (Abstract), 1962.

Goodrich RD, Emerick RJ and Embry LB: Effect of sodium nitrate on the vitamin A nutrition of sheep. *J Anim Sci*, 23:100-104, 1964.

Green LC, Tannenbaum SR, and Fox JG: Nitrate in human and canine milk. *N Eng J Med*, 306:1367-1368, 1982.

Green LC, Ralt D, and Tannenbaum SR: Nitrate, nitrite and N-nitroso compounds: Biochemistry, metabolism, toxicity and carcinogenicity. In Neuberger and Jukes (editors). *Human Nutrition*, Jack K. Burgess, Inc., Englewood, New Jersey: 87-140, 1982.

Greene I and Hiatt EP: Renal excretion of nitrate and its effect on excretion of sodium and chloride. *Am J Physiol*, 180:179-182, 1955.

Greene I and Hiatt EP: Behavior of the nitrate ion in the dog. *Am J Physiol*, 176:463-467, 1954.

Grudziński I and Szymański A: The effect of subchronic poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:447-452, 1991.

Grudziński I and Szymański A: The effect of exercise associated with subchronic poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:462-467, 1991.

Grudziński I and Szymański A: The effect of acute poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:453-461, 1991.

Grudziński I: Studies on the mechanism of the toxic action of sodium nitrite on intestinal absorption in rats. *Arch Environ Contam Toxicol*, 21:475-479, 1991.

Grudziński I, Szymański A, and Chomiczewski K: The effect of exercise associated with acute poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:468-474, 1991.

Gruener N, Shuval HI, Behroozi K, Cohen S, and Shechter H: Methemoglobinemia induced by transplacental passage of nitrites in rats. *Bull Environ Contam Toxicol*, 9:44-48, 1973.

Gwatkin R and Plummer PJG: Toxicity of certain salts of sodium and potassium for swine. *Can J Comp Med*, 10:183-190, 1946.

Hainline A Jr: Methemoglobin. In Meites (editor), *Standard Methods of Clinical Chemistry, Volume 5*. Academic Press, New York: 143-157, 1965.

Hale WH, Hubbert F Jr, and Taylor RE: Effect of energy level and nitrate on hepatic vitamin A and performance of fattening steers. *Proc Soc Exper Biol Med*, 109:289-290, 1962.

Hale WH, Hubbert F Jr, and Taylor RE: The effect of concentrate level and nitrate addition on hepatic vitamin A stores and performance of fattening steers. *J Anim Sci*, 20:934 (Abstract), 1961.

Haliburton JC and Edwards WC: Nitrate poisoning in Oklahoma cattle during the winter of 1977-1978. *Vet Human Toxicol*, 20:401-403, 1978.

Hambitzer R, Velke H, Ramm K, and Sommer H: Bewertung der nitratbelastung von laktierenden und trockenstehenden milchkühen anhand der nitratwerte im blutplasma und eines blutprofils. *Tierärztliche Umschau*, 42:1-12, 1987.

Hansen MA: An outbreak of toxic liver injury in ruminants. *Nord Vet*, 16:323-342, 1964.

Hartman PE: Nitrates and nitrites: Ingestion, pharmacodynamics, and toxicology. In de Serres and Hollaender (editors). *Chemical Mutagens, volume 7*, Plenum Press, N.Y., pp. 211-294, 1982.

Hatfield EE and Smith GS: Nitrate and urea in rations of feeder lambs. *J Anim Sci*, 22:1122 (Abstract), 1963.

Hatfield EE, Smith GS, Neumann AL, Forbes RM, Garrigus US and Ross OB: Interactions of nitrite, alpha-tocopherol and tapazole upon the vitamin A nutrition of lambs fed "high nitrate" silage. *J Anim Sci*, 20:676 (Abstract), 1961.

Hiatt EP: Extreme hypochloremia in dogs induced by nitrate administration. *Am J Physiol*, 129:597-609, 1940.

Hibbs CM, Stencel EL, and Hill RM: Nitrate toxicosis in cattle. *Vet Human Toxicol*, 20:401-403, 1978.

Hoar DW, Embry LB, King HR, and Emerick RJ: Urea-nitrate interrelationships in sheep under feedlot conditions. *J Anim Sci*, 27:557-561, 1968.

Holst WO, Flynn LM, Garner GB and Pfander WH: Dietary nitrite vs. sheep performance. *J Anim Sci*, 20:936 (Abstract), 1961.

Holtenius P: Nitrite poisoning in sheep, with special reference to the detoxification of nitrite in the rumen: An experimental study. *Acta Agricul Scand*, 7:113-163, 1957.

Hueper WC and Landsberg JW: Experimental studies in cardiovascular pathology. *Arch Pathol*, 29:633-648, 1940.

Inui N, Nishi Y, Taketomi M, and Mori M: Transplacental action of sodium nitrite on embryonic cells of Syrian Golden hamster. *Mutation Res*, 66:149-158, 1979.

Inui N, Nishi Y, Taketomi M, Mori M, Yamamoto M, Yamada T, and Tanimura A: Transplacental mutagenesis of products formed in the stomach of golden hamsters given sodium nitrite and morpholine. *Int J Cancer*, 24:365-372, 1979.

Jainudeen MR, Hansel W and Davison KL: Nitrate toxicity in dairy heifers. 2. Erythropoietic responses to nitrate ingestion during pregnancy. *J Dairy Sci*, 47:1382-1387, 1964.

Jainudeen MR, Hansel W and Davison KL: Nitrate toxicity in dairy heifers. 3. Endocrine responses to nitrate ingestion during pregnancy. *J Dairy Sci*, 48:217-221, 1965.

Jamieson ND: Adverse effect of nitrate metabolic products on sheep growth. *Nature*, 181:1601-1602, 1958.

Jamieson ND: Nitrate reduction in the rumen of the grazing sheep. *NZ J Agricul Res*, 2:96-106, 1959.

Johnson CJ, Bonrud PA, Dosch TL, Kilness AW, Senger KA, Busch DC, and Meyer MR: Fatal outcome of methemoglobinemia in an infant. *J Am Med Assoc*, 257:2796-2797, 1987.

Johnson JL, Schneider NR, Kelling CL, and Doster AR: Nitrate exposure in perinatal beef calves. In *Proc 26th Ann Mtg Am Assoc of Vet Lab Diagnos*, pp. 167-180, 1983.

Johnson RM and Baumann CA: The effect of thyroid on the conversion of carotene into vitamin A. *J Biol Chem*, 171:513-521, 1947.

Jones IR, Weswig PH, Bone JF, Peters MA, and Alban SO: Effect of high-nitrate consumption on lactation and vitamin A nutrition of dairy cows. *J Dairy Sci*, 49:491-499, 1966.

Jordan HA, Neumann AL, Smith GS, Zimmerman JE and Vatthauer RJ: Vitamin A status of steers fed "high nitrate" corn silages, and a study of subsequent effects upon carotene utilization. *J Anim Sci*, 20:937-938 (Abstract), 1961.

Jordan HA, Smith GS, Neumann AL, Zimmerman JE and Breniman GW: Vitamin A nutrition of beef cattle fed corn silages. *J Anim Sci*, 22:738-745, 1963.

Juszkiewicz T, and Kowalski B: Passage of nitrosamines from rumen into milk in goats. In Bogovski, P. and Walker, E.A., (editors), *N-Nitroso Compounds in the Environment*. Lyon, IARC Publication 9: 173-176, 1974.

Juszkiewicz T and Kowalski B: An investigation of the possible presence or formation of nitrosamines in animal feeds. In Walker et al (editors), *Environmental N-nitroso Compounds Analysis and Formation*. IARC, Lyons: 375-383, 1976.

Kahler LW, Jorgensen NA, Satter LD, Thler WJ, Crowley JW, and Finner MF: Effect of nitrate in drinking water on reproductive and productive efficiency of dairy cattle. *J Dairy Sci*, 58:771 (Abstract), 1975.

Kahraman M: Nitrat ve nitrit'in farelerde immunosupresif etkileri üzerinde araştırmalar. *Veteriner Fakültesi Dergisi*, 6:251-254, 1988.

Kammerer M, Siliart, B: Toxicité à moyen terme des nitrates: évaluation expérimentale des effets sur les fonctions de reproduction chez la lapine. *Ann Rech Vet*, 24:434-444.

Kammerer M, Pinault L, and Pouliquen H: Teneur en nitrate du lait. Relation avec sa concentration dans l'eau d'abreuvement. *Ann Rech Vét*, 23:131-138, 1992.

Kearley EO, Garner GB, Bloomfield RA, and Muhrer ME: Physiological pathways of nitrate in ruminants. *J Anim Sci*, 21:1002 (Abstract), 1962.

Keating EK, Hale WH, and Hubbert F Jr: *In vitro* degradation of vitamin A and carotene by rumen liquor. *J Anim Sci*, 23:111-117, 1964.

Keith NM, Whelan M, and Bannick EG: The action and excretion of nitrates. *Arch Int Med*, 46:797-832, 1930.

Keleştimur H, Pirinççi I, and Servi K: Deneysel olarak nitrat zehirlenmesi oluşturulan koyunlarda bazı hematolojik araştırmalar. Doğa, 12:52-57, 1988.

Kelley ST, Oehme FW, and Hoffman SB: Effect of chronic dietary nitrates on canine thyroid function. Toxicol Appl Pharmacol, 27:200-203, 1974.

Kendrick JW, Tucker J and Peoples SA: Nitrate poisoning in cattle due to ingestion of variegated thistle, *Silybum marianum*. J Am Vet Med Assoc, 126:53-56, 1955.

Ketchum JL, Garner GB, Flynn LM, and Pfander WH: The role of butylated hydroxy anisole and sodium meta bisulfite in the utilization of rations containing nitrate. J Anim Sci, 16:1092-1093 (Abstract), 1957.

Kienholz EW, Enos HL, and McPherron TA: The effects of some water sources and treatments upon turkey performance. Poultry Sci, 44:1390 (Abstract), 1965.

Kilgore L, Almon L and Gieger M: The effects of dietary nitrate on rabbits and rats. J Nutr, 69:39-44, 1959.

Kilgore L, Stasch AR, and Barrentine BF: Relation of ascorbic acid to nitrate content of turnip greens and to methemoglobin formation. Am J Clin Nutr, 14:52-55, 1964.

Knight AP: The toxicology of sulphur and nitrate in ruminants. Bov Pract, 20:121-123, 1985.

Koch BA, Parrish DB, and Sukhonthasarnpa S: Effects of dietary NO₂ and NO₃ on growing swine. J Anim Sci, 22:840 (Abstract), 1963.

Kociba RJ and Sleight SD: Nitrite toxicosis in the ascorbic acid-deficient Guinea pig. Toxicol Appl Pharmacol, 16:424-429, 1970.

Koppang N, Slagsvold P, Aas Hansen M, Sögnen E, and Svenkerud R: Feeding experiments with meal produced from herring preserved with sodium nitrite and formalin: Possible connection between nitrite preservation and toxic hepatitis in sheep. Nord Vet, 16:343-362, 1964.

Koppang N: A severe progressive liver disease in fur animals I. Symptoms and organ changes. Nord Vet, 18:205-209, 1966.

Koppang N: An outbreak of toxic liver injury in ruminants. Case reports pathological-anatomical investigations, and feeding experiments. Nord Vet, 16:305-322, 1964.

Koppang N and Helgebostad A: Toxic hepatitis in fur animals II. The etiology elucidated by feeding experiments. Nord Vet, 18:210-215, 1964.

Kretschmer AE Jr: Nitrate accumulation in everglades forages. Agron J, 50:314-316, 1958.

Lewis D: The metabolism of nitrate and nitrite in the sheep. 2. Hydrogen donors in nitrate reduction by rumen micro-organisms *in vitro*. Biochem J, 49:149-153, 1951.

Lewis D: The metabolism of nitrate and nitrite in the sheep. 1. The reduction of nitrate in the rumen of the sheep. Biochem J, 48:175-180, 1951.

Lindsey GA and Rhines CM: The production of hydroxylamine by the reduction of nitrates and nitrites by various pure cultures of bacteria. J Bacteriol, 24:489-492, 1932.

Lippsmeyer BC, Tracy ML, Moller G: Ion-exchange liquid chromatographic determination of nitrite and nitrite in biological fluids. J Assoc Off Analyt Chem, 73:457-462, 1990.

London WT, Henderson W, and Cross RF: An attempt to produce chronic nitrite toxicosis in swine. J Am Vet Med Assoc, 150:398-402, 1967.

Magee PN: Nitrogen as a potential health hazard. Philos Trans R Soc Lond, 296:543-550, 1982.

Malone P: Monensin sodium toxicity in cattle. Vet Rec, 103:477-478, 1978.

Marrett LE, and Sunde ML: The use of turkey poults and chickens as test animals for nitrate and nitrite toxicity. Poultry Sci, 47:511-519, 1968.

Mayo NS: Cattle poisoning by nitrate of potash. In Kansas Agricultural Experiment Station bulletin 49, 3-11, 1895.

McIlwain PK and Schipper IA: Toxicity of nitrate nitrogen to cattle. J Am Vet Med Assoc, 142:502-505, 1963.

Metcalf WK: The sensitivity of intracorporeal haemoglobin to oxidation by nitrite ions. Phys Med Biol, 6:427-435, 1962.

Miller RW, Moore LA, Waldo DR, and Wren TR: Effect of nitrate on utilization of corn silage carotene by calves. J Anim Sci, 24:898 (Abstract), 1965.

Mitchell GE Jr, Little CO and Hayes BW: Pre-intestinal destruction of vitamin A by ruminants fed nitrate. *J Anim Sci*, 26:827-829, 1967.

Mitchell GE Jr, Little CO, Hayes BW, and Tucker RE: Preintestinal destruction of vitamin A in steers fed nitrate. *J Anim Sci*, 24:898 (Abstract), 1965.

Morris MP, Cancel B and González-Más A: Toxicity of nitrates and nitrites to dairy cattle. *J Dairy Sci*, 41:694-696, 1958.

Mortensen RA: The effect of diet on methemoglobin levels of nitrite-injected rats. *Arch Biochem Biophys*, 46:241-243, 1953.

Muhrer ME, Garner GB, Pfander WH and O'Dell BL: The effect of nitrate on reproduction and lactation. *J Anim Sci*, 15:1291-1292 (Abstract), 1956.

Muhrer ME, Case AA, Garner GB and Pfander WH: Toxic forages produced in a drought area. *J Anim Sci*, 14:1251 (Abstract), 1955.

Murdock FR, and Hodgson AS: Utilization of nitrates by dairy cows. *J Dairy Sci*, 55:640-642, 1972.

Nakamura Y, Yoshida J, Nakamura R, and Horie H: Nitrate metabolism of microorganisms in the rumen of sheep fed high nitrate forages. *Japan J Zootech Sci*, 47:63-67, 1976.

National Academy of Sciences--National Research Council, Subcommittee on nutrient and toxic elements in water: Nutrients and toxic substances in water for livestock and poultry. *Nat Acad Sci*, Washington, D.C., 1974.

National Academy of Sciences--National Research Council, Assembly of Life Sciences: The health effects of nitrate, nitrite and N-nitroso compounds. *Nat Acad Sci*, Washington, D.C., 1981.

Nelson PA, Paulson GD, and Feil VJ: The effect of nitrite on ¹⁴C-sulphathiazole (4-amino-N-2-thiazolyl[U-¹⁴C]benzenesulphonamide) metabolism in the rat. *Xenobiotica*, 17:829-838, 1987.

Newsom IE, Stout EN, Thorp F Jr, Barber CW and Groth AH: Oat hay poisoning. *J Am Vet Med Assoc*, 43:66-75, 1937.

Nicholls TJ and Miles EJ: Nitrate/nitrite poisoning of cattle on ryegrass pasture. *Aust Vet J*, 56:95-96, 1980.

O'Dell BL, Ereik Z, Flynn L, Garner GB, and Huhner ME: Effects of nitrite containing rations in producing vitamin A deficiencies in rats. *J Anim Sci*, 19:1280 (Abstract), 1960.

O'Hara PJ and Fraser AJ: Nitrate poisoning in cattle grazing crops. *NZ Vet J*, 23:45-53, 1975.

Olson OE, Nelson DL, and Emerick RJ: Effect of nitrate and some of its reduction products on carotene stability. *Agric Food Chem*, 11:140-143, 1963.

Olson OE and Moxon AL: Nitrate reduction in relation to oat hay poisoning. *J Am Vet Med Assoc*, Vol. C, 782:403-406, 1942.

Olson JR, Oehme RW, and Carnahan DL: Relationship of nitrate levels in water and livestock feeds to herd health problems on 25 Kansas farms. *Vet Med Sm Anim Clin*, 67:257-260, 1972.

Osweiler GD, Carson TL, Buck WB and Van Gelder GA: Urea and nonprotein nitrogen. In *Clin Diag Vet Toxicol*, Third Edition, pages 160-166, 171-173, 1976.

Osweiler GD, Carson TL, Buck WB, Van Gelder GA: Nitrates, nitrites, and related problems. In *Clinical and Diagnostic Veterinary Toxicology*, Third Edition. Kendall/Hunt, Dubuque, Iowa: 460-467, 1985.

Page RD, Gilson WD, Guthrie LD, Mertens DR, and Hatch RC: Serum progesterone and milk production and composition in dairy cows fed two concentrations of nitrate. *Vet Human Toxicol*, 32:27-31, 1990.

Paulson GD: The effect of dietary nitrite and nitrate on the metabolism of sulphonamethazine in the rat. *Xenobiotica*, 16:53-61, 1986.

Paulson GD and Feil VJ: Evidence for diazotization of ¹⁴C-sulfamethazine {4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene[U-¹⁴C]sulfonamide} in swine. *Drug Metab Disp*, 15:841-845, 1987.

Paulson GD: The influence of nitrite on sulfonamide drug metabolism in animals. *Drug Metabol Rev*, 18:137-162, 1987.

Paulson GD and Aschbacher PW: Effect of high dietary nitrate on the disposition of sulfamethazine [4-amino-N-(4,6-dimethyl-2-pyrimidinyl) benzenesulfonamide] in swine. *J Agric Food Chem*, 38:308-310, 1990.

Pfander WH, Garner GB, Ellis WC and Muhrer ME: The etiology of "nitrate poisoning" in sheep. University of Missouri Agricultural Experiment Station, Bulletin 637, 1957.

Phillips WEJ. Nitrate content of foods - Public health implications: Can Inst Food Technol J, 1:98-103, 1968.

Pirinççi I and Keleştimur H: Koyunlarda nitrat ve nitrit zehirlenmesi üzerine deneysel çalışmalar. Doğa, 11:255-265, 1987.

Pirinççi I, Keleştimur H, Servi K, and Karahan İ: Koyunlarda nitrat zehirlenmesinin tedavisi üzerine deneysel çalışmalar. Doğa, 12:78-84, 1988.

Prewitt RD and Merilan CP: Effect of potassium nitrate intake on dairy calves. J Dairy Sci, 41:807-811, 1958.

Pugh DL, Garner GB, Bloomfield RA and Muhrer ME: Carotene-vitamin A destruction by nitrite *in vitro*. J Anim Sci, 21:1009 (Abstract), 1962.

Reddy BS and Thomas JW: Interrelationship between thyroid status and nitrate on carotene conversion. J Anim Sci, 21:1010 (Abstract), 1962.

Ridder WE and Oehme FW: Nitrates as an environmental, animal, and human hazard. Clin Toxicol, 7:145-159, 1974.

Riggs CW: Nitrite poisoning from ingestion of plants high in nitrate. Am J Vet Res, 6:194-197, 1945.

Roberts WK and Sell JL: Vitamin A destruction by nitrite *in vitro* and *in vivo*. J Anim Sci, 22:1081-1085, 1963.

Rose DP: Micronutrients in carcinogenesis. In Wynder et al. (editors), *Environmental Aspects of Cancer - The Role of Macro and Micro Components of Foods*. Food Nutr Press, Inc., Connecticut: 128-139, 1983.

Rubenchik BL, Khmel'nitsky GA, Tiktin LA, Tsapenko VF, Vovk CM, Glavin AA, Dan'ko NF, Skarinskays EY, and Glushknow PV: [Content of N-nitrosamine and their precursors in milk and blood of cattle]. Veterinariia, 7:59-61, 1988.

Ruhr LP and Osweiler GD: Nitrate accumulators. In Howard (editor), *Current Veterinary Therapy - Food Animal Practice 2*. W.B. Saunders Co., Philadelphia, PA: 392-394, 1986.

Sakshaug J, Sögnen E, Aas Hansen, M, and Koppang N: Dimethylnitrosamine; its hepatotoxic effect in sheep and its occurrence in toxic batches of herring meal. *Nature*, 206:1261-1262, 1965.

Sapiro ML, Hoflund S, Clark R, and Quin JI: Studies on the alimentary tract of the Merino sheep in South Africa. XVI.- The fate of nitrate in ruminal ingesta as studied *in vitro*. Onderstepoort J Vet Sci Anim Indust, 22:357-372, 1949.

Savage A: Nitrate poisoning from sugar beet tops. *Can J Comp Med*, 13:9-10, 1949.

Schmitz JT: Methemoglobinemia - A cause of abortions? Preliminary Report. *Obstet Gynecol*, 17:413-415, 1961.

Schneider NR and Yeary RA: Nitrite and nitrate pharmacokinetics in the dog, sheep, and pony. *Am J Vet Res*, 36:941-947, 1975.

Schneider NR and Yeary RA: Measurement of nitrite and nitrate in blood. *Am J Vet Res*, 34:133-135, 1973.

Schwarte LH, Eveleth DF and Biester HE: Studies on the so-called corn stalk poisoning in cattle. *Vet Med*, 34:648-651, 1939.

Scragg RKR, Dorsch MM, McMichael AJ, and Baghurst PA: Birth defects and household water supply. *Med J Aust*, 2:577-579, 1982.

Sebaugh TP, Lane AG and Campbell JR: Effects of two levels of nitrate and energy on lactating cows receiving urea. *J Anim Sci*, 31:142-144, 1970.

Seerley RW, Emerick RJ, Embry LB and Olson OE: Effect of nitrate or nitrite administered continuously in drinking water for swine and sheep. *J Anim Sci*, 24:1014-1019, 1965.

Sell JL and Roberts WK: Effects of dietary nitrite on the chick: Growth, liver vitamin A stores and thyroid weight. *J Nutr*, 79:171-178, 1963.

Setchell BP and Williams AJ: Plasma nitrate and nitrite concentration in chronic and acute nitrate poisoning in sheep. *Aust Vet J*, 38:58-62, 1962.

Shuval HI and Gruener N: Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am J Public Health*, 62:1045-1052, 1972.

Simon J, Sund JM, Douglas FD, Wright MJ and Kowalczyk T: The effect of nitrate or nitrite when placed in the rumens of pregnant dairy cattle. J Am Vet Med Assoc, 135:311-314, 1959.

Simon J, Sund JM, Wright MJ, Winter A and Douglas FD: Pathological changes associated with the lowland abortion syndrome in Wisconsin. J Am Vet Med Assoc, 132:164-169, 1958.

Simon J, Sund JM, Wright MJ and Douglas FD: Prevention of noninfectious abortion in cattle by weed control and fertilization practices on lowland pastures. J Am Vet Med Assoc, 135:315-317, 1959.

Sinclair KB and Jones DIH: Nitrite toxicity in sheep. Res in Vet Sci, 8:65-70, 1967.

Sinclair KB and Jones DIH: The effect of nitrate on blood composition and reproduction in the ewe. Br Vet J, 120:78-86, 1964.

Singer RH: Environmental nitrates and animal health. Southwest Vet, 22:13-18, 1968.

Singer RH: The nitrate poisoning complex. Proc of the 76th Annual Meeting US Anim Health Assoc, p. 310-322. Miami Beach, Florida, 1972.

Sinha DP and Sleight SD: Pathogenesis of abortion in acute nitrite toxicosis in Guinea pigs. Toxicol Appl Pharmacol, 18:340-347, 1971.

Slanina L', Slivka P, and Struháriková: Transmamárny prestup dusičnanov a dusitanov u prežúvavcov a úroveň methemoglobinémie v krvi mláďat a ich matiek. Veterinárni Medicina, 35:647-656, 1990.

Sleight SD and Atallah OA: Reproduction in the Guinea pig as affected by chronic administration of potassium nitrate and potassium nitrite. Toxicol Appl Pharmacol, 12:179-185, 1968.

Sleight SD, Sinha DP, and Uzoukwu M: Effect of sodium nitrite on reproductive performance of pregnant sows. J Am Vet Med Assoc, 161:819-823, 1972.

Slennings BD, Galey FD and Anderson M: Forage-related nitrate toxicoses possibly confounded by nonprotein nitrogen and monensin in the diet used at a commercial dairy heifer replacement operation. J Am Vet Med Assoc, 198:867-870, 1991.

Sloan KW: Nitrates and animal health. In Proc of the Second Allerton Conference, December, 1971, pp. 25-27. College of Agriculture, University of Illinois, special publication 26, 1972.

Smith HC, Lovell VE, Reppert R, and Griswold D: Nitrate poisoning in swine. Vet Med, 54:547-550, 1959.

Smith RA and Suleiman A: Toxic plant case reports. Vet Human Toxicol, 33:349-350, 1991.

Smith GS, Neumann AL, and Hatfield EE: Carotene utilization and vitamin A nutrition as influenced by dietary nitrite and "high nitrate" silage: Laboratory studies. J Anim Sci, 20:683-684 (Abstract), 1961.

Smith GS, Hatfield EE, Durdle WM, and Neumann AL: Vitamin A status of cattle and sheep as affected by nitrate added to rations of hay or silage and by supplementation with carotene or preformed vitamin A. J Anim Sci, 22:1013-1014 (Abstract), 1962.

Cline TR, Hatfield EE and Garrigus US: Effects of potassium nitrate, alpha-tocopherol, thyroid treatments, and vitamin A on weight gain and liver storage of vitamin A in fattening lambs. J Anim Sci, 22:911-913, 1963.

Smith GS: Diagnosis and causes of nitrate poisoning. J Am Vet Med Assoc, 147:365-366, 1965.

Smith JE and Beutler E: Methemoglobin formation and reduction in man and various animal species. Am J Physiol, 210:347-350, 1986.

Sokolowski JH, Garrigus US and Hatfield EE: Effects of inorganic sulfur on KNO_3 utilization by lambs. J Anim Sci, 20:953 (Abstract), 1961.

Spicer SS: Species differences in susceptibility to methemoglobin formation. J Pharmacol Exp Ther, 99:185-194, 1950.

Stewart GA, and Merilan CP: Effect of potassium nitrate intake on lactating dairy cows. University of Missouri Agricultural Experiment Station, Bulletin 650, 1958.

Stoewsand GS, Anderson JL, and Lee CY: Nitrite-induced methemoglobinemia in Guinea pigs: Influence of diets containing beets with varying amounts of nitrate, and the effect of ascorbic acid, and methionine. *J Nutr*, 103:419-424, 1973.

Struble CB and Paulson GD: The metabolism and deamination of [^{14}C]-sulphamethazine in a germ-free pig: The influence of nitrate and nitrite. *Food Chem Toxicol*, 26:797-801, 1988.

Stuart LD and Oehme FW: Environmental factors in bovine and porcine abortion. *Vet Human Toxicol*, 24:435-441, 1982.

Sund JM, Wright MJ, and Simon J: Weeds containing nitrates cause abortion in cattle. *Agron J*, 49:278-279, 1957.

Sunde ML: The use of the turkey poult as a test animal for nitrite toxicity. *Poultry Sci*, 43:1368 (Abstract), 1964.

Super M, De V Heese H, MacKenzie D, Dempster WS, Du Plessis J, and Ferreira JJ: An epidemiological study of well-water nitrates in a group of South West African/Namibian infants. *Water Res*, 15:1265-1270, 1981.

Takács S: Nitrate content of drinking water and tumors of the digestive organs. *Zentralb Bakter Mikrobiol* 184: 269-279, 1987.

Takahashi J and Young BA: Prophylactic effect of L-cysteine on nitrate-induced alterations in respiratory exchange and metabolic rate in sheep. *Anim Feed Sci Technol*, 35:105-113, 1991.

Tannenbaum SR, Weisman M, and Fett D: The effect of nitrate intake on nitrite formation in human saliva. *Food Cosmetics Toxicol*, 14:549-552, 1976.

Tannenbaum SR, Sinskey AJ, Weisman M and Bishop W: Nitrite in human saliva. Its possible relationship to nitrosamine formation. *J Nat Cancer Inst*, 53:79-84, 1974.

Tillman AD, Sheriha GM and Sirny RJ: Nitrate reduction studies with sheep. *J Anim Sci*, 24:1140-1146, 1965.

Tillman AD, Sheriha GM, Goodrich RD and Nelson EC: Effect of injected hydroxylamine upon vitamin A status of sheep. *J Anim Sci*, 24:1136-1139, 1965.

Tollett JT, Becker DE, Jensen AH and Terrill SW: Effect of dietary nitrate on growth and reproductive performance of swine. J Anim Sci, 19:1297 (Abstract), 1960.

van 't Klooster ATh, Malestein A, Kemp A, Geurink JH, and Schyt G: Nitrate intoxication in cattle. In Proc to the XIIth World Congress on Diseases of Cattle, pp.398-403. The Netherlands, September 7-10, 1982.

Vermunt J and Visser R: Nitrate toxicity in cattle. NZ Vet J, 35:136-137, 1987.

Walker R: Naturally occurring nitrate/nitrite in foods. J Sci Food Agric, 26:1735-1742, 1975.

Wallace JD, Raleigh RJ and Weswig PH: Performance and carotene conversion in Hereford heifers fed different levels of nitrate. J Anim Sci, 23:1042-1045, 1964.

Wang LC, Garcia-Rivera J and Burris RH: Metabolism of nitrate by cattle. Biochem J, 81:237-242, 1961.

Wanntorp H and Swahn O: The toxicity of whey for swine in conjunction with bacterial reduction of nitrate to nitrite. Proc of the 15th Int Vet Congress, Part 1:496-501, 1953.

Watts H, Webster M, Chappe A, and Leaver DD: Laboratory diagnosis of nitrite poisoning in sheep and cattle. Aust Vet J, 45:492, 1969.

Weichenthal BA, Embry LB, Emerick RJ and Whetzal FW: Influence of sodium nitrate, vitamin A and protein level on feedlot performance and vitamin A status of fattening cattle. J Anim Sci, 22:979-984, 1963.

Welsch C, Bloomfield RA, Garner GB, and Muhrer ME: Effect of dietary nitrate on thyroid and adrenal gland weight. J Anim Sci, 20:981 (Abstract), 1961.

Welsch CW, Bloomfield RA, Garner GB and Muhrer ME: Response of rats to low temperature and nitrate. J Anim Sci, 21:1032 (Abstract)1962.

Wendel WB: The control of methemoglobinemia with methylene blue. J Clin Invest, 18:179-185, 1939.

Whitehead JE: Potassium nitrate poisoning in a dog -- A case report. J Am Vet Med Assoc, 123:232-233, 1953.

Whitehead EI and Moxson AL: Nitrate poisoning. South Dakota Agricultural Experiment Station bulletin 424, 1952.

Williams MC: Accumulation of nitrate by annual goldeneye and showy goldeneye. J Range Mgmt, 42:196-198, 1989.

Wilson JK: Nitrate in foods and its relation to health. Agron J, 41:20-22, 1989.

Wilson JK: Nitrate in plants: Its relation to fertilizer injury, changes during silage making, and indirect toxicity to animals. Agron J, 35:279-290, 1943.

Winks WR, Sutherland AK, and Salisbury RM: Nitrite poisoning of pigs. Queensland J Agric Sci, 7:1-14, 1950.

Winter AJ: Studies on nitrate metabolism in cattle. Am J Vet Res, 23:500-505, 1962.

Winter AJ and Hokanson JF: Effects of long-term feeding of nitrate, nitrite, or hydroxylamine on pregnant dairy heifers. Am J Vet Res, 125:353-361, 1964.

Wood RD, Chaney CH, Waddill DG, and Garrison GW: Effect of adding nitrate or nitrite to drinking water on the utilization of carotene by growing swine. J Anim Sci, 26:510-513, 1967.

Wood PA: The molecular pathology of chronic nitrate intoxication in domestic animals: A hypothesis. Vet Human Toxicol, 22:26-27, 1980.

Woolley JL Jr, and Sigel CW: The role of dietary nitrate and nitrite in the reductive deamination of sulfadiazine by the rat, Guinea pig, and neonatal calf. Life Sci, 30:2229-2234, 1982.

Wright MJ and Davison KL: Nitrate accumulation in crops and nitrate poisoning in animals. Adv Agron, 16:197-247, 1964.

Würlmli R, Wolffram S, and Scharrer E: Inhibition of chloride absorption from the sheep rumen by nitrate. J Vet Med, 34:476-479, 1987.

Wyngaarden JB, Wright BM, and Ways P: The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland. Endocrinology, 50:537-549, 1952.

Yadav KP, Garner GB, O'Dell BL, Flynn LM, Bloomfield RA, and Muhrer ME: Iodine and nitrate as factors in vitamin A storage. J Anim Sci, 21:1017 (Abstract), 1962.

Yong C, Brandow RA, and Howlett P: An unusual cause of nitrate poisoning in cattle. Can Vet J, 31:118, 1990.

Zemla B: A possible association between quality of drinking water and stomach cancer incidence among native and immigrant populations of a selected industrial city. Neoplasma 27: 55-61, 1980.

Zimmerman JE, Neumann AL, Durdle WM, and Smith GS: Nutritive value of corn silages as affected by soil fertilization, with further observations of impaired carotene utilization in silage-fed steers. J Anim Sci, 21:1018 (Abstract), 1962.

CHAPTER 4

THE EFFECTS OF NITRATE CONTAINED IN DRINKING WATER ON THE PRODUCTIVITY AND HEALTH OF FARROWING SWINE

ABSTRACT

A cross-sectional study design was used to determine the effects of nitrate contained in drinking water on farrowing swine health and productivity. The study was conducted from November 1989 through February 1991 on randomly selected swine farms in the United States. The study consisted of 571 farms containing 27,207 farrowing swine. At the beginning and end of each farm's 3-month monitoring period, the drinking water provided to the farrowing swine was tested for the levels of nitrate and 15 other elements and compounds. Nitrate was detected in 53.2% (304/571) of the well-water samples with a mean concentration of 17.9 ppm and a median of 2.1 ppm. Pre-tested questionnaires administered to farm operators by trained personnel, were used to collect data on management, housing, and environmental parameters. During the farm's monitoring period, data on farrowing swine health and productivity were observed and recorded daily by the animal caretaker.

The data were analyzed on a farm basis using stratified analysis, and multiple linear regression or multiple logistic regression. No association was seen between the nitrate concentration of drinking water and farrowing swine productivity as measured by average litter size, average percentage of the litter stillborn, or the risk of having an

above median percentage of the litter born mummified. No association was seen between nitrate and the health of farrowing swine as measured by the risk of having an above median (>0) percentage of swine ill or dead due to farrowing problems, other reproductive problems, other known health problems, or unknown health problems. The statistical power of this study was .90 (at $\alpha=.05$) to detect if nitrate in the drinking water explained 3% (partial $R^2=.03$) of the variation in either farm average litter size or percent stillborn. Statistical power was sufficient to have detected an odds ratio (at $\alpha=0.05$, $\beta=.20$) as low as 1.3 for farrowing illness; 1.4 for illness due to reproductive, known, or unknown problems; 1.5 for death due to farrowing or known problems; 1.8 due to unknown problems; and 2.5 for death due to reproductive problems.

INTRODUCTION

Nitrate affects both animal (Bruning-Fann and Kaneene, 1993a) and human (Bruning-Fann and Kaneene, 1993b) health in a variety of ways. Methemoglobinemia from the consumption of nitrate-contaminated water was first reported in humans in 1945 (Comly, 1945). Cases continue to occur with the latest U.S. fatality reported in 1987 (Johnson et al., 1987). Many episodes of methemoglobinemia and abortion in animals have been attributed to acute nitrate toxicosis (Carrigan and Gardner, 1982; Haliburton and Edwards, 1978; Hibbs et al., 1978; Nicholls and Miles, 1980; Ruhr and Osweiler, 1986; Vermunt and Visser, 1987). Nitrates are suspected of causing "lowland abortion syndrome" in cattle (Simon et al., 1959a; Sund et al., 1957). When pregnant dairy cattle were given large doses of potassium nitrate, fetal death occurred followed by abortion (Simon et al., 1959b).

While the effects of acute nitrate toxicosis have been well described, the effects of chronic, low-dose exposure in humans and animals are unclear. Two epidemiological studies in humans found the consumption of drinking water from wells to be associated with congenital malformations and suggested nitrate contained in the water may have been responsible (Scragg et al., 1982; Dorsch et al., 1984). However, a review of nitrate in regard to teratogenic effects concluded that no such association has been shown (Fan et al., 1987). Some

animal studies found decreased conception rates (Davison et al., 1964) and increased stillbirths (Sleight and Atallah, 1968) attributable to nitrate. However, other studies reported no effect from nitrate exposure, on the maintenance of pregnancy (Davison et al., 1965; Eppson et al., 1960; Winter and Hokanson, 1964) or birth weights (Davison et al., 1965; Eppson et al., 1960). High levels of nitrate (3000 mg/L) in drinking water have been associated with thinning and dilation of coronary arteries in rats (Shuval and Gruener, 1972). In addition, nitrate has been linked to decreased immune response in rats (Kahraman, 1988; De Saint Blanquat, et al., 1983) and chickens (Atef et al., 1991).

Relatively little is known about the effects of nitrate in swine. Gastritis (Gwatkin and Plummer, 1946; Smith et al., 1959), abortion, stillbirths and deformities have been associated with the consumption of high levels of nitrate (Case, 1957). Nitrate ingestion has been linked to reduced weight gain (Tollett et al., 1960) and decreased liver storage of vitamin A in some studies (Koch et al., 1963; Garrison et al., 1966; Wood et al., 1967) but not in others (Anderson and Stothers, 1978; Seerley et al., 1965). Conflicting results were also reported on the effects of nitrate on farrowing swine productivity. One study reported an apparent decrease in the number of strong pigs born and their ability to survive when sows were given 2% potassium nitrate in their rations compared to control sows (Garner et al., 1958). However,

another study found no effect on corpora lutea number, percent implantation, ovary weight, embryo weight or placenta weight when up to 3.17% nitrate was added to the ration of gilts (Tollett et al., 1960). A third study reported no difference in litter size or average birth weight of pigs when sows were given drinking water containing up to 300 ppm (parts per million) nitrate (Seerley et al., 1965).

Although the toxic effects of large doses of nitrate have been clearly established in humans and animals, much controversy remains as to the effects of chronic low-level nitrate exposure. With nitrate levels increasing in groundwater (Fraser and Chilvers, 1981; Shuval and Gruener, 1972; Vigil et al., 1965; Holländer and Sander, 1987; Møller et al., 1989), it is important to establish the significance of chronic low-level nitrate exposure to health. The objective of this study was to determine if nitrate, at levels currently found on swine farms, adversely affects the productivity or health of farrowing swine.

Hypotheses Tested:

The nitrate level of drinking water is associated with the farm:

- 1) average number of pigs born per litter,
- 2) average percentage of the litter stillborn,

The nitrate level of drinking water is associated with the risk of a farm experiencing:

- 3) above median percentage of the litter born mummified,
- 4) above median percentage of swine with farrowing problems,
- 5) above median percentage of swine with reproductive problems other than farrowing problems,
- 6) above median percentage of farrowing swine with other health problems,
- 7) above median percentage of farrowing swine with unknown health problems,
- 8) above median mortality rate due to farrowing problems,
- 9) above median mortality rate due to reproductive problems other than farrowing problems,
- 10) above median mortality rate due to other known problems, and
- 11) above median mortality rate due to unknown problems.

MATERIALS AND METHODS

National Swine Survey

The data used in this report are a portion of the data collected during the National Swine Survey (NSS) conducted by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service's, National Animal Health Monitoring System (NAHMS). The NSS was conducted on swine farms located in 18 states (Alabama, California, Colorado, Georgia, Iowa, Illinois, Indiana, Maryland, Michigan,

Minnesota, Nebraska, North Carolina, Ohio, Oregon, Pennsylvania, Tennessee, Virginia and Wisconsin) from November 1989 through February 1991. The NSS was a hybrid epidemiologic study utilizing retrospective, prospective and cross-sectional study designs. The data used in this analysis were collected in a cross-sectional manner. Further information on the entire NSS is contained in the NSS technical report (USDA, 1992).

Sample Selection

The sample size was determined primarily by the desire to estimate preweaning mortality within 1% of true mortality using the following formula:

$$n = \frac{s^2 z^2}{d^2}$$

where:

n = the sample size

s² = the variance of the proportion

z² = z value for the appropriate confidence level

d² = the acceptable bound on the proportion estimate

Preweaning mortality was thought to be approximately 13% with a usual range of 0% to 35%. The variance for the proportion of preweaning mortality was estimated using three different methods (s²=pq; s²=AB² [using the Gamma distribution where A identifies the shape of the distribution and B is the

mean]; $s^2 = (2p \text{ bar})^2$ [assumes the relationship of the standard deviation to the mean is 2]). Ultimately, it was decided that 1,400 farms would be sufficient to estimate the true preweaning pig mortality rate with a confidence level of 99%.

The sampling process began with the selection of States to be included in the NSS. Thirteen States were preselected for inclusion in the study because of their prior involvement in NAHMS projects. These States are Alabama, California, Colorado, Georgia, Iowa, Illinois, Maryland, Michigan, Ohio, Oregon, Tennessee, Virginia, and Wisconsin. Indiana and North Carolina were also preselected because of their large swine populations and strong interest in participating in the project. All remaining States which contained 2% or more of the nation's swine population were eligible to be included in the NSS. Remaining States under consideration were placed in 1 of 2 strata by average herd size.

Stratum 1: Kansas, Minnesota, Missouri, Nebraska, and South Dakota (states with larger average herd sizes; criteria for farm eligibility was 10 or more sows expected to farrow).

Stratum 2: Kentucky, Pennsylvania, South Carolina, and Texas (states with smaller average herd sizes; criteria for farm eligibility was 1 or more sows expected to farrow).

A proportionate sample was selected based on the number of hogs in the state. This resulted in the selection of Minnesota, Nebraska, and Pennsylvania for participation in the NSS. The 15 preselected States plus the 3 probability selected States accounted for 62% of the swine operations and 81% of the hogs in the nation. Because the probability selected States are representative of the States in their respective strata, Minnesota and Nebraska represent the 3 remaining Stratum 1 States and Pennsylvania represents the 3 remaining Stratum 2 States. Therefore, the 18 selected States represent a total of 24 States which contain 84% of the U.S. swine operations and 95% of the swine population.

Potential study participants were identified by the USDA's National Agricultural Statistics Service (NASS). Criteria for farm eligibility in the study were designed to account for differences in average herd sizes among the States. In States with a small average herd size (Alabama, California, Colorado, Maryland, North Carolina, Oregon, Pennsylvania and Virginia), farms with one or more sows expected to farrow during the study period were eligible to participate in the NSS. In the remaining 10 States (Georgia, Illinois, Indiana, Iowa, Michigan, Minnesota, Nebraska, Ohio, Tennessee and Wisconsin), farms were eligible if 10 or more sows were expected to farrow during the study period.

Swine farms to be included in the NSS were selected by NASS using a multiple frame sampling technique. This consists

of using both list (a roster of all eligible units) and area frames (the actual land area is divided into sections, all swine farms in chosen sections are sampled) to select farms for participation in the NSS.

Farm population = farms from list estimate + farms from area estimate that are not included on list estimate

NASS personnel contacted randomly selected farms and explained the NSS. During this visit, the General Swine Survey was completed. Out of 3,184 farms initially identified by NASS, 2,965 were located and met the criteria for eligibility in the NSS. Of the 2,965 eligible farms, 1,320 refused to participate in the NSS and 3 farms were mislaid prior to arrival at the NAHMS office. Of the 1,642 farm contacts received by NAHMS from NASS, 746 operators refused to participate in the study, 65 were ineligible, 16 were never visited, and 815 agreed to participate (52.2% [815/1,561]). Of the 815 farm operators who started the NSS, 103 left the study prior to completion. The response rate for completion of the NSS was 45.6% (712/1561). Although a total of 712 farms completed the study, data were excluded from 141 farms because a water source other than a well was used ($n = 106$) or because 2 well-water samples were not obtained ($n = 35$).

Ultimately, data from 571 swine farms were utilized in this analysis.

Data Collection

Individual swine farms were monitored for 3 months with the period of surveillance staggered throughout the year. In this way, approximately equal numbers of farms were monitored during each month of the study year. To ensure comparability of data, training of NASS and NAHMS personnel was accomplished prior to the first farm visit.

NASS personnel completed a General Swine Farm Report during the initial visit to the swine producer. This questionnaire emphasized current management practices and other descriptive farm information. Farms whose operators agreed to participate in the NSS were assigned to federal and state veterinarians associated with NAHMS. These veterinarians visited the farms on a monthly basis to collect the farrowing diary cards and administer three more questionnaires. Examples of the questionnaires and the farrowing diary cards can be found in the NSS technical report (USDA, 1992).

Completion of a Swine Health Report by a NAHMS veterinarian during the second visit to each farm initiated the start of the farm's monitoring period. This questionnaire dealt with current vaccination and other preventive practices,

biosecurity and health problems experienced on the farm during the past year.

A NAHMS veterinarian completed a Swine Facilities and Feed Report on the third farm visit. Housing during all phases of swine production was delineated with emphasis on the monitored farrowing facilities. In addition, the composition of all the feeds used on the swine farm during all phases of production was described in detail.

A Swine Ending Inventory and Economics Report was completed on the last farm visit by a NAHMS veterinarian, which occurred at the end of each farm's monitoring period. Information was gathered concerning purchases and sales of swine, operating expenses, the amount of labor used in the operation and the current swine inventory.

On farms expecting less than 100 farrowings during the 3 month monitoring period, all farrowing sows were monitored; on farms expecting 100 or more farrowings, only sows entering selected farrowing units were monitored. The NAHMS staff selected farrowing units to be monitored using simple random sampling. Data from a total of 27,207 sows on 571 farms in 18 states were used in this analysis.

A Farrowing Diary Card was completed for each monitored sow and her litter. The animal caretaker recorded on the diary cards all birth events, health events and preventive practices as they occurred to the sow and her litter. Cases of illness or death were classified into one of eight broad

categories of disease. Cards were initiated for each sow in the monitored farrowing facility at the start of the farm's monitoring period and for each sow that entered the monitored facility during that period. Only sows that entered the farrowing facility, farrowed and weaned their litters during the farm's monitoring period are included in this analysis.

Water Sampling

Water samples were collected from participating farms at the first and last interviews (3 months between samples). Samples from the water supply that served the farrowing unit were collected as close to the point of water consumption as possible. To ensure that the water sample was representative of the well, the pipes were flushed by running the water for at least one minute prior to sampling. All samples were shipped by priority mail (delivery by the second day) to the USDA's National Veterinary Services Laboratories (NVSL) for analysis. Instructions for water sample collection and shipment were provided to the veterinarians.

Laboratory Testing of Water

NVSL analyzed the water samples for a number of elements and compounds using an inductively coupled argon plasma emission spectrophotometer and an ion chromatograph.

Calibration standards were included at the beginning and end of every batch, and control samples with known ion concentrations were analyzed after every 10 samples. In cases where the values were extremely high or the results were in question, the analyses were repeated.

Data Analysis

Measures of Health and Productivity

Using the farm as the unit of comparison, various measures were calculated in order to assess sow productivity and health. Sow productivity was measured by determining the average litter size (Avg Litter Size), the average percentage of pigs stillborn (Avg % Stillborn), and the average percentage of pigs born mummified (Avg % Mummified). The average litter size was calculated by dividing the total number of pigs born by the number of swine which farrowed. Similarly, the average percentage of pigs stillborn (or born mummified) was calculated by dividing the total number of pigs stillborn (or born mummified) by the total number of pigs born. The percentage of sows on each farm ill or dead were calculated for each of the following disease categories: farrowing problems (% Ill-Farrowing), reproductive problems other than farrowing (% Ill-Other Repro. Prob.), other known health problems (problems recognized by the animal caretaker

that are not included in other disease categories [% Other Known Illness]), and unknown health problems (% Unknown Illness). The percentage of sows ill for each disease category was calculated by dividing the number of sows experiencing each disease problem by the number of sows at risk for experiencing that particular disease problem during the 3-month monitoring period. Similarly, the percentage of sows dead in each disease problem category was determined by dividing the number of sows dead in each disease category by the number at risk. For use in stratified analysis and logistic regression, the farm percentage of mummies and sows ill or dead for each disease category were dichotomized as above the median or not. These variables and their descriptions are provided in Tables 4.1a.

Variables

To quantify the impact of nitrate (the independent variable of interest) on the various measures of sow health and productivity (the dependent variables), other factors presumed likely to influence the dependent variables were controlled in the analyses. A review of the literature suggested that in addition to nitrate, a number of other factors might affect farrowing sow productivity or health (Leman et al., 1988; National Academy of Sciences, 1974; Fraser et al., 1990). These potentially confounding and/or

effect modifying variables are the levels of ammonia, barium and nitrite in the drinking water, the total number of swine on the farm (TtlSwn), the years of continuous swine farm experience (YrsSwn), and the farm average sow parity (AvgPar). Additional variables include how the farrowing unit was managed (all-in all-out or continuous farrowing [TypeFar]), vaccination against *Actinobacillus pleuropneumonia* (Actino), atrophic rhinitis (Rhinitis), parvovirus (Parvo), leptospirosis (Lepto), pseudorabies (Pseudo), *Escherichia coli*, rota virus (Rota V), *Clostridium perfringens* (Clos), erysipelas (Erysip), transmissible gastroenteritis (TGE), other diseases (Other V), the use of antibiotics in the feed (Antibio F), and whether the farm manager administers anthelmintics (Anthel). These variables and their descriptions are provided in Tables 4.1b.

Statistical Analysis

The data were examined using descriptive statistics, Spearman correlations, univariate logistic regression, stratified analysis, multiple linear regression, and multiple logistic regression (SAS Institute Inc., 1988; SAS Institute Inc., 1990).

Descriptive statistics allow comparison of the composition of water and selected characteristics of the swine population with other studies. Generation and examination of

these statistics afforded familiarity with the data, suggested appropriate points at which to stratify the continuous variables, and allowed determination of whether the data met certain assumptions. Because most of the data were not normally distributed, nonparametric statistics were employed.

Spearman correlations were used to examine the relationships between the individual independent and dependent variables and between nitrate and other independent variables. Those variables associated with both nitrate and the dependent variable(s) had the potential to confound the relationships between nitrate and the dependent variables. To control for confounding, those correlations with a statistical significance of $P \leq 0.2$ were deemed portentous (Maldonado and Greenland, 1993) and were included in stratified analysis and multiple logistic regression.

It is recommended that univariate logistic regression be accomplished prior to multiple logistic regression (Hosmer and Lemeshow, 1989). Univariate logistic regression was performed on all independent variables hypothesized to be associated with the dependent variables. This procedure aided in the determination of whether any important predictor variables had been missed with correlation analysis.

Stratified analysis was performed to assess effect modification and to control confounding. Unadjusted odds ratios estimated only the effect of nitrate on the dependent variables without controlling (adjusting) for the effect of

other independent variables. Adjusted odds ratios estimated the effect of nitrate while controlling (adjusting) for the effect of other independent (predictor) variables on sow health and productivity. The association between nitrate and decreased sow productivity was assessed as the odds ratios for 1) below-median farm average litter size, 2) above-median farm percentage stillborn, and 3) above-median farm percentage born mummified (Table 4.1a). Similarly, the association between nitrate and sow health was measured by the odd ratios for above-median (>0) percent illness and mortality for each of the categories (4.1a). Because only 25 farms had nitrate levels ≥ 100 ppm, the human limit of 45 ppm was used during stratified analysis when control variables were included.

Multiple linear and logistic regression were utilized to examine the effect of nitrate on sow productivity and health. This allowed the use of nitrate as a continuous variable while controlling for the effect of other predictor variables on the dependent variables. While it is difficult to control for more than a few variables with stratified analysis (due to vacant cells), multivariate regression procedures can simultaneously control many independent variables. In doing so, joint confounding can be controlled. With multiple logistic regression, odds ratios were calculated for the association of nitrate with sow productivity and health by exponentiating the maximum likelihood-derived coefficients (Hosmer and Lemeshow, 1989).

RESULTS

Results of water analysis revealed that the mean concentration of nitrate in well-water during the NSS was 17.9 ppm with a median of 2.1 ppm (Table 4.2). No nitrate was detected in 46.8% (267/571) of the well-water samples. Tables 4.3a-4.3b describe the size of swine farms, years of swine experience, and frequency of selected management practices on farms which participated in the NSS. The general state of farrowing swine productivity and health of the study farms is presented in Table 4.3c.

Spearman correlations among the independent and dependent variables are presented in Tables 4.4a-4.4f. There are statistically significant correlations ($P \leq 0.05$) between nitrate and several other independent variables (ammonia, nitrite, the total number of swine on the farm and vaccination against atrophic rhinitis and pseudorabies). However, the largest correlation between these independent variables is $-.251$, providing little evidence that multicollinearity among the independent variables would be a problem. Numerous independent variables were correlated ($P \leq .20$) with the dependent variables. Nitrate and independent variables correlated with the dependent variables were included in stratified analysis and multiple logistic regression. Univariate logistic regression did not disclose any additional

independent variables to include in stratified analysis and multiple logistic regression.

A comparison of swine farms stratified by drinking-water nitrate levels (45 ppm) revealed no significant differences in the distribution of other independent variables except for ammonia and vaccination of the sow against parvovirus or leptospirosis (Tables 4.5a-4.5b). There are no significant differences in sow health or productivity between farms with levels of nitrate ≥ 45 ppm and those < 45 ppm (Table 4.5c).

With stratified analysis, the unadjusted odds ratios for the various measures of sow health and productivity on farms exposed to nitrate ≥ 45 ppm, and nitrate ≥ 100 ppm are displayed in Table 4.6. Neither concentration of nitrate demonstrates any significant association with sow health or productivity.

The results of stratified analyses for the associations between nitrate ≥ 45 ppm and the various measures of sow health and productivity while controlling (adjusting) for other factors are contained in Tables 4.7a-4.7k. Mantel-Haenszel estimators provided adjusted summary odds ratio estimators which did not significantly differ from the unadjusted odds ratios, thereby providing no evidence of confounding. The Breslow-Day statistic tests the null hypothesis that there is no difference in odds ratios between any of the strata. Lack of significance of the Breslow-Day tests implies uniformity of the stratum-specific odds ratios providing evidence that effect modification is not occurring (Breslow and Day, 1980).

Multiple linear regression models did not reveal any association between nitrate contained in drinking water and sow productivity as assessed by the farm average litter size (Table 4.8a), or percentage of the litter stillborn (Table 4.8b). This study could have detected if nitrate in the drinking water explained 3% of the variation in either farm average litter size or percent stillborn, with a power of .90 at $\alpha = .05$. Multiple logistic regression revealed no association between nitrate and the risk of a farm experiencing an above median percentage of the litter born mummified (Table 4.8c). Similarly, no effect was seen on the health of sows as measured by the risk of a farm experiencing above median farrowing swine morbidity and mortality due to farrowing problems (Tables 4.8d-4.8e), reproductive problems other than farrowing problems (Tables 4.8f-4.8g), other known health problems (Tables 4.8h-4.8i) or unknown health problems (Tables 4.8j-4.8k).

The statistical power of this study was determined by utilizing the tables published by Cohen (1988) for linear regression and those by Hsieh (1989) for logistic regression. In regards to logistic regression, with the sample size of this study (571), the smallest detectable difference (odds ratio) for several levels of power can be found in Table 4.9. The level of statistical power varies with the probability of the event occurring. With a sample size of 571, an odds ratio as low as 1.3 for illness due to farrowing problems could have

been detected (if one existed) at $\alpha=0.05$ with a statistical power of 80% (Table 4.9). Similarly, statistical power was sufficient to have detected an odds ratio (at $\alpha=0.05$, $\beta=.20$) as low as 1.4 for illness due to reproductive, known, or unknown problems; 1.5 for death due to farrowing or known problems; 1.8 due to unknown problems; and 2.5 for death due to reproductive problems.

DISCUSSION

The NSS was a large swine study designed to reflect the swine population and swine farms of the U.S. However, not all swine farms initially selected for the study, chose to participate. A comparison of respondents versus nonrespondents (based on data previously reported to NASS regarding herd size, litter size, and litter mortality) revealed no significant differences in regards to litter size or mortality. However, both total herd and farrowing herd sizes were larger with NSS participants compared to nonparticipants. Therefore, the herd sizes reported with the NSS are larger than those which would have been reported, had all selected farms chosen to participate.

Investigators have reported dystocia affecting 2.9% (103 farrowings on 5 farms) (Randall, 1972a), 0.25% (772 farrowings) (Jones, 1966), and 1.54%-2.50% (70 farms) (Lingaas and Rønnigen, 1991) of farrowing sows. The overall farm

average percentage of sows ill due to farrowing problems in this investigation is 2.1%. Because this category of illness includes dystocia plus other farrowing-related problems (i.e. prolapsed uterus), this figure is not immediately comparable with the previously mentioned investigations. However, it is thought that dystocia is by far the largest portion of farrowing related problems.

Previous studies have reported 1.8% (24/125) (Randall, 1972b) and 1.6% (228/14,390) (Billie et al., 1974) of all pigs born were mummified; 5% (67/125) (Randall, 1972b) and 4.3% (622/14,390) (Billie et al., 1974) stillborn. Another study reported that a total of 7.6% (4,366/57,195) of the pigs born were stillborn or mummified (Partlow et al., 1993). These findings are similar to the average farm rates of 1.4% mummified and 6.9% stillborn observed in this study. Comparing my study with other investigations is somewhat tenuous in that there are several differences between the studies. The previous studies were conducted in England, Canada, Norway, and Denmark while this investigation was done in the U.S. This study is much larger than the other studies as it involves 27,207 farrowings on 571 swine farms. Also, unlike the previously mentioned studies which were performed on an individual animal basis, this study utilized the farm as the unit of analysis.

The presence of water samples with no nitrate detected is an impediment to statistical analysis. The occurrence of

samples with values below detection limits is common in environmental water sampling and can be handled in a variety of ways (Newman et al., 1989; Hurd, 1993). In this study, values below the detection level were recorded as 0. This method will, to some extent, bias the estimate of the mean downward while increasing the estimate of the standard deviation.

Nitrate levels measured in this study exceeded the E.P.A.'s maximum concentration of 45 ppm for human drinking water in 12.1% (69/571) of the wells sampled. This figure is higher than estimates of 6.4% by the U.S. Geological Survey or 2.4% by the E.P.A. (U.S.D.A., 1991). However, the level of 12.1% found in this study compares favorably with the 10% reported by the Monsanto Agricultural Products Company (USDA, 1991). The differences in estimates of nitrate contamination of wells is probably related to the different criteria for well selection. The U.S. Geological Survey and the E.P.A. sampled household wells, whereas the NSS and the Monsanto study sampled wells on farms.

In order to assume that no difference exists when there is insufficient evidence to reject the null hypothesis, the statistical power (power = $1 - \beta$ where β is the type II error) of a study must be sufficiently large. Statistical power is the probability of detecting a difference if, in fact, one exists. Without sufficient power a study may not detect any effect from an exposure, although in reality the factor does

affect the outcome of interest. Adjustment for covariates in logistic regression either has no effect or results in a loss of precision (Robinson and Jewell, 1991). To maintain the same level of precision, the sample size may need to be increased. The amount of increase in sample size is directly related to the amount of correlation between the covariate of interest (nitrate) and the remaining covariates. To calculate the increase in sample size needed, the following formula may be used (Hsieh, 1989):

$$n_1 = n_0 / (1 - \rho^2)$$

n_0 = number of samples needed when only the covariate of interest is included in the model,

n_1 = number of samples needed after adjustment for other covariates and

ρ = multiple correlation coefficient relating the specific covariate of interest to the remaining covariates.

From the formula, it can be seen that the greater the correlations among covariates, the larger the sample size must be to achieve the same level of power to detect an identical amount of difference (difference in this study refers to the odds ratio). Because the amount of correlation between covariates in this study is small, ρ^2 is also small. Consequently, no modification of these tables was deemed necessary to adjust for covariates.

Had any of the covariates been effect modifiers, the odds ratios would not have remained constant across the strata. No evidence of confounding was seen as the odds ratios did not appreciably change with adjustment for other covariates.

When included in multivariate analysis, nitrate was not associated with farrowing sow health or productivity. Controlling for potentially confounding and/or effect modifying factors did not change the results. The power level achieved with this study was sufficiently high to have detected even a moderate level of effect from nitrate, if such an effect existed.

The lack of any detectable effect from nitrate in a study this powerful, indicates that the concentration of nitrate seen in well-water during the NSS does not affect the health or productivity of farrowing swine as measured by the criteria utilized in this investigation. These findings agree with studies that found no effect from nitrate on sow productivity (Seerley et al., 1965; Tollett et al., 1960). A previous study linking nitrate to decreased productivity (Garner et al., 1958) was conducted with a small number of animals, so those results may be unreliable.

Because of the paucity of information on the effects of low levels of nitrate on swine health and productivity, collaborative studies would serve to solidify these conclusions. In addition, this study only examined the effects of nitrate contained in drinking water on farrowing

sow health. Further research is needed to determine the effects of nitrate on swine during other stages of production.

Table 4.1a: Dependent Variables Utilized In Statistical Analysis

Variable	Description
AvgLitSize	Farm average number of pigs born per litter
%StillBorn	Farm average % of litter stillborn
%Mummies	Above-median average % of litter born mummified
% Ill-Farrowing	Above-median % of swine with problems at farrowing
% Ill-Other Repro. Prob.	Above-median % of swine with reproductive problems other than farrowing problems
% Ill-Other Known Illness	Above-median % of farrowing swine with other known (recognized) health problems
% Ill-Unknown Illness	Above-median % of farrowing swine with unknown health problems
% Mort-Farrowing	Above-median mortality rate due to farrowing problems
% Mort- Other Repro. Prob.	Above-median mortality rate due to reproductive problems other than farrowing problems
% Mort-Other Known Cause	Above-median mortality rate due to other known health problems
% Mort-Unknown Cause	Above-median mortality rate due to unknown causes

Table 4.1b: Independent Variables Utilized In Statistical Analysis

Variable	Description	Coding
Nitrate	Nitrate level of water in ppm	Continuous
Ammonia	Ammonia level of water in ppm	Continuous
Barium	Barium level of water in ppm	Continuous
Nitrite	Nitrite level of water in ppm	Continuous
YrsSwn	Number of years of continuous swine farming	Continuous
TtlSwn	Total number of swine on the farm	Continuous
AvgPar	Average sow parity	Continuous
TypeFar	Type of farrowing operation (all in/ all out or continuous farrowing)	Categorical
Vaccination against:		
Clos	Clostridium perfringens	0, 1
E. coli	Escherichia coli	0, 1
Erysip	Erysipelas	0, 1
Actino	Actinobacillus pleuropneumonia	0, 1
Lepto	Leptospirosis	0, 1
Parvo	Parvovirus	0, 1
Pseudo	Pseudorabies	0, 1
Rhinitis	Atrophic rhinitis	0, 1
Rota V	Rotavirus	0, 1
TGE	Transmissible gastroenteritis	0, 1
Other V	Other diseases	0, 1
Use of:		
Anthel	Anthelmintics	0, 1
Antibio F	Antibiotics in the feed	0, 1

Table 4.2: Results of water analysis on swine farms: National Swine Survey, 1989 - 1991.

Variable	# Detected	Mean	Median	Range	S.D.
Ammonia	152	0.3	0	0-18.9	1.1
Barium	270	0.1	0	0-1.8	0.2
Bromide	*				
Calcium	571	71.4	68.7	0.3-580.5	52.1
Chloride	473	20.1	7.6	0-715.1	48.4
Fluoride	*				
Iron	54	0.1	0	0-4.6	0.3
Lithium	*				
Magnesium	570	36.7	39.2	0-152.9	20.0
Nitrate	304	17.9	2.1	0-460.6	39.0
Nitrite	8	0.1	0	0-18.9	0.9
Phosphate	103	0.1	0	0-1.8	0.1
Potassium	484	5.3	2.8	0-173	5.3
Sodium	571	67.1	21.2	3-1,318.2	138.4
Sulfate	491	83.2	25.9	0-2,240.8	187.1
Zinc	90	0.1	0	0-2.1	0.2

All concentrations are measured in parts per million; # Detected = the number of wells with detectable concentrations of each substance; S.D. = standard deviation; * = all samples were below detection limits; a value of zero was used in calculating the mean and median when the concentration of a substance was below detection limits.

Table 4.3a: Selected characteristics of swine farms: National Swine Survey, 1989 - 1991.

Variable	Mean	Median	Range	S.D.
Total Number of Swine	1642	787	3-174,735	7513.1
Years of Farming	25.8	20	0-150	22.3
Average Sow Parity*	3	3	1-11	1.2
Nitrate (ppm)	17.9	2.1	0-460.6	39.0
Nitrite (ppm)	0.1	0	0-18.9	0.9
Ammonia (ppm)	0.3	0	0-18.9	1.1
Barium (ppm)	0.1	0	0-1.8	0.2

S.D. = standard deviation; * N = 546 farms; ppm = parts per million.

Table 4.3b: Prevalence of selected practices on swine farms: National Swine Survey, 1989 - 1991.

Variable	%
Vaccinate for:	
Actinobacillus	9.5
Atrophic Rhinitis	45.2
Parvovirus	69.5
Leptospirosis	77.8
Pseudorabies	22.2
E. coli	52.9
Rota virus	18.9
Clostridium	26.3
Erysipelas	68.7
Transmissible	
gastroenteritis	29.9
Other vaccines	18.7
Type Farrowing	
(all-in all-out)	55.5
(continuous)	44.5
Use of Antibiotics	
in the Feed	41.2
Use of Anthelmintics	86.2

**Table 4.3c: Measures of sow health and productivity on swine farms:
National Swine Survey, 1989 - 1991.**

Variable	Mean	Median	Range	S.D.
Average Litter Size	10.3	10.5	5-16.2	1.43
Percentage Stillborn	6.9	6.3	0-50	4.31
Percentage Mummified	1.4	0.8	0-66.7	3.26
% Illness-Farrowing	2.1	0	0-100	7.99
% Illness-Other Reproductive	1.9	0	0-63.9	5.02
% Other Known Illness	0.5	0	0-25	1.69
% Unknown Illness	0.8	0	0-76.8	4.11
% Mortality-Farrowing	0.2	0	0-20	1.15
% Mortality-Other Reproductive	0.1	0	0-7.7	0.47
% Mortality-Other Known Cause	0.2	0	0-10	0.85
% Mortality-Unknown Cause	0.1	0	0-4.6	0.46

S.D. = standard deviation.

Table 4.4a: Spearman correlations between nitrate, potential confounders and measures of sow productivity on swine farms: National Swine Survey, 1989-1991.

	Nitrate	Barium	Ammonia	Nitrite	TtlSwN	YrSwN	AvgPart	TypePar
Nitrate	.036 (.385)*	-.251 (.001)	-.056 (.168)	-.115 (.006)	-.001 (.998)	-.014 (.737)	.050 (.233)	
Avg Litter Size	.040 (.335)	-.379 (.375)	-.078 (.064)	.029 (.494)	.111 (.008)	.034 (.412)	.139 (.001)	-.110 (.008)
Avg % Stillborn	-.026 (.530)	.068 (.106)	-.035 (.399)	.029 (.492)	.029 (.492)	.049 (.241)	.062 (.150)	.031 (.462)
Avg % Mummified	-.002 (.957)	.016 (.703)	-.081 (.052)	.031 (.458)	.075 (.002)	-.087 (.928)	.110 (.052)	-.103 (.014)

† N = 546, * numbers in parentheses = P value, bolded are significant at P≤0.2.

Table 4.4b: Spearman correlations between nitrate, potential confounders and measures of sow productivity on swine farms: National Swine Survey, 1989-1991.

	TGE	E.coli	Rota V	Clos	Actino	Erysip	Rhinitis
Nitrate	-.008 (.848)	-.051 (.223)	.005 (.911)	-.025 (.559)	.003 (.945)	-.006 (.891)	-.115 (.006)
Avg Litter Size	.076 (.070)	.047 (.261)	.034 (.417)	.074 (.076)	-.028 (.506)	.088 (.036)	-.008 (.847)
Avg % Stillborn	-.031 (.453)	-.058 (.165)	-.088 (.035)	-.025 (.551)	.016 (.695)	-.013 (.758)	-.001 (.984)
Avg % Mummified	-.022 (.603)	.044 (.291)	-.014 (.732)	.052 (.216)	-.036 (.387)	.028 (.510)	.064 (.125)

* numbers in parentheses = P value, bolded are significant at $P \leq 0.2$.

Table 4.4c: Spearman correlations between independent variables and measures of sow productivity: National Swine Survey, 1989-1991.

	Parvo	Lepto	Pseudo	Other V	Anthel†	Antibio F
Nitrate	-.034 (.368)	.017 (.682)	-.091 (.023)	.019 (.657)	.010 (.804)	-.003 (.950)
Avg Litter Size	.083 (.046)	.113 (.007)	-.021 (.611)	-.072 (.085)	.015 (.722)	.027 (.525)
Avg % Stillborn	-.010 (.812)	.031 (.456)	-.041 (.334)	-.047 (.260)	-.004 (.926)	-.007 (.866)
Avg % Mummified	.040 (.337)	-.005 (.903)	-.009 (.835)	.005 (.912)	.024 (.569)	-.001 (.987)

† N = 492, * numbers in parentheses = P value, bolded are significant at $P \leq 0.2$.

Table 4.4d: Spearman correlations between independent variables and measures of sow health: National Swine Survey, 1989-1991.

	Nitrate	Barium	Ammonia	Nitrite	TtlSwN	YrsSwN	AvgPart	TypeFar
% Ill-Farrowing	-.001 (.983)	-0.00 (1.00)	.007 (.870)	.065 (.118)	.130 (.002)	-.004 (.928)	.083 (.052)	-.076 (.071)
% Ill-Other Repro. Prob	-.023 (.583)	-.022 (.601)	.016 (.710)	.064 (.126)	.044 (.295)	-.013 (.751)	.001 (.998)	-.059 (.160)
% Other Known Illness	-.090 (.032)	-.063 (.134)	.012 (.772)	.110 (.009)	.120 (.004)	-.016 (.670)	.028 (.508)	-.015 (.728)
% Unknown Illness	.002 (.963)	-.052 (.217)	.030 (.471)	.014 (.736)	.196 (.001)	-.072 (.084)	.111 (.010)	-.051 (.225)
% Mort-Farrowing	-.050 (.229)	-.024 (.570)	.104 (.013)	.062 (.141)	.164 (.001)	.059 (.157)	.082 (.056)	-.047 (.260)
% Mort-Other Repro. Prob.	-.023 (.591)	-.045 (.284)	.063 (.134)	-.017 (.677)	.036 (.392)	-.006 (.889)	-.055 (.197)	.065 (.118)
% Mort-Other Known Cause	-.072 (.084)	-.028 (.507)	-.029 (.494)	.069 (.102)	.148 (.001)	-.048 (.253)	-.029 (.492)	-.068 (.104)
% Mort-Unkn. Cause	-.006 (.877)	.020 (.635)	.108 (.010)	.119 (.005)	.108 (.010)	-.091 (.030)	.049 (.252)	-.012 (.782)

† N = 546, * numbers in parentheses = P value, **bolded** are significant at $P \leq 0.2$.

Table 4.4e: Spearman correlations between independent variables and measures of sow health: National Swine Survey, 1989-1991.

	TGE	E.coli	Rota V	Clos	Actino	Erysip	Rhinitis
% Ill-Farrowing	.003 (.948)	.077 (.068)	-.004 (.924)	.047 (.263)	.082 (.052)	.081 (.052)	.047 (.258)
% Ill-Other Repro. Prob.	-.024 (.563)	-.008 (.840)	-.053 (.204)	.021 (.615)	-.020 (.631)	.035 (.402)	-.026 (.538)
% Other Known Illness	-.042 (.313)	.007 (.871)	-.020 (.640)	.003 (.945)	-.033 (.428)	.060 (.156)	.055 (.189)
% Unknown Illness	-.044 (.292)	.052 (.215)	-.014 (.744)	-.008 (.854)	.071 (.092)	.115 (.006)	.045 (.279)
% Mort-Farrowing	.002 (.957)	.038 (.366)	-.007 (.870)	.014 (.740)	.051 (.220)	.090 (.031)	.053 (.210)
% Mort-Other Repro. Prob.	-.016 (.705)	-.033 (.435)	-.009 (.837)	-.032 (.442)	.036 (.389)	.046 (.276)	.063 (.132)
% Mort-Other Known Cause	-.042 (.322)	-.009 (.837)	-.030 (.473)	-.046 (.276)	-.073 (.082)	.058 (.167)	.013 (.752)
% Mort-Unknown Cause	-.003 (.945)	.058 (.168)	-.011 (.791)	.055 (.192)	.020 (.632)	.065 (.122)	.090 (.031)

* numbers in parentheses = P value, bold are significant at $P \leq 0.2$.

Table 4.4f: Spearman correlations between independent variables and measures of sow health: National Swine Survey, 1989-1991.

	Parvo	Lepto	Pseudo	Other V	Anthel†	Antibio F
% Ill-Farrowing	.061 (.143)	.069 (.102)	.011 (.796)	.018 (.669)	-.019 (.650)	.001 (.986)
% Ill-Other Repro. Prob.	.002 (.958)	.006 (.878)	-.044 (.289)	-.040 (.338)	.024 (.574)	.003 (.938)
% Other Known Illness	.033 (.431)	.070 (.093)	.022 (.606)	-.015 (.721)	-.007 (.866)	-.053 (.210)
% Unknown Illness	.050 (.229)	.066 (.115)	.093 (.026)	-.026 (.535)	-.057 (.168)	-.041 (.330)
% Mort-Farrowing	.052 (.211)	.102 (.015)	.091 (.029)	.019 (.644)	.014 (.742)	.067 (.110)
% Mort-Other Repro. Prob.	-.009 (.832)	.020 (.638)	-.049 (.243)	-.039 (.347)	-.047 (.259)	.050 (.233)
% Mort-Other Known Cause	.015 (.729)	.080 (.056)	.043 (.306)	-.030 (.471)	-.034 (.420)	-.038 (.359)
% Mort-Unknown Cause	.082 (.051)	.090 (.031)	.161 (.001)	.034 (.413)	.008 (.852)	.038 (.360)

† N = 492, * numbers in parentheses = P value, bolded are significant at P≤0.2.

Table 4.5a: Selected characteristics of swine farms stratified according to the level of nitrate in the drinking water: National Swine Survey, 1989-1991.

Variable	<45 ppm Nitrate 502 farms			>45 ppm Nitrate 69 farms			Wilcoxon
	Mean	Median	Range	Mean	Median	Range	
Total Swine	1,539	802.5	3-174,735	934	698	21-4,526	.221
Years Farming	25	20	0-150	25	20	1-130	.744
Avg Sow Parity	2.9†	3.0	1-11	2.9†	2.7	1-7.6	.448
Nitrate (ppm)	6.8	0	0-44.5	98.4	79.3	45.8-460.7	-
Nitrite (ppm)	0.1	0	0-18.9	0	0	0-0	.293
Ammonia (ppm)	0.3	0	0-18.9	0.1	0	0-1.6	.001
Barium (ppm)	0.1	0	0-1.8	0.2	0.1	0-1.8	.091

Wilcoxon = P value for the Wilcoxon rank sum test; † N = 479 farms; ‡ N = 67 farms; ppm = parts per million.

Table 4.5b: Prevalence of selected practices on swine farms stratified according to the level of nitrate in the drinking water: National Swine Survey, 1989 - 1991.

Variable	<45 ppm Nitrate†		≥45 ppm Nitrate†		Wilcoxon
	%		%		
Vaccinate for:					
Actinobacillus	10.2		4.3		.123
Atrophic Rhinitis	45.6		42.0		.575
Parvovirus	68.1		79.7		.051
Leptospirosis	76.3		88.4		.024
Pseudorabies	22.3		21.7		.915
E. coli	52.4		56.5		.520
Rota virus	18.1		24.6		.197
Clostridium	26.7		23.2		.536
Erysipelas	67.9		73.9		.316
Transmissible gastroenteritis	29.1		36.2		.225
Other vaccines	19.5		13.0		.197
Type Farrowing (all-in all-out)	56.2		50.7		.394
(continuous)	43.8		49.3		.394
Use of Antibiotics in the Feed	41.0		42.0		.876
Use of Anthelmintic	86.3		85.5		.867

Wilcoxon = P value for the Wilcoxon rank sum test; † N = 502 farms; ‡ N = 69 farms.

Table 4.5c: Measures of sow productivity and health on swine farms stratified according to the level of nitrate in the drinking water: National Swine Survey, 1989-1991.

Variable	<45 ppm Nitrate			502 farms			>45 ppm Nitrate			69 farms		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Average Litter Size	10.3	10.5	5-16.1	10.4	10.8	5.4-13						
Percentage Stillborn	6.9	6.3	0-50	6.7	6.0	0-25.5						
Percentage Mummified	1.2	0.8	0-19.5	2.3	0.8	0-66.7						
% Illness -Farrowing	2.2	0	0-100	1.8	0	0-28.6						
% Illness -Other Repro.	1.3	0	0-63.9	0.7	0	0-7.7						
% Other Known Illness	0.5	0	0-25	0.3	0	0-10						
% Unknown Illness	0.8	0	0-76.8	1.1	0	0-22						
% Mortality -Farrowing	0.2	0	0-7.1	0.5	0	0-20						
% Mortality -Other Repro.	0.1	0	0-7.7	0.1	0	0-2.6						
% Mortality-Other Known Cause	0.2	0	0-8.3	0.2	0	0-10						
% Mortality -Unknown Cause	0.1	0	0-4.6	0.1	0	0-3						

Wilcoxon = p value for the Wilcoxon rank sum test

Table 4.6: Unadjusted stratified analysis of detectable levels of nitrate, nitrate ≥ 45 ppm and nitrate ≥ 100 ppm on farrowing sow productivity and health: National Swine Survey, 1989-1991.

Variable	Nitrate ≥ 45 ppm		Nitrate ≥ 100 ppm	
	Odds Ratio	C.I.	Odds Ratio	C.I.
Average Litter Size (below median)	.86	.52-1.42	1.55	.69-3.48
% Stillborn (above median)	.80	.48-1.33	.55	.24-1.26
% Mummified (above median)	.98	.59-1.62	.79	.35-1.76
>0% Farrowing Illness	.71	.41-1.26	.39	.14-1.11
>0% Other Reproductive Illness	1.29	.68-2.42	.66	.19-2.22
>0% Other Known Illness	.62	.28-1.40	.49	.12-2.07
>0% Unknown Illness	.93	.47-1.85	1.25	.46-3.41
>0% Farrowing Mortality	.69	.27-1.79	*	-
>0% Other Reproductive Mortality	1.47	.32-6.79	*	-
>0% Other Known Mortality	.89	.34-2.31	.99	.23-4.35
>0% Unknown Cause Mortality	1.04	.30-3.59	2.07	.47-9.07

C.I. = 95% confidence interval, * unable to calculate due to zero cell.

Table 4.7a: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Average Litter Size: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	OR ₄	Mantel-Haenszel		Breslow-Day	
					OR ₁	χ^2	χ^2	P
None	-	-	-	-	.86*	.35	.552	
Ammonia	.82	2.09	-	-	.91	.14	.704	.286
TtlSwm	.71	.89	.70	.95	.81	.69	.405	.962
AvgPar	.78	.85	.77	.57	.78	.88	.348	.986
TypeFar	.99	.71	-	-	.84	.45	.505	.533
Parvo	.53	1.04	-	-	.90	.16	.691	.277
Lepto	.59	.99	-	-	.93	.09	.767	.503
Clos	.89	.72	-	-	.85	.40	.527	.725
Erysip	.59	1.02	-	-	.88	.26	.613	.341
Other V	1.00	.36	-	-	.88	.27	.605	.183

OR₁=summary odds ratio, * crude odds ratio. The variables are categorized as ammonia (0,>0); total swine (≤ 49 , >49 to ≤ 300 , >300 to $\leq 1,000$, >1000); average parity (<2, ≥ 2 to <3, ≥ 3 to <5, ≥ 5); type of farrowing (all-in all-out, continuous); all vaccinations as (not vaccinated, vaccinated).

Table 4.7b: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and the Percentage of Stillbirths: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	OR ₄	Mantel-Haenszel		Breslow-Day	
					OR ₅	X ²	X ²	P
None	-	-	-	-	.80*	.73	.395	
Barium _a	.95	.68	-	-	.79	.85	.42	.518
Barium _b	.95	.78	.56	-	.79	.81	.62	.734
AvgPar	.61	.71	1.55	.19	.80	.77	4.68	.197
E. coli	.57	1.07	-	-	.81	.67	1.45	.229
Clos	.68	1.37	-	-	.80	.73	1.32	.250

OR₅=summary odds ratio, * crude odds ratio. The variables are categorized as barium_a (0, >0); barium_b (0, >0 to ≤ 2 , >2); average parity (<2, ≥ 2 to <3, ≥ 3 to <5, ≥ 5); all vaccinations as (not vaccinated, vaccinated).

Table 4.7c: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and the Percentage of Mummies: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	OR ₄	Mantel-Haenszel		Breslow-Day
					OR ₅	χ^2 P	χ^2 P
None	-	-	-	-	.98*	.01	.935
Ammonia	1.01	.47	-	-	.94	.07	.797
Barium ₀	1.02	.94	-	-	.98	.01	.928
Barium ₆	1.02	.81	1.13	-	.97	.02	.902
TtlSw	2.00	.96	.95	1.16	1.03	.01	.906
AvgPar	.99	.94	.82	5.00	1.01	.01	.952
TypeFar	1.08	.91	-	-	.99	.01	.980
Avg Lit Siz	.86	1.10	-	-	.96	.02	.887
Rhinitis	1.03	.94	-	-	.99	.01	.962
							.78
							.03
							.26
							.32
							2.36
							.10
							.24
							.03

OR₅=summary odds ratio, * crude odds ratio. The variables are categorized as ammonia (0,>0); barium₀ (0, >0); barium₆ (0, >0 to <.2, $\geq$.2); total swine ($\leq$ 49, >49 to $\leq$ 300, >300 to $\leq$ 1,000, >1000); average parity (<2, $\geq$ 2 to <3, $\geq$ 3 to <5, $\geq$ 5); type of farrowing (all-in all-out, continuous); avg lit siz as (>10.5, $\leq$ 10.5); vaccination as (not vaccinated, vaccinated).

Table 4.7d: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Illness Due to Farrowing Problems: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	Mantel-Haenszel		Breslow-Day χ^2	P
				OR ₄	χ^2		
None	-	-	-	.71*	1.35	.245	
Nitrite	.73	@	-	.73	1.18	.278	@
TtlSw	1.36	.62	.72	.77	.84	.361	1.12 .572
AvgPar	1.20	.90	.46	.73	1.16	.282	1.90 .387
TypeFar	.80	.65	-	.73	1.20	.274	.13 .723
E. coli	.80	.64	-	.70	1.52	.218	.14 .710
Hemoph	.75	.61	-	.74	1.08	.298	.03 .874
Erysip	.59	.72	-	.69	1.63	.202	.08 .778
Parvo	1.09	.61	-	.69	1.71	.191	.70 .403
Lepto	2.04	.57	-	.67	1.91	.167	2.58 .108

OR₄=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as nitrite (0, >0); total swine (≤ 300 , >300 to $\leq 1,000$, >1000); average parity (<2, ≥ 2 to <3, ≥ 3); type of farrowing (all-in all-out, continuous); all vaccinations as (not vaccinated, vaccinated).

Table 4.7e: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Mortality Due to Farrowing Problems: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	Mantel-Haenszel		Breslow-Day	
				OR ₄	X ²	X ²	P
None	-	-	-				
Ammonia	.75	.98	-	.69*	.58	.446	.827
Barium	1.34	.24	-	.79	.23	.633	.05
Nitrite	.71	@	-	.71	.52	.471	2.48
TtlSwm	2.42	1.21	.27	.71	.50	.480	@
AvgPar	1.14	.82	.54	.77	.29	.591	2.64
YrsSwm	.25	1.23	-	.71	.49	.485	.36
Erysip	.99	.61	-	.69	.58	.446	2.10
Pseudo	.87	.37	-	.66	.73	.395	.17
Lepto	4.11	.50	-	.69	.57	.450	.51
Antibio F.	.57	.80	-	.63	.95	.329	3.46
				.69	.60	.439	.12
							.734

OR₁=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as ammonia (0,>0); barium (0, >0); nitrite (0, >0); total swine (≤ 300 , >300 to $\leq 1,000$, >1000); average parity (<2, ≥ 2 to <3, ≥ 3); years swine farming (<10, ≥ 10); all vaccinations as (not vaccinated, vaccinated); antibiotics in feed as (no, yes).

Table 4.7f: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Illness Due to Reproductive Problems Other Than Farrowing: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	Mantel-Haenszel		Breslow-Day	
			OR _c	$\frac{X^2}{P}$	$\frac{X^2}{P}$	$\frac{X^2}{P}$
None	-	-	1.29*	.61	.436	
Nitrite	1.32	@	1.32	.73	.393	@
TypeFar	1.57	1.01	1.31	.69	.405	.43
						.511

OR_c=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as nitrite (0, >0); type of farrowing (all-in all-out, continuous).

Table 4.7g: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Mortality Due to Reproductive Problems Other Than Farrowing: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	Mantel-Haenszel		Breslow-Day	
			OR ₁	χ^2	χ^2	P
None	-	-				
Ammonia	.96	5.88	1.47*	.24	1.49	.223
AvgPar	.80	4.40	1.38	.50	1.23	.267
TypeFar	2.74	.92	1.40	.18	.49	.483
Rhinitis	.99	1.76	1.28	.18	.79	.375

OR₁=summary odds ratio, * crude odds ratio, θ not calculated due to zero cell. The variables are categorized as ammonia (0,>0); average parity (<3, ≥ 3); type of farrowing (all-in all-out, continuous); vaccination as (not vaccinated, vaccinated).

Table 4.7h: Summary of adjusted stratified analyses (adjusted) for the association between Nitrate ≥ 45 ppm and Illness Due to Other Known Causes: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	Mantel-Haenszel		Breslow-Day	
				OR ₄	X ²	X ²	p
None	-	-	-	.62*	1.30	.254	
Barium	.75	.54	-	.64	1.13	.289	.16
Nitrite	.65	@	-	.65	1.06	.302	@
Tt1Sw	1.45	.22	.92	.69	.82	.366	2.47
Erysip	.44	.65	-	.61	1.45	.229	.12
Rhinitis	.97	.33	-	.63	1.24	.266	1.49
Lepto	1.40	.52	-	.59	1.70	.192	.71
							.398

OR₄=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as barium (0, >0); nitrite (0, >0); total swine (≤ 300 , >300 to $\leq 1,000$, >1000); all vaccinations as (not vaccinated, vaccinated).

Table 4.7i: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Mortality Due to Other Known Causes: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	Mantel-Haenszel		Breslow-Day
				OR ₄	χ^2	χ^2 p
None	-	-	-	.88*	.07	.792
Nitrite	@	.91	-	.91	.04	.851
TtlSwm	3.67	.38	1.28	.97	.01	.947
YrsSwm	.27	1.87	-	.88	.07	.785
TypeFar	1.22	.45	-	.90	.04	.837
Hemoph	.84	@	-	.83	.14	.710
Erysip	.99	.82	-	.85	.10	.747
Lepto	4.11	.66	-	.81	.19	.662
						2.45
						.188

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OR₄=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as ammonia (0,>0); nitrite (0, >0); total swine (≤ 300 , >300 to $\leq 1,000$, >1000); years swine farming (<10, ≥ 10); type of farrowing (all-in all-out, continuous); all vaccinations as (not vaccinated, vaccinated).

Table 4.7j: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Illness Due to Unknown Causes: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	OR ₄	OR ₅	Mantel-Haenszel		Breslow-Day	
						χ^2	P	χ^2	P
None	-	-	-	-	.93*	.04	.837		
TtlSwm	2.14	.84	.99	-	1.03	.01	.923	.90	.637
AvgPar	.49	1.16	1.05	2.00	1.02	.01	.963	.80	.851
YrsSwm	1.18	1.31	.56	-	.92	.05	.820	1.19	.552
Hemoph	.94	1.46	-	-	.97	.01	.932	.11	.736
Erysip	.50	.98	-	-	.89	.11	.743	.37	.542
Lepto	@	.98	-	-	.88	.13	.715	1.11	.293
Pseudo	.84	1.20	-	-	.93	.04	.844	.23	.630
Anthel	1.68	.80	-	-	.93	.05	.830	.78	.378

OR₅=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as total swine (≤ 300 , >300 to $\leq 1,000$, >1000); average parity (<2 , ≥ 2 to <3 , ≥ 3 to <5 , ≥ 5); years swine farming (<10 , ≥ 10 to <20 , ≥ 20); all vaccinations as (not vaccinated, vaccinated); anthelmintic usage as (no, yes).

Table 4.7k: Summary of adjusted stratified analyses for the association between Nitrate ≥ 45 ppm and Mortality Due to Unknown Causes: National Swine Survey, 1989-1991.

Control Variables	OR ₁	OR ₂	OR ₃	Mantel-Haenszel		Breslow-Day	
				OR ₁	X ²	X ²	P
None	-	-	-	1.04*	<.01		
Ammonia	1.97	@	-	1.37	.23	1.14	.285
Nitrite	1.14	@	-	1.14	.04	@	
TtlSw	1.76	.72	-	1.19	.07	.46	.499
YrsSw	1.04	.86	1.26	1.03	<.01	.06	.969
E. coli	@	1.60	-	1.02	<.01	1.53	.216
Clos	1.07	1.05	-	1.06	.01	<.01	.988
Erysip	3.10	.73	-	1.00	<.01	1.18	.277
Rhinitis	@	1.92	-	1.08	.01	2.05	.153
Parvo	@	1.04	-	.94	.01	.28	.600
Lepto	@	.94	-	.92	.02	.06	.801
Pseudo	1.63	.60	-	1.05	.01	.59	.442

OR₁=summary odds ratio, * crude odds ratio, @ not calculated due to zero cell. The variables are categorized as ammonia (0,>0); nitrite (0,>0); total swine ($\leq 1,000$, >1000); years swine farming (≤ 2 , >2 to <10, ≥ 10); all vaccinations as (not vaccinated, vaccinated).

Table 4.8a: Results of a multiple linear regression model for the effect of Nitrate on the farm average litter size: National Swine Survey, 1989-1991.

Variable	Coef.	Std. Error	T for H₀ Parameter=0	P
Intercept	10.337	.272	38.018	<.001
Nitrate	0.086	.051	1.698	.090
Ammonia	-0.056	.056	-0.985	.325
TtlSwm	0.000001	.000008	0.125	.901
AvgPar	0.086	.051	1.698	.964
TypeFar	-0.226	.126	-1.789	.955
Vaccination:				
Parvo	0.042	.192	0.221	.826
Lepto	0.060	.227	0.264	.792
Clos	0.224	.151	1.487	.138
Erysip	0.054	.170	0.315	.753
Other V	-0.477	.165	-2.893	.004

Model F-value = 1.758, P-value = .066, adjusted R² = .014.

Table 4.8b: Results of a multiple linear regression model for the effect of Nitrate on the farm average percentage stillborn: National Swine Survey, 1989-1991.

Variable	Coeff.	Std. Error	T for H ₀ Parameter=0	P
Intercept	6.132	.530	11.564	<.0001
Nitrate	-0.003	.005	-0.747	.456
Barium	1.896	.938	2.022	.044
AvgPar	0.304	.146	2.080	.038
Vaccination:				
E. coli	-0.698	.418	-1.672	.095
Clos	0.182	.471	0.387	.699

Model F-value = 2.269, P-value = .046, adjusted R² = .012.

Table 4.8c: Results of a multiple logistic regression model for the effect of Nitrate on the farm average percentage born mummified: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.99, 1.00
Ammonia	.96	.90, 1.13
Barium	1.44	.59, 3.56
TtlSwn	1.16	1.02, 1.31
AvgPar	1.00	1.00, 1.00
Avg. Litter Size	1.19	1.03, 1.38
TypeFar	1.22	.86, 1.74
Vaccination for: Rhinitis	1.29	.91, 1.82

CI = Confidence Interval

Table 4.8d: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sows ill due to farrowing problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.99, 1.00
Nitrite	1.23	.86, 1.74
TtlSwn	1.00	1.00, 1.00
AvgPar	1.09	.94, 1.27
TypeFar	1.19	.81, 1.74
Vaccination for:		
E. coli	1.27	.84, 1.93
Hemoph	1.40	.77, 2.57
Erysip	1.30	.77, 2.19
Parvo	.88	.50, 1.56
Lepto	1.11	.56, 2.23

CI = Confidence Interval

Table 4.8e: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sow mortality due to farrowing problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	.99	.98, 1.01
Ammonia	1.09	.89, 1.32
Barium	.21	.03, 1.80
Nitrite	1.17	.92, 1.49
TtlSwn	1.00	1.00, 1.00
AvgPar	1.18	.95, 1.47
YrsSwn	1.01	1.00, 1.02
Vaccination for:		
Erysip	1.34	.58, 3.02
Lepto	1.40	.74, 2.65
Pseudo	1.85	.62, 5.52
Antibio F	1.40	.78, 2.53

CI = Confidence Interval

Table 4.8f: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sows with reproductive illness other than farrowing problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	1.00, 1.01
Nitrite	1.31	.95, 1.80
TypeFar	1.37	.87, 2.16

CI = Confidence Interval

Table 4.8g: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sow mortality due to reproductive illness other than farrowing problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.98, 1.01
Ammonia	1.08	.76, 1.52
AvgPar	.66	.36, 1.21
TypeFar	.37	.10, 1.30
Vaccination for: Rhinitis	2.36	.67, 8.31

CI = Confidence Interval

Table 4.8h: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sows ill with other known problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.99, 1.00
Barium	.18	.03, 1.15
Nitrite	1.58	1.06, 2.35
TtlSwn	1.00	1.00, 1.00
Vaccination for:		
Rhinitis	1.33	.78, 2.28
Erysip	1.62	.75, 3.49
Lepto	1.10	.56, 2.18

CI = Confidence Interval

Table 4.8i: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sow mortality due to other known problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.99, 1.01
Nitrite	1.33	.89, 2.00
TtlSwn	1.00	1.00, 1.00
YrsSwn	.99	.98, 1.01
TypeFar	1.48	.77, 2.85
Vaccination for:		
Hemoph	.15	.02, 1.28
Erysip	2.10	.33, 6.29
Lepto	1.27	.55, 2.96

CI = Confidence Interval

Table 4.8j: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sows ill with unknown problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.99, 1.01
TtlSwn	1.00	1.00, 1.00
AvgPar	1.10	.92, 1.32
YrsSwn	.99	.98, 1.00
Vaccination for:		
Hemop	1.52	.76, 3.04
Erysip	.85	.40, 1.79
Lepto	1.74	.88, 3.44
Pseudo	1.67	.99, 2.81
Anthel	.63	.33, 1.17

CI = Confidence Interval

Table 4.8k: Results of a multiple logistic regression model for the effect of Nitrate on the farm percentage of sows mortality due to unknown problems: National Swine Survey, 1989-1991.

Variable	Odds Ratio	95% CI
Nitrate	1.00	.99, 1.01
Ammonia	.80	.38, 1.73
Nitrite	1.75	.94, 3.28
TtlSwn	1.00	1.00, 1.00
YrsSwn	.97	.95, 1.00
Vaccination for:		
E. coli	.94	.31, 2.87
Clos	.99	.34, 2.90
Rhinitis	2.37	.82, 6.89
Parvo	.92	.21, 4.01
Erysip	.83	.23, 2.96
Lepto	3.36	.30, 37.83
Pseudo	3.98	1.56, 10.13

CI = Confidence Interval

Table 4.9: The approximate minimum detectable odds ratio when $\alpha=0.05$ (one-tailed), for an overall event proportion (Prop) with a sample size of 571.

<u>Prop</u>	Power ($1-\beta$)				
	70%	80%	90%	95%	95%*
.01	2.5	3.0			
.02	2.0	2.5	2.5	3.0	
.03	1.8	1.9	2.5	2.5	3.0
.04	1.7	1.8	1.9	2.0	2.5
.05	1.6	1.7	1.8	1.9	2.5
.06	1.5	1.6	1.7	1.9	2.5
.07	1.5	1.6	1.7	1.8	2.0
.08	1.5	1.5	1.6	1.7	1.9
.09	1.4	1.5	1.6	1.7	1.9
.10	1.4	1.5	1.6	1.7	1.8
.12	1.4	1.4	1.5	1.6	1.8
.14	1.4	1.4	1.5	1.6	1.7
.16	1.4	1.4	1.5	1.5	1.7
.18	1.3	1.4	1.5	1.5	1.6
.20	1.3	1.4	1.4	1.5	1.6
.25	1.3	1.3	1.4	1.5	1.6
.30	1.3	1.3	1.4	1.4	1.5
.35	1.3	1.3	1.4	1.4	1.5
.40	1.3	1.3	1.4	1.4	1.5
.45	1.3	1.3	1.3	1.4	1.5
.50	1.3	1.3	1.3	1.4	1.5

* $1-\beta=95\%$, $\alpha=0.01$ (one-tailed).

REFERENCES

- Anderson DM and Stothers SC: Effects of saline water high in sulfates, chlorides and nitrates on the performance of young weanling pigs. *J Anim Sci*, 47:900-907, 1978.
- Atef M, Abo-Norage MAM, Hanafy MSM, and Agag AE: Pharmacotoxicological aspects of nitrate and nitrite in domestic fowls. *Br Poultry Sci*, 32:399-404, 1991.
- Billie N, Nielsen, NC, Larsen, JL and Svendsen J: Preweaning mortality in pigs. 2. The perinatal period. *Nord Vet Med*, 26:294-313, 1974.
- Breslow NE and Day NE: Statistical methods in cancer research. IARC Publications: Lyon, France, chapter 4, 1980.
- Bruning-Fann CS and Kaneene JB: The effects of nitrate, nitrite, and N-nitroso compounds on animal health. *Vet Hum Toxicol*, 35:237-253, 1993a.
- Bruning-Fann CS and Kaneene JB: The effects of nitrate, nitrite, and N-nitroso compounds on human health: a review. *Vet Hum Toxicol*, 35:521-538, 1993b.
- Carrigan MJ and Gardner IA: Nitrate poisoning in cattle fed sudax (*sorghum* sp. hybrid) hay. *Australian Vet J*, 59:155-157, 1982.
- Case AA: Abortion in swine due to excessive nitrate. *J Am Vet Med Assoc*, 131:226, 1957.
- Cohen J: *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates, Hillsdale, NJ: 567 pgs., 1988.
- Comly HH: Cyanosis in infants caused by nitrates in well water. *J Am Vet Med Assoc*, 129:112-116, 1945.
- Davison KL, Hansel W, Krook L, McEntee K, and Wright MJ: Nitrate toxicity in dairy heifers. I. Effects on reproduction, growth, lactation, and vitamin A nutrition. *J Dairy Sci*, 47:1065-1073, 1964.
- Davison KL, McEntee K and Wright MJ: Responses in pregnant ewes fed forages containing various levels of nitrate. *J Dairy Sci*, 48:968-977, 1965.
- De Saint Blanquat G, Fritsch P, Cazottes C: Effects of dietary nitrite and nitrate on experimentally-induced inflammation in the rat. *Int J Tissue Reac*, 2:173-180, 1983.

Dorsch MM, Scragg RKR, McMichael AJ, Baghurst PA and Dyer KF: Congenital malformations and maternal drinking water supply in rural south Australia: A case-control study. *J Epidemiol*, 119:473-486, 1984.

Eppson HF, Glenn MW, Ellis WW and Gilbert CS: Nitrate in the diet of pregnant ewes. *J Am Vet Med Assoc*, 137:611-614, 1960.

Fan AM, Willhite CC, and Book SA: Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Reg Toxicol Pharmacol*, 7:135-148, 1987.

Fraser P and Chilvers C: Health aspects of nitrate in drinking water. *Sci Total Environ*, 18:103-116, 1981.

Fraser D, Patience JF, Phillips, PA, McLeese, JM: Water for piglets and lactating sows: Quantity, Quality and quandaries. In Haresign and Cole (editors), *Recent Advances in Animal Nutrition*. Butterworths, Seven Oaks, UK: 137-160, 1990.

Garner GB, O'Dell BL, Radar P and Muhrer ME: Further studies on the effects of nitrate upon reproduction and vitamin A storage with rats and swine. *J Anim Sci*, 17:1213 (Abstract), 1958.

Garrison GW, Wood RD, Chaney CH, and Waddill DG: Effects of nitrate and nitrite in drinking water on the utilization of carotene in swine. *Kentucky Agricul Exper Station Progress Report* 164:85-87, 1966.

Gwatkin R and Plummer PJG: Toxicity of certain salts of sodium and potassium for swine. *Can J Comp Med*, 10:183-190, 1946.

Haliburton JC, and Edwards WC: Nitrate poisoning in Oklahoma cattle during the winter of 1977-1978. *Vet Hum Toxicol*, 20:401-403, 1978.

Hibbs CM, Stencel EL, and Hill RM: Nitrate toxicosis in cattle. *Vet Hum Toxicol*, 20:401-403, 1978.

Holländer R and Sander J: Nitratkonzentrationen von trinkwässern in Südwest-Niedersachsen. *Zentralbl Bakter Mikrobiol Hyg*, 184:287-296, 1987.

Hosmer DW and Lemeshow S: *Applied logistic regression*. New York: John Wiley and Sons, 82-134, 1989.

Hsieh FY: Sample size tables for logistic regression. *Stat Med*, 8:795-802, 1989.

Hurd S: An analysis of methods used to report water quality results from a National sample: National Swine Survey. Animal Health Insight, Summer:1-4, 1993.

Johnson CJ, Bonrud PA, Dorsch TL, Kilness AW, Senger KA, Busch DC, and Meyer MR: Fatal outcome of methemoglobinemia in an infant. J Am Med Assoc, 257:2796-2797, 1987.

Jones JET: Observations on parturition in the sow. The parturient and post-parturient phases. Br Vet J, 122:471-478, 1966.

Kahraman M: Nitrat ve nitriti'in farelerde immunosporesif etkileri üzerinde arastirmalar. Veteriner Fakultesi Dergisi, 6:251-254, 1988.

Koch BA, Parrish DG, and Sukhonthasarnpa S Effects of dietary NO₂ and NO₃ on growing swine. J Anim Sci, 22:840 (Abstract), 1963.

Leman AD, Straw B, Glock RD, Mengeling WL, Penny RHC, Scholl E (editors): *Diseases of Swine - Sixth Edition*. Iowa State University Press, Iowa, 1986.

Lingaas F and Rønnigen K: Epidemiological and genetical studies in Norwegian pig herds. II. Overall disease incidence and seasonal variation. Acta Vet Scand, 32:89-96, 1991.

Maldonado G and Greenland S: Simulation study of confounder-selection strategies. Amer J Epidemiol, 138:923-936, 1993.

Møller H, Landt J, Jensen P, Pedersen E, Autrup H, and Møller Jensen, O: Nitrate exposure from drinking water and diet in a Danish rural population. Int J Epidemiol, 18:206-212, 1989.

National Academy of Sciences-National Research Council, subcommittee on nutrient and toxic elements in water: Nutrients and toxic substances in water for livestock and poultry. Nat Acad Sci, Washington, D.C, 1974.

Newman MC, Dixon PM, Looney BB, and Pinder JE: Estimating mean and variance for environmental samples with below detection limit observations. Water Resources Bull, 25:905-916, 1989.

Nicholls TJ and Miles EJ: Nitrate/nitrite poisoning of cattle on ryegrass pasture. Australian Vet J, 56:95-96, 1980.

Partlow GD, Fisher KRS, Page PD, MacMillan K, and Walker AF: Prevalence and types of birth defects in Ontario swine determined by mail survey. *Can J Vet Res*, 57:67-73.

Randall GCB: Observations on parturition in the sow. I. Factors associated with the delivery of the piglets and their subsequent behaviour. *Vet Rec*, 90:178-182, 1972a.

Randall GCB: Observations on parturition in the sow. II. Factors influencing stillbirth and perinatal mortality. *Vet Rec*, 90:183-186, 1972b.

Robinson LD and Jewell JP: Some surprising results about covariate adjustment in logistic regression models. *Int Stat Rev*, 58:227-240, 1991.

Ruhr LP and Osweiler GD: Nitrate accumulators. In: Howard (editor), *Current Veterinary Therapy - Food Animal Practice 2*, pp. 392-394. WB Saunders Co, Philadelphia, PA, 1986.

SAS Institute Inc: *SAS/STAT® User's Guide*, Release 6.03 Edition. Cary, N.C. SAS Institute Inc, 1988.

SAS Institute Inc: *SAS® Technical Report P-200, SAS/STAT® Software: CALIS and LOGISTIC Procedures*, Release 6.04, Cary, NC: SAS Institute Inc., 1990.

Scragg RKR, Dorsch MM, McMichael AJ, and Baghurst PA: Birth defects and household water supply. *Med J Australia*, 2:577-579, 1982.

Seerley RW, Emerick RJ, Embry LB and Olson OE: Effect of nitrate or nitrite administered continuously in drinking water for swine and sheep. *J Anim Sci*, 24:1014-1019, 1965.

Shuval HI and Gruener N: Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am J Pub Health*, 62:1045-1052, 1972.

Simon J, Sund JM, Wright MJ and Douglas FD: Prevention of noninfectious abortion in cattle by weed control and fertilization practices on lowland pastures. *J Am Vet Med Assoc*, 135:315-317, 1959a.

Simon J, Sund JM, Douglas FD, Wright MJ and Kowalczyk T: The effect of nitrate or nitrite when placed in the rumens of pregnant dairy cattle. *J Am Vet Med Assoc*, 135:311-314, 1959b.

Sleight SD, and Atallah OA: Reproduction in the guinea pig as affected by chronic administration of potassium nitrate and potassium nitrite. *Toxicol Appl Pharmacol*, 12:179-185, 1968.

Smith HC, Lovell VE, Reppert R, and Griswold D: Nitrate poisoning in swine. Vet Med, 54:547-550, 1959.

Sund JM, Wright MJ, and Simon J: Weeds containing nitrates cause abortion in cattle. Agron J, 49:278-279, 1957.

Tollett JT, Becker DE, Jensen AH and Terrill SW: Effect of dietary nitrate on growth and reproductive performance of swine. J Anim Sci, 19:1297 (Abstract), 1960.

United States Department of Agriculture, Animal and Plant Health Inspection Service: National Swine Survey: data collection: 1990 [unnumbered technical report]. US Dept of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System, Fort Collins, CO., 1992.

United States Department of Agriculture: Nitrate occurrence in U.S. Waters (and related questions). United States Department of Agriculture, Washington, D.C., pp. 1-35, 1991.

United States Environmental Protection Agency: Proposed criteria for water quality. Volume I. Quality of water for livestock. Environ Rep Current Develop, 4:663-664, 1973.

Vermunt J, and Visser R: Nitrate toxicity in cattle. N Z Vet J, 35:136-137, 1987.

Vigil J, Warburton S, Haynes WS and Kaiser LR: Nitrates in municipal water supply cause methemoglobinemia in infant. Pub Health Rep, 80:1119-1121, 1965.

Winter AJ and Hokanson JF: Effects of long-term feeding of nitrate, nitrite, or hydroxylamine on pregnant dairy heifers. Am J Vet Res, 125:353-361, 1964.

Wood RD, Chaney CH, Waddill DG and Garrison GW: Effect of adding nitrate or nitrite to drinking water on the utilization of carotene by growing swine. J Anim Sci, 26:510-513, 1967.

CHAPTER 5

DISCUSSION

Chapter 3 reviews the known effects of nitrate on animal health and productivity. Because nitrate is frequently found in groundwater, it is important to determine what effects the consumption of nitrate-contaminated drinking water may have.

The objectives of this study were to investigate the effects of nitrate contained in drinking water on farrowing swine health and productivity. Data used in this study were taken from a much larger study entitled the "National Swine Survey" (NSS). The NSS is a large epidemiological study, designed to permit inferences to be made about the swine population of the U.S. The portion of data from the NSS used in my analyses was collected in a cross-sectional manner.

Sow health was assessed by determining the rates of illness or death attributed to; farrowing problems, reproductive problems other than farrowing, "other known" problems, and unknown problems. Sow productivity was measured by calculating the percentage of pigs born alive, born mummified, and stillborn.

These measures of swine health and productivity, the nitrate concentration of drinking water, and potentially confounding and/or effect modifying variables were examined using descriptive statistics, correlation analysis, univariate logistic regression, stratified analysis and multiple logistic regression.

This study has many strengths. The study population was selected by the NASS, an agency whose mission is to select and

sample agricultural populations in order to make inferences about the U.S. It is large (571 farms sampled), powerful study (there is a high probability of detecting an association between nitrate and sow productivity or health if one really did exist). With the sample size of this study, there is an 80% probability of detecting an odds ratio as low as 1.3, and a 95% probability of detecting an odds ratio of 1.5.

All questionnaires were administered by specifically trained personnel. These interviewers had no preconceived ideas to be proven or dispelled by this study, thereby minimizing interviewer bias. All water samples were collected and analyzed at one laboratory using established protocols diminishing measurement error.

With a cross-sectional study, exposure and disease status are evaluated at the same time. It is assumed that the exposure to nitrate remained at a constant level and occurred throughout the sow's life. The levels of nitrate measured in the drinking water are unlikely to vary much (due to the length of time required for surface deposits of nitrate to enter groundwater). However, nitrate is also found in many foodstuffs, suggesting the possibility that the swine were exposed to higher levels of nitrate than those indicated by the water analysis. In this case, misclassification of exposure status would have occurred. It is doubtful that the level of nitrate exposure has been underestimated to any substantial degree because the foodstuffs most likely to

contain high levels of nitrate (green leafy vegetable material) are not generally fed to swine in the U.S.

The concentration of the drinking water provided to the sows was measured in the farrowing unit. Misclassification of exposure status could also have occurred if the sows were exposed to a different level of nitrate in drinking water when they were not in the farrowing unit or if the level of nitrate changed during the monitoring period. To minimize this problem, those farms which changed the water source during the study were excluded. It is unlikely that many sows were exposed to more than one concentration of nitrate on the farm because most farms had only one water source. Regarding the length of time the sows were exposed to nitrate in the drinking water, information concerning the origin of the sows was not available and it is possibly that at least some of the 27,207 sows may have been purchased and brought onto the study farm. In this case, their exposure period would be much less than that indicated by their parity. There is no evidence to suggest that if misclassification of exposure status occurred, that it differed between the exposed and nonexposed sample. Nondifferential misclassification would tend to bias the results towards the null. However, it seem doubtful that the results of this study were significantly affected by misclassification.

The nitrate contained in well-water did not adversely effect the health and productivity of farrowing swine as

assessed by my criteria. While the levels of nitrate seen in well-water during the NSS did not effect the health and productivity of farrowing swine, this does not mean that nitrate does not have any affect on these parameters. Rather, it may indicate that nitrate levels were not yet high enough to cause problems. It is widely believed that nitrate levels are rising in the U.S. In this circumstance, repeating this study at a future time could result in different conclusions. Also, this study examined only the effects of nitrate on farrowing swine health. Further research is needed to determine the effects of nitrate on swine during other stages of production. In addition, groundwater is the primary source of drinking water for approximately 85% of the rural population and 50% of the entire U.S. population. With so many humans and animals dependent on groundwater, it is imperative to determine the factors that contribute to nitrate contamination in order to prevent further degradation of this resource.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adams AW: Effects of nitrate in drinking water of Japanese quail: Poultry Sci, 53:832-834, 1974.

Adams AW, Emerick RJ, and Carlson CW: Effects of nitrate and nitrite in the drinking water on chicks, poults and laying hens. Poultry Sci, 45:1215-1222, 1966.

Adams AW, West JL and Kahrs AJ: Some effects on turkeys of nitrate in the drinking water. Poultry Sci, 48:1222-1229, 1969.

Anderson DM and Stothers SC: Effects of saline water high in sulfates, chlorides and nitrates on the performance of young weanling pigs. J Anim Sci, 47:900-907, 1978.

Anonymous: MAFF Investigations Service Report--Nitrate poisoning. Vet Rec, 102:183, 1978.

Arends LG, Kienholz EW, and Nockels CF: Growth stimulation effects of sodium nitrate for bronze turkeys. Poultry Sci, 46:1228 (abstract), 1967.

Armstrong JG, McKendry JBJ, and Slemon KW: Methaemoglobinaemia in infancy. Can Med Assoc, 79:392-395, 1958.

Asbury AC and Rhode EA: Nitrite intoxication in cattle: The effects of lethal doses of nitrite on blood pressure. Am J Vet Res, 25:1010-1013, 1964.

Atef M, Abo-Norage MAM, Hanafy MSM, and Agag AE: Pharmacotoxicological aspects of nitrate and nitrite in domestic fowls. Br Poultry Sci, 32:399-404, 1991.

Beath OA, Gilbert CS, Eppson HF and Rosenfeld I: Oat-hay and oat-straw poisoning. In Wyoming Agricultural Experiment Station bulletin 324, 46-48, 1953.

Beck EG: Toxic effects from bismuth subnitrate with reports of cases to date. J Am Med Assoc, 52:14-18, 1909.

Bentley AB, Kinder QB, Garner GB, and Savage JE: Effect of nitrate in water on performance of laying hens. Poultry Sci, 44:1351 (abstract), 1965.

Billie N, Nielsen, NC, Larsen, JL and Svendsen J: Preweaning mortality in pigs. 2. The perinatal period. Nord Vet Med, 26:294-313, 1974.

Bjornson CB, McIlwain P, Eveleth DF, and Bolin FM: Sources of nitrate intoxication. *Vet Med*, 56:198-200, 1961.

Bjornson CB: Sources of nitrate intoxications in North Dakota. *North Dakota Farm Research Bimonthly Bulletin*, 23:16-20, 1964.

Blood DC, Radostits OM, and Henderson JA: *Vet Med, Sixth Edition*. Bailliere Tindall, Philadelphia, 1983.

Bloomfield RA, Garner BB, Welsch C, and Muhrer ME: Effect of dietary nitrate on urea utilization *in vitro* and *in vivo*. *J Anim Sci*, 20:925-926 (Abstract), 1961.

Bloomfield RA, Welsch CW, Garner GB, and Muhrer ME: Effect of dietary nitrate on thyroid function. *Science*, 134:1690, 1961.

Bloomfield RA, Welsch CW, Garner GB and Muhrer ME: Thyroidal I¹³¹ metabolism in nitrate fed sheep. *J of Anim Sci*, 21:988 (Abstract), 1962.

Bodansky O and Gutmann H: Treatment of methemoglobinemia. *J Pharmacol and Exp Therap*, 90:46-55, 1947.

Bodansky O: Methemoglobinemia and methemoglobin-producing compounds. *Pharm Rev*, 3:144-196, 1951.

Boermans HJ: Diagnosis of nitrate toxicosis in cattle, using biological fluids and a rapid ion chromatographic method. *Am J Vet Res*, 51:491-495, 1990.

Bouchard DC, Williams MK, and Surampalli RY: Nitrate contamination of groundwater: Sources and potential health effects. *J Am Water Works Assoc*, 85:85-90, 1992.

Bouwkamp FT, and Counotte GHM: Verslag van een onderzoek naar de gevolgen van extra nitraattoevoeging aan drinkwater voor mestvarkens en gespeende biggen. *Tijdschr Diergeneeskd*, 113:737-747, 1988.

Bradley WB, Eppson HF and Beath OA: Nitrate as the cause of oat hay poisoning. *J Am Vet Med Assoc*, 94:541-542, 1939.

Bradley WB, Beath OA and Eppson HF: Oat hay poisoning. *Science*, 89:365, 1939.

Bradley WB, Eppson HF and Beath OA: Livestock poisoning by oat hay and other plants containing nitrate. In *University of Wyoming Agricultural Experiment Station bulletin* 241, 3-20, 1940.

Bradley WB, Eppson HF and Beath OA: Methylene blue as an antidote for poisoning by oat hay and other plants containing nitrates. J Am Vet Med Assoc, 96:41-42, 1940.

Breslow NE and Day NE: Statistical methods in cancer research. Lyon, France: IARC Publications, chapter 4, 1980.

Brown CM, Burrows GE, and Edwards WC: Nitrate Intoxication. Vet Human Toxicol, 32:481-482, 1990.

Bruning-Fann C and Kaneene JB: The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Vet Human Toxicol, 35:237-253, 1993a.

Bruning-Fann C and Kaneene JB: The effects of nitrate, nitrite, and N-nitroso compounds on human health: A review. Vet Human Toxicol, 35:521-538, 1993b.

Buck WB: Laboratory toxicologic tests and their interpretation. J Am Vet Med Assoc, 155:1928-1941, 1969.

Buck WB: Diagnosis of feed-related toxicoses. J Am Vet Med Assoc, 156:1434-1443, 1970.

Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, Bonaguri C, Cipriani F, Cocco P, Giacosa A, Marubini E, Minacci C, Puntoni R, Russo A, Vindigni C, Faumeni JF Jr, Blot WJ: A case-control study of gastric cancer and diet in Italy: II. Association with nutrients. Int J Cancer 45: 896-901, 1990.

Burrows GE: Nitrate intoxication. J Am Vet Med Assoc, 177:82-83, 1980.

Campbell JB, Davis AN and Myhr PJ: Methaemoglobinaemia of livestock caused by high nitrate contents of well water. Can J Comp Med, 18:93-101, 1954.

Carlson DJ and Shapiro FL: Methemoglobinemia from well water nitrates: A complication of home dialysis. Ann Int Med, 73:757-759, 1970.

Carrigan MJ, and Gardner IA: Nitrate poisoning in cattle fed sudax (*sorghum* sp. hybrid) hay. Aust Vet J, 59:155-157, 1982.

Carter RL, Percival WH and Roe FJC: Exceptional sensitivity of mink to the hepatotoxic effects of dimethylnitrosamine. J Pathol, 97:79-88, 1969.

Carver LA and Pfander WH: Some metabolic aspects of urea and/or potassium nitrate utilization by sheep. J Anim Sci, 38:410-416, 1974.

Carver LA and Pfander WH: Urea utilization by sheep in the presence of potassium nitrate. *J Anim Sci*, 36:581-587, 1973.

Case AA: Abortion in swine due to excessive nitrate. *J Am Vet Med Assoc*, 131:226, 1957.

Case AA: Some aspects of nitrate intoxication in livestock. *J Am Vet Med Assoc*, 130:323-329, 1957.

Case AA: Nitrate poisoning from plants. *N Am Vet*, 38:46A, 1957.

Case AA: Corn stalk intoxication. *Sheep Breeder*, 74:14-15, 1954.

Cawley GD, Collings DF, and Dyson DA: Nitrate poisoning. *Vet Rec*, 101:305-306, 1977.

Cheng KJ, Phillippe RC, Kozub GC, Majak W, and Costerton JW: Induction of nitrate and nitrite metabolism in bovine rumen fluid and the transfer of this capacity to untreated animals. *Can J Anim Sci*, 65:647-652, 1985.

Christian HA: Diuretic action of nitrates and their use in the treatment of dropsy. *J Am Med Assoc*, 91:121 (Society Proc), 1928.

Clark R, and Quin JI: Studies on the alimentary tract of the Merino sheep in South Africa. XXIII.--The effect of supplementing poor quality grass hay with molasses and nitrogenous salts. *Onderstepoort J Vet Res*, 25:93-103, 1951.

Clark JL, Pfander WH, Bloomfield RA, Krause GF and Thompson GB: Nitrate containing rations for cattle supplemented with either urea or soybean meal. *J Anim Sci*, 31:961-966, 1970.

Cohen J: *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates, Hillsdale, NJ: 567 pgs., 1988.

Comly HH: Cyanosis in infants caused by nitrates in well water. *J Am Med Assoc*, 129:112-116, 1945.

Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M: A model for gastric cancer epidemiology. *Lancet* 12: 58-60, 1975.

Correa P: Modulation of gastric carcinogenesis: Updated model based on intragastric nitrosation. In Bartsch et al, eds: *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms*. IARC Scientific Publications No. 84, IARC, Lyons, : 485-491, 1987.

Council for Agricultural Science and Technology: Agriculture and groundwater quality. Council for Agricultural Science and Technology Report No. 103., p. 62, May, 1985.

Crawford RF, Kennedy WK and Davison KL: Factors influencing the toxicity of forages that contain nitrate when fed to cattle. Cornell Vet, 56:3-17, 1966.

Cuello C, Correa P, Haenszel W, Gordillo G, Brown C, Archer M, Tannenbaum S: Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. J Natl Cancer Inst 57: 1015-1020, 1976.

Davidson WB, Doughty JL and Bolton JL: Nitrate poisoning of livestock. Can J Comp Med, 5:303-313, 1941.
Thorp F Jr: Further observations on oat hay poisoning. J Am Vet Med Assoc, 92:159-170, 1938.

Davison KL, Hansel W, Krook L, McEntee K and Wright MJ: Nitrate toxicity in dairy heifers. I. Effects on reproduction, growth, lactation, and vitamin A nutrition. J Dairy Sci, 47:1065-1073, 1964.

Davison KL, McEntee K and Wright MJ: Responses in pregnant ewes fed forages containing various levels of nitrate. J Dairy Sci, 48:968-977, 1965.

Davison KL and Seo J: Influence of nitrate upon carotene destruction during in vitro fermentation with rumen liquor. J Dairy Sci, 46:862-864, 1963.

Davison KL, Hansel W, McEntee K, and Wright MJ: Reproduction, growth and lactation of heifers fed nitrate. J Anim Sci, 22:835 (Abstract), 1963.

Deeney J, Murdock ET, and Rogan JJ: Familial idiopathic methaemoglobinaemia. Br Med J, 1:721-723, 1943.

De Saint Blanquat G, Fritsch P, Cazottes C: Effects of dietary nitrite and nitrate on experimentally-induced inflammation in the rat. Int J Tissue Reac, 2:173-180, 1983.

Diven RH, Reed RE, Trautman RJ, Pistor WJ and Watts RE: Experimentally induced nitrite poisoning in sheep. Am J Vet Res, 23:494-496, 1962.

Diven RH, Pistor WJ, Reed RE, Trautman RJ and Watts RE: The determination of serum or plasma nitrate and nitrite. Am J Vet Res, 23:497-499, 1962.

Diven RH, Pahnish OF, Roubicek CB, Erwin ES, and Page HM: Vitamin A and carotenoid inter-relationships in bovine plasma and liver. *J Dairy Sci*, 43:1632-1638, 1960.

Dodd DC and Coup MR: Poisoning of cattle by certain nitrate-containing plants. *NZ Vet J*, 5:51-54, 1957.

Dollahite JW and Holt EC: Nitrate Poisoning. *Southwest Vet*, 23:23-28, 1969.

Dorsch MM, Scragg RKR, McMichael AJ, Baghurst PA and Dyer KF: Congenital malformations and maternal drinking water supply in rural south Australia: A case-control study. *J Epidemiol*, 119:473-486, 1984.

Ellis NR: Salt tolerance and salt poisoning of swine. In *Yearbook of Agriculture*. United States Government Printing Office, Washington, D.C.: 803-809, 1942.

Emerick RJ, Embry LB and Seerley RW: Rate of formation and reduction of nitrite-induced methemoglobin *in vitro* and *in vivo* as influenced by diet of sheep and age of swine. *J Anim Sci*, 24:221-230, 1965.

Emerick RJ and Olson OE: Effect of nitrate and nitrite on vitamin A storage in the rat. *J Nutr*, 78:73-77, 1962.

Emerick RJ: Consequences of high nitrate levels in feed and water supplies. *Fed Proc*, 33:1183-1187, 1974.

Eppson HF, Glenn MW, Ellis WW and Gilbert CS: Nitrate in the diet of pregnant ewes. *J Am Vet Med Assoc*, 137:611-614, 1960.

Eusterman BB and Keith NM: Transient methemoglobinemia following administration of ammonium nitrate. *Med Clin N Am*, 12:1489-1496, 1929.

Evelyn KA and Malloy HT: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. *J Biol Chem*, 126:655-662, 1938.

Fan AM, Willhite CC, and Book SA: Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Reg Toxicol Pharmacol*, 7:135-148, 1987.

Fincher MG: Sodium nitrate poisoning; a report of cases. *Cornell Vet*, 26:271-273, 1936.

Fraser P and Chilvers C: Health aspects of nitrate in drinking water. *Sci Total Environ*, 18:103-116, 1981.

Fraser D, Patience JF, Phillips, PA, McLeese, JM: Water for piglets and lactating sows: Quantity, Quality and quandaries. In Haresign and Cole (editors), *Recent Advances in Animal Nutrition*. Butterworths, Seven Oaks, UK: 137-160, 1990.

Fritsch P and De Saint Blanquat G: Excretion of nitrates and nitrites in saliva and bile in the dog. *Food Chem Toxicol*, 23:655-659, 1985.

Garner GB: Learn to live with nitrates. University of Missouri Agricultural Experiment Station Bulletin 708, 1958.

Garner GB, O'Dell BL, Radar P, and Muhrer ME: Further studies on the effects of nitrate upon reproduction and vitamin A storage with rats and swine. *J Anim Sci*, 17:1213 (Abstract), 1958.

Garrison GW, Wood RD, Chaney CH, and Waddill DG: Effects of nitrate and nitrites in drinking water on the utilization of carotene in swine. Kentucky Agricultural Experimental Station Progress Report 164, pages 85-87, 1966.

Gelperin A, Jacobs EE, and Kletke LS: The development of methemoglobin in mothers and newborn infants from nitrate in water supplies. *Illinois Med J*, 140:42-44, 82, 1971.

Gilli G, Corrao G, Favilli S: Concentrations of nitrates in drinking water and incidence of gastric carcinomas: First descriptive study of the Piemonte region, Italy. *Sci Total Environ*. 34: 35-48, 1984.

Globus M and Samuel D: Effect of maternally administered sodium nitrite on hepatic erythropoiesis in fetal CD-1 mice. *Teratology*, 18:367-378, 1978.

Goodrich RD, Emerick RJ, and Embry LB: Effects of sodium nitrate on vitamin A status of sheep. *J Anim Sci*, 21:997 (Abstract), 1962.

Goodrich RD, Emerick RJ and Embry LB: Effect of sodium nitrate on the vitamin A nutrition of sheep. *J Anim Sci*, 23:100-104, 1964.

Green LC, Tannenbaum SR, and Fox JG: Nitrate in human and canine milk. *N Eng J Med*, 306:1367-1368, 1982.

Green LC, Ralt D, and Tannenbaum SR: Nitrate, nitrite and N-nitroso compounds: Biochemistry, metabolism, toxicity and carcinogenicity. In Neuberger and Jukes (editors). *Human Nutrition*, Jack K. Burgess, Inc., Englewood, New Jersey: 87-140, 1982.

Greene I and Hiatt EP: Renal excretion of nitrate and its effect on excretion of sodium and chloride. *Am J Physiol*, 180:179-182, 1955.

Greene I and Hiatt EP: Behavior of the nitrate ion in the dog. *Am J Physiol*, 176:463-467, 1954.

Grudziński I and Szymański A: The effect of subchronic poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:447-452, 1991.

Grudziński I and Szymański A: The effect of exercise associated with subchronic poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:462-467, 1991.

Grudziński I and Szymański A: The effect of acute poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:453-461, 1991.

Grudziński I: Studies on the mechanism of the toxic action of sodium nitrite on intestinal absorption in rats. *Arch Environ Contam Toxicol*, 21:475-479, 1991.

Grudziński I, Szymański A, and Chomiczewski K: The effect of exercise associated with acute poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose in rats. *Arch Environ Contam Toxicol*, 21:468-474, 1991.

Gruener N, Shuval HI, Behrooz K, Cohen S, and Shechter H: Methemoglobinemia induced by transplacental passage of nitrites in rats. *Bull Environ Contam Toxicol*, 9:44-48, 1973.

Gwatkin R and Plummer PJG: Toxicity of certain salts of sodium and potassium for swine. *Can J Comp Med*, 10:183-190, 1946.

Hainline A Jr: Methemoglobin. In Meites (editor), *Standard Methods of Clinical Chemistry*, Volume 5. Academic Press, New York: 143-157, 1965.

Hale WH, Hubbert F Jr, and Taylor RE: Effect of energy level and nitrate on hepatic vitamin A and performance of fattening steers. *Proc Soc Exper Biol Med*, 109:289-290, 1962.

Hale WH, Hubbert F Jr, and Taylor RE: The effect of concentrate level and nitrate addition on hepatic vitamin A

stores and performance of fattening steers. J Anim Sci, 20:934 (Abstract), 1961.

Haliburton JC and Edwards WC: Nitrate poisoning in Oklahoma cattle during the winter of 1977-1978. Vet Human Toxicol, 20:401-403, 1978.

Hambitzer R, Velke H, Ramm K, and Sommer H: Bewertung der nitratbelastung von laktierenden und trockenstehenden milchkühen anhand der nitratwerte im blutplasma und eines blutprofils. Tierärztliche Umschau, 42:1-12, 1987.

Hansen MA: An outbreak of toxic liver injury in ruminants. Nord Vet, 16:323-342, 1964.

Hartman PE: Nitrates and nitrites: Ingestion, pharmacodynamics, and toxicology. In de Serres and Hollaender (editors). Chemical Mutagens, volume 7, Plenum Press, N.Y., pp. 211-294, 1982.

Hatfield EE and Smith GS: Nitrate and urea in rations of feeder lambs. J Anim Sci, 22:1122 (Abstract), 1963.

Hatfield EE, Smith GS, Neumann AL, Forbes RM, Garrigus US and Ross OB: Interactions of nitrite, alpha-tocopherol and tapazole upon the vitamin A nutrition of lambs fed "high nitrate" silage. J Anim Sci, 20:676 (Abstract), 1961.

Hiatt EP: Extreme hypochloremia in dogs induced by nitrate administration. Am J Physiol, 129:597-609, 1940.

Hibbs CM, Stencel EL, and Hill RM: Nitrate toxicosis in cattle. Vet Human Toxicol, 20:401-403, 1978.

Hoar DW, Embry LB, King HR, and Emerick RJ: Urea-nitrate interrelationships in sheep under feedlot conditions. J Anim Sci, 27:557-561, 1968.

Holländer R and Sander J: Nitratkonzentrationen von trinkwässern in Südwest-Niedersachsen. Zentralbl Bakter Mikrobiol Hyg, 184:287-296, 1987.

Holst WO, Flynn LM, Garner GB and Pfander WH: Dietary nitrite vs. sheep performance. J Anim Sci, 20:936 (Abstract), 1961.

Holtenius P: Nitrite poisoning in sheep, with special reference to the detoxification of nitrite in the rumen: An experimental study. Acta Agric Scand, 7:113-163, 1957.

Hosmer DW and Lemeshow S: applied logistic regression. New York: John Wiley and Sons, pp.82-134, 1989.

Hsieh FY: Sample size tables for logistic regression. Stat Med, 8:795-802, 1989.

Hueper WC and Landsberg JW: Experimental studies in cardiovascular pathology. Arch Pathol, 29:633-648, 1940.

Hurd S: An analysis of methods used to report water quality results from a National sample: National Swine Survey. Animal Health Insight, Summer:1-4, 1993.

Inui N, Nishi Y, Taketomi M, and Mori M: Transplacental action of sodium nitrite on embryonic cells of Syrian Golden hamster. Mutation Res, 66:149-158, 1979.

Inui N, Nishi Y, Taketomi M, Mori M, Yamamoto M, Yamada T, and Tanimura A: Transplacental mutagenesis of products formed in the stomach of golden hamsters given sodium nitrite and morpholine. Int J Cancer, 24:365-372, 1979.

Jainudeen MR, Hansel W and Davison KL: Nitrate toxicity in dairy heifers. 2. Erythropoietic responses to nitrate ingestion during pregnancy. J Dairy Sci, 47:1382-1387, 1964.

Jainudeen MR, Hansel W and Davison KL: Nitrate toxicity in dairy heifers. 3. Endocrine responses to nitrate ingestion during pregnancy. J Dairy Sci, 48:217-221, 1965.

Jamieson ND: Adverse effect of nitrate metabolic products on sheep growth. Nature, 181:1601-1602, 1958.

Jamieson ND: Nitrate reduction in the rumen of the grazing sheep. NZ J Agricul Res, 2:96-106, 1959.

Johnson CJ, Bonrud PA, Dosch TL, Kilness AW, Senger KA, Busch DC, and Meyer MR: Fatal outcome of methemoglobinemia in an infant. J Am Med Assoc, 257:2796-2797, 1987.

Johnson JL, Schneider NR, Kelling CL, and Doster AR: Nitrate exposure in perinatal beef calves. In Proc 26th Ann Mtg Am Assoc of Vet Lab Diagnos, pp. 167-180, 1983.

Johnson RM and Baumann CA: The effect of thyroid on the conversion of carotene into vitamin A. J Biol Chem, 171:513-521, 1947.

Jones IR, Weswig PH, Bone JF, Peters MA, and Alpan SO: Effect of high-nitrate consumption on lactation and vitamin A nutrition of dairy cows. *J Dairy Sci*, 49:491-499, 1966.

Jones JET: Observations on parturition in the sow. The parturient and post-parturient phases. *Br Vet J*, 122:471-478, 1966.

Jordan HA, Neumann AL, Smith GS, Zimmerman JE and Vathauer RJ: Vitamin A status of steers fed "high nitrate" corn silages, and a study of subsequent effects upon carotene utilization. *J Anim Sci*, 20:937-938 (Abstract), 1961.

Jordan HA, Smith GS, Neumann AL, Zimmerman JE and Breniman GW: Vitamin A nutrition of beef cattle fed corn silages. *J Anim Sci*, 22:738-745, 1963.

Juszkiewicz T, and Kowalski B: Passage of nitrosamines from rumen into milk in goats. In Bogovski, P. and Walker, E.A., (editors), *N-Nitroso Compounds in the Environment*. Lyon, IARC Publication 9: 173-176, 1974.

Juszkiewicz T and Kowalski B: An investigation of the possible presence or formation of nitrosamines in animal feeds. In Walker et al (editors), *Environmental N-nitroso Compounds Analysis and Formation*. IARC, Lyons: 375-383, 1976.

Kahler LW, Jorgensen NA, Satter LD, Thler WJ, Crowley JW, and Finner MF: Effect of nitrate in drinking water on reproductive and productive efficiency of dairy cattle. *J Dairy Sci*, 58:771 (Abstract), 1975.

Kahraman M: Nitrat ve nitrit'in farelerde immunosupresif etkileri üzerinde araştırmalar. *Veteriner Fakültesi Dergisi*, 6:251-254, 1988.

Kammerer M, Siliart, B: Toxicité à moyen terme des nitrates: évaluation expérimentale des effets sur les fonctions de reproduction chez la lapine. *Ann Rech Vet*, 24:434-444.

Kammerer M, Pinault L, and Pouliquen H: Teneur en nitrate du lait. Relation avec sa concentration dans l'eau d'abreuvement. *Ann Rech Vét*, 23:131-138, 1992.

Kearley EO, Garner GB, Bloomfield RA, and Muhrer ME: Physiological pathways of nitrate in ruminants. *J Anim Sci*, 21:1002 (Abstract), 1962.

Keating EK, Hale WH, and Hubbert F Jr: *In vitro* degradation of vitamin A and carotene by rumen liquor. *J Anim Sci*, 23:111-117, 1964.

Keith NM, Whelan M, and Bannick EG: The action and excretion of nitrates. Arch Int Med, 46:797-832, 1930.

Keleştimur H, Pirinççi I, and Servi K: Deneysel olarak nitrat zehirlenmesi oluşturulan koyunlarda bazı hematolojik araştırmalar. Doğa, 12:52-57, 1988.

Kelley ST, Oehme FW, and Hoffman SB: Effect of chronic dietary nitrates on canine thyroid function. Toxicol Appl Pharmacol, 27:200-203, 1974.

Kendrick JW, Tucker J and Peoples SA: Nitrate poisoning in cattle due to ingestion of variegated thistle, *Silybum marianum*. J Am Vet Med Assoc, 126:53-56, 1955.

Ketchum JL, Garner GB, Flynn LM, and Pfander WH: The role of butylated hydroxy anisole and sodium meta bisulfite in the utilization of rations containing nitrate. J Anim Sci, 16:1092-1093 (Abstract), 1957.

Kienholz EW, Enos HL, and McPherron TA: The effects of some water sources and treatments upon turkey performance. Poultry Sci, 44:1390 (Abstract), 1965.

Kilgore L, Almon L and Gieger M: The effects of dietary nitrate on rabbits and rats. J Nutr, 69:39-44, 1959.

Kilgore L, Stasch AR, and Barrentine BF: Relation of ascorbic acid to nitrate content of turnip greens and to methemoglobin formation. Am J Clin Nutr, 14:52-55, 1964.

Knight AP: The toxicology of sulphur and nitrate in ruminants. Bov Pract, 20:121-123, 1985.

Koch BA, Parrish DB, and Sukhonthasarnpa S: Effects of dietary NO₂ and NO₃ on growing swine. J Anim Sci, 22:840 (Abstract), 1963.

Kociba RJ and Sleight SD: Nitrite toxicosis in the ascorbic acid-deficient Guinea pig. Toxicol Appl Pharmacol, 16:424-429, 1970.

Koppang N, Slagsvold P, Aas Hansen M, Sögnen E, and Svenkerud R: Feeding experiments with meal produced from herring preserved with sodium nitrite and formalin: Possible connection between nitrite preservation and toxic hepatitis in sheep. Nord Vet, 16:343-362, 1964.

Koppang N: A severe progressive liver disease in fur animals I. Symptoms and organ changes. Nord Vet, 18:205-209, 1966.

Koppang N: An outbreak of toxic liver injury in ruminants. Case reports pathological-anatomical investigations, and feeding experiments. Nord Vet, 16:305-322, 1964.

Koppang N and Helgebostad A: Toxic hepatitis in fur animals II. The etiology elucidated by feeding experiments. Nord Vet, 18:210-215, 1964.

Kretschmer AE Jr: Nitrate accumulation in everglades forages. Agron J, 50:314-316, 1958.

Leman AD, Straw B, Glock RD, Mengeling WL, Penny RHC, Scholl E (editors): *Diseases of Swine - Sixth Edition*. Iowa State University Press, Iowa, 1986.

Lewis D: The metabolism of nitrate and nitrite in the sheep. 2. Hydrogen donors in nitrate reduction by rumen micro-organisms in vitro. Biochem J, 49:149-153, 1951.

Lewis D: The metabolism of nitrate and nitrite in the sheep. 1. The reduction of nitrate in the rumen of the sheep. Biochem J, 48:175-180, 1951.

Lindsey GA and Rhines CM: The production of hydroxylamine by the reduction of nitrates and nitrites by various pure cultures of bacteria. J Bacteriol, 24:489-492, 1932.

Lingaas F and Rønnigen K: Epidemiological and genetical studies in Norwegian pig herds. II. Overall disease incidence and seasonal variation. Acta Vet Scand, 32:89-96, 1991.

Lippsmeyer BC, Tracy ML, Moller G: Ion-exchange liquid chromatographic determination of nitrite and nitrite in biological fluids. J Assoc Off Analyt Chem, 73:457-462, 1990.

London WT, Henderson W, and Cross RF: An attempt to produce chronic nitrite toxicosis in swine. J Am Vet Med Assoc, 150:398-402, 1967.

Magee PN: Nitrogen as a potential health hazard. Philos Trans R Soc Lond, 296:543-550, 1982.

Maldonado G and Greenland S: Simulation study of confounder-selection strategies. Amer J Epidemiol, 138:923-936, 1993.

Malone P: Monensin sodium toxicity in cattle. Vet Rec, 103:477-478, 1978.

Marrett LE, and Sunde ML: The use of turkey poults and chickens as test animals for nitrate and nitrite toxicity. *Poultry Sci*, 47:511-519, 1968.

Mayo NS: Cattle poisoning by nitrate of potash. In *Kansas Agricultural Experiment Station bulletin* 49, 3-11, 1895.

McIlwain PK and Schipper IA: Toxicity of nitrate nitrogen to cattle. *J Am Vet Med Assoc*, 142:502-505, 1963.

Metcalf WK: The sensitivity of intracorpuseular haemoglobin to oxidation by nitrite ions. *Phys Med Biol*, 6:427-435, 1962.

Miller RW, Moore LA, Waldo DR, and Wren TR: Effect of nitrate on utilization of corn silage carotene by calves. *J Anim Sci*, 24:898 (Abstract), 1965.

Mitchell GE Jr, Little CO and Hayes BW: Pre-intestinal destruction of vitamin A by ruminants fed nitrate. *J Anim Sci*, 26:827-829, 1967.

Mitchell GE Jr, Little CO, Hayes BW, and Tucker RE: Preintestinal destruction of vitamin A in steers fed nitrate. *J Anim Sci*, 24:898 (Abstract), 1965.

Morris MP, Cancel B and González-Más A: Toxicity of nitrates and nitrites to dairy cattle. *J Dairy Sci*, 41:694-696, 1958.

Mortensen RA: The effect of diet on methemoglobin levels of nitrite-injected rats. *Arch Biochem Biophys*, 46:241-243, 1953.

Møller H, Landt J, Jensen P, Pedersen E, Autrup H, and Møller Jensen O: Nitrate exposure from drinking water and diet in a Danish rural population. *Int J Epidemiol*, 18:206-212, 1989.

Muhrer ME, Garner GB, Pfander WH and O'Dell BL: The effect of nitrate on reproduction and lactation. *J Anim Sci*, 15:1291-1292 (Abstract), 1956.

Muhrer ME, Case AA, Garner GB and Pfander WH: Toxic forages produced in a drought area. *J Anim Sci*, 14:1251 (Abstract), 1955.

Murdock FR, and Hodgson AS: Utilization of nitrates by dairy cows. *J Dairy Sci*, 55:640-642, 1972.

Nakamura Y, Yoshida J, Nakamura R, and Horie H: Nitrate metabolism of microorganisms in the rumen of sheep fed high nitrate forages. *Japan J Zootech Sci*, 47:63-67, 1976.

National Academy of Sciences--National Research Council, Subcommittee on nutrient and toxic elements in water: Nutrients and toxic substances in water for livestock and poultry. Nat Acad Sci, Washington, D.C., 1974.

National Academy of Sciences--National Research Council, Assembly of Life Sciences: The health effects of nitrate, nitrite and N-nitroso compounds. Nat Acad Sci, Washington, D.C., 1981.

Nelson PA, Paulson GD, and Feil VJ: The effect of nitrite on ¹⁴C-sulphathiazole (4-amino-N-2-thiazolyl[U-¹⁴C]benzenesulphonamide) metabolism in the rat. Xenobiotica, 17:829-838, 1987.

Newman MC, Dixon PM, Looney BB, and Pinder JE: Estimating mean and variance for environmental samples with below detection limit observations. Water Resources Bull, 25:905-916, 1989.

Newsom IE, Stout EN, Thorp F Jr, Barber CW and Groth AH: Oat hay poisoning. J Am Vet Med Assoc, 43:66-75, 1937.

Nicholls TJ and Miles EJ: Nitrate/nitrite poisoning of cattle on ryegrass pasture. Aust Vet J, 56:95-96, 1980.

O'Dell BL, Erek Z, Flynn L, Garner GB, and Huhner ME: Effects of nitrite containing rations in producing vitamin A deficiencies in rats. J Anim Sci, 19:1280 (Abstract), 1960.

O'Hara PJ and Fraser AJ: Nitrate poisoning in cattle grazing crops. NZ Vet J, 23:45-53, 1975.

Olson OE, Nelson DL, and Emerick RJ: Effect of nitrate and some of its reduction products on carotene stability. Agric Food Chem, 11:140-143, 1963.

Olson OE and Moxon AL: Nitrate reduction in relation to oat hay poisoning. J Am Vet Med Assoc, Vol. C, 782:403-406, 1942.

Olson JR, Oehme RW, and Carnahan DL: Relationship of nitrate levels in water and livestock feeds to herd health problems on 25 Kansas farms. Vet Med Sm Anim Clin, 67:257-260, 1972.

Osweiler GD, Carson TL, Buck WB and Van Gelder GA: Urea and nonprotein nitrogen. In *Clin Diag Vet Toxicol*, Third Edition, pages 160-166, 171-173, 1976.

Osweiler GD, Carson TL, Buck WB, Van Gelder GA: Nitrates, nitrites, and related problems. In *Clinical and Diagnostic*

Veterinary Toxicology, Third Edition. Kendall/Hunt, Dubuque, Iowa: 460-467, 1985.

Page RD, Gilson WD, Guthrie LD, Mertens DR, and Hatch RC: Serum progesterone and milk production and composition in dairy cows fed two concentrations of nitrate. *Vet Human Toxicol*, 32:27-31, 1990.

Partlow GD, Fisher KRS, Page PD, MacMillan K, and Walker AF: Prevalence and types of birth defects in Ontario swine determined by mail survey. *Can J Vet Res*, 57:67-73.

Paulson GD: The effect of dietary nitrite and nitrate on the metabolism of sulphamethazine in the rat. *Xenobiotica*, 16:53-61, 1986.

Paulson GD and Feil VJ: Evidence for diazotization of ¹⁴C-sulfamethazine {4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene[U-¹⁴C]sulfonamide} in swine. *Drug Metab Disp*, 15:841-845, 1987.

Paulson GD: The influence of nitrite on sulfonamide drug metabolism in animals. *Drug Metabol Rev*, 18:137-162, 1987.

Paulson GD and Aschbacher PW: Effect of high dietary nitrate on the disposition of sulfamethazine [4-amino-N-(4,6-dimethyl-2-pyrimidinyl) benzenesulfonamide] in swine. *J Agric Food Chem*, 38:308-310, 1990.

Pfander WH, Garner GB, Ellis WC and Muhrer ME: The etiology of "nitrate poisoning" in sheep. University of Missouri Agricultural Experiment Station, Bulletin 637, 1957.

Phillips WEJ. Nitrate content of foods - Public health implications: *Can Inst Food Technol J*, 1:98-103, 1968.

Pirinççi I and Keleştimur H: Koyunlarda nitrat ve nitrit zehirlenmesi üzerine deneysel çalışmalar. *Doğa*, 11:255-265, 1987.

Pirinççi I, Keleştimur H, Servi K, and Karahan İ: Koyunlarda nitrat zehirlenmesinin tedavisi üzerine deneysel çalışmalar. *Doğa*, 12:78-84, 1988.

Prewitt RD and Merilan CP: Effect of potassium nitrate intake on dairy calves. *J Dairy Sci*, 41:807-811, 1958.

Pugh DL, Garner GB, Bloomfield RA and Muhrer ME: Carotene-vitamin A destruction by nitrite *in vitro*. *J Anim Sci*, 21:1009 (Abstract), 1962.

Randall GCB: Observations on parturition in the sow. I. Factors associated with the delivery of the piglets and their subsequent behaviour. *Vet Rec*, 90:178-182, 1972a.

Randall GCB: Observations on parturition in the sow. II. Factors influencing stillbirth and perinatal mortality. *Vet Rec*, 90:183-186, 1972b.

Reddy BS and Thomas JW: Interrelationship between thyroid status and nitrate on carotene conversion. *J Anim Sci*, 21:1010 (Abstract), 1962.

Ridder WE and Oehme FW: Nitrates as an environmental, animal, and human hazard. *Clin Toxicol*, 7:145-159, 1974.

Riggs CW: Nitrite poisoning from ingestion of plants high in nitrate. *Am J Vet Res*, 6:194-197, 1945.

Roberts WK and Sell JL: Vitamin A destruction by nitrite *in vitro* and *in vivo*. *J Anim Sci*, 22:1081-1085, 1963.

Robinson LD and Jewell JP: Some surprising results about covariate adjustment in logistic regression models. *Int Stat Rev*, 58:227-240, 1991.

Rose DP: Micronutrients in carcinogenesis. In Wynder et al. (editors), *Environmental Aspects of Cancer - The Role of Macro and Micro Components of Foods*. Food Nutr Press, Inc., Connecticut: 128-139, 1983.

Rubenchik BL, Khmel'nitsky GA, Tiktin LA, Tsapenko VF, Vovk CM, Glavin AA, Dan'ko NF, Skarinskays EY, and Glushknow PV: [Content of N-nitrosamine and their precursors in milk and blood of cattle]. *Veterinariia*, 7:59-61, 1988.

Ruhr LP and Osweiler GD: Nitrate accumulators. In Howard (editor), *Current Veterinary Therapy - Food Animal Practice 2*. W.B. Saunders Co., Philadelphia, PA: 392-394, 1986.

Sakshaug J, Sögnen E, Aas Hansen, M, and Koppang N: Dimethylnitrosamine; its hepatotoxic effect in sheep and its occurrence in toxic batches of herring meal. *Nature*, 206:1261-1262, 1965.

Sapiro ML, Hoflund S, Clark R, and Quin JI: Studies on the alimentary tract of the Merino sheep in South Africa. XVI.-The fate of nitrate in ruminal ingesta as studied *in vitro*. *Onderstepoort J Vet Sci Anim Indust*, 22:357-372, 1949.

SAS Institute Inc: *SAS/STAT® User's Guide*, Release 6.03 Edition. Cary, N.C. SAS Institute Inc, 1988.

SAS Institute Inc: SAS® Technical Report P-200, *SAS/STAT® Software: CALIS and LOGISTIC Procedures*, Release 6.04, Cary, NC: SAS Institute Inc, 236 pp., 1990.

Savage A: Nitrate poisoning from sugar beet tops. *Can J Comp Med*, 13:9-10, 1949.

Schmitz JT: Methemoglobinemia - A cause of abortions? Preliminary Report. *Obstet Gynecol*, 17:413-415, 1961.

Schneider NR and Yeary RA: Nitrite and nitrate pharmacokinetics in the dog, sheep, and pony. *Am J Vet Res*, 36:941-947, 1975.

Schneider NR and Yeary RA: Measurement of nitrite and nitrate in blood. *Am J Vet Res*, 34:133-135, 1973.

Schwarte LH, Eveleth DF and Biester HE: Studies on the so-called corn stalk poisoning in cattle. *Vet Med*, 34:648-651, 1939.

Scragg RKR, Dorsch MM, McMichael AJ, and Baghurst PA: Birth defects and household water supply. *Med J Aust*, 2:577-579, 1982.

Sebaugh TP, Lane AG and Campbell JR: Effects of two levels of nitrate and energy on lactating cows receiving urea. *J Anim Sci*, 31:142-144, 1970.

Seerley RW, Emerick RJ, Embry LB and Olson OE: Effect of nitrate or nitrite administered continuously in drinking water for swine and sheep. *J Anim Sci*, 24:1014-1019, 1965.

Sell JL and Roberts WK: Effects of dietary nitrite on the chick: Growth, liver vitamin A stores and thyroid weight. *J Nutr*, 79:171-178, 1963.

Setchell BP and Williams AJ: Plasma nitrate and nitrite concentration in chronic and acute nitrate poisoning in sheep. *Aust Vet J*, 38:58-62, 1962.

Shuval HI and Gruener N: Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am J Public Health*, 62:1045-1052, 1972.

Simon J, Sund JM, Douglas FD, Wright MJ and Kowalczyk T: The effect of nitrate or nitrite when placed in the rumens of pregnant dairy cattle. *J Am Vet Med Assoc*, 135:311-314, 1959.

Simon J, Sund JM, Wright MJ, Winter A and Douglas FD: Pathological changes associated with the lowland abortion syndrome in Wisconsin. *J Am Vet Med Assoc*, 132:164-169, 1958.

Simon J, Sund JM, Wright MJ and Douglas FD: Prevention of noninfectious abortion in cattle by weed control and fertilization practices on lowland pastures. J Am Vet Med Assoc, 135:315-317, 1959.

Sinclair KB and Jones DIH: Nitrite toxicity in sheep. Res in Vet Sci, 8:65-70, 1967.

Sinclair KB and Jones DIH: The effect of nitrate on blood composition and reproduction in the ewe. Br Vet J, 120:78-86, 1964.

Singer RH: Environmental nitrates and animal health. Southwest Vet, 22:13-18, 1968.

Singer RH: The nitrate poisoning complex. Proc of the 76th Annual Meeting US Anim Health Assoc, p. 310-322. Miami Beach, Florida, 1972.

Sinha DP and Sleight SD: Pathogenesis of abortion in acute nitrite toxicosis in Guinea pigs. Toxicol Appl Pharmacol, 18:340-347, 1971.

Slanina L', Slivka P, and Struháriková: Transmamárny prestup dusičnanov a dusitanov u prežúvavcov a úroveň methemoglobínémie v krvi mláďat a ich matiek. Veterinárni Medicina, 35:647-656, 1990.

Sleight SD and Atallah OA: Reproduction in the Guinea pig as affected by chronic administration of potassium nitrate and potassium nitrite. Toxicol Appl Pharmacol, 12:179-185, 1968.

Sleight SD, Sinha DP, and Uzoukwu M: Effect of sodium nitrite on reproductive performance of pregnant sows. J Am Vet Med Assoc, 161:819-823, 1972.

Slenning BD, Galey FD and Anderson M: Forage-related nitrate toxicoses possibly confounded by nonprotein nitrogen and monensin in the diet used at a commercial dairy heifer replacement operation. J Am Vet Med Assoc, 198:867-870, 1991.

Sloan KW: Nitrates and animal health. In Proc of the Second Allerton Conference, December, 1971, pp. 25-27. College of Agriculture, University of Illinois, special publication 26, 1972.

Smith HC, Lovell VE, Reppert R, and Griswold D: Nitrate poisoning in swine. Vet Med, 54:547-550, 1959.

Smith RA and Suleiman A: Toxic plant case reports. Vet Human Toxicol, 33:349-350, 1991.

Smith GS, Neumann AL, and Hatfield EE: Carotene utilization and vitamin A nutrition as influenced by dietary nitrite and "high nitrate" silage: Laboratory studies. J Anim Sci, 20:683-684 (Abstract), 1961.

Smith GS, Hatfield EE, Durdle WM, and Neumann AL: Vitamin A status of cattle and sheep as affected by nitrate added to rations of hay or silage and by supplementation with carotene or preformed vitamin A. J Anim Sci, 22:1013-1014 (Abstract), 1962.

Cline TR, Hatfield EE and Garrigus US: Effects of potassium nitrate, alpha-tocopherol, thyroid treatments, and vitamin A on weight gain and liver storage of vitamin A in fattening lambs. J Anim Sci, 22:911-913, 1963.

Smith GS: Diagnosis and causes of nitrate poisoning. J Am Vet Med Assoc, 147:365-366, 1965.

Smith JE and Beutler E: Methemoglobin formation and reduction in man and various animal species. Am J Physiol, 210:347-350, 1986.

Sokolowski JH, Garrigus US and Hatfield EE: Effects of inorganic sulfur on KNO_3 utilization by lambs. J Anim Sci, 20:953 (Abstract), 1961.

Spicer SS: Species differences in susceptibility to methemoglobin formation. J Pharmacol Exp Ther, 99:185-194, 1950.

Stewart GA, and Merilan CP: Effect of potassium nitrate intake on lactating dairy cows. University of Missouri Agricultural Experiment Station, Bulletin 650, 1958.

Stoewsand GS, Anderson JL, and Lee CY: Nitrite-induced methemoglobinemia in Guinea pigs: Influence of diets containing beets with varying amounts of nitrate, and the effect of ascorbic acid, and methionine. J Nutr, 103:419-424, 1973.

Struble CB and Paulson GD: The metabolism and deamination of [^{14}C]-sulphamethazine in a germ-free pig: The influence of nitrate and nitrite. Food Chem Toxicol, 26:797-801, 1988.

Stuart LD and Oehme FW: Environmental factors in bovine and porcine abortion. Vet Human Toxicol, 24:435-441, 1982.

Sund JM, Wright MJ, and Simon J: Weeds containing nitrates cause abortion in cattle. Agron J, 49:278-279, 1957.

Sunde ML: The use of the turkey poult as a test animal for nitrite toxicity. Poultry Sci, 43:1368 (Abstract), 1964.

Super M, De V Heese H, MacKenzie D, Dempster WS, Du Plessis J, and Ferreira JJ: An epidemiological study of well-water nitrates in a group of South West African/Namibian infants. Water Res, 15:1265-1270, 1981.

Takács S: Nitrate content of drinking water and tumors of the digestive organs. Zentralb Bakter Mikrobiol 184: 269-279, 1987.

Takahashi J and Young BA: Prophylactic effect of L-cysteine on nitrate-induced alterations in respiratory exchange and metabolic rate in sheep. Anim Feed Sci Technol, 35:105-113, 1991.

Tannenbaum SR, Weisman M, and Fett D: The effect of nitrate intake on nitrite formation in human saliva. Food Cosmetics Toxicol, 14:549-552, 1976.

Tannenbaum SR, Sinskey AJ, Weisman M and Bishop W: Nitrite in human saliva. Its possible relationship to nitrosamine formation. J Nat Cancer Inst, 53:79-84, 1974.

Tillman AD, Sheriha GM and Sirny RJ: Nitrate reduction studies with sheep. J Anim Sci, 24:1140-1146, 1965.

Tillman AD, Sheriha GM, Goodrich RD and Nelson EC: Effect of injected hydroxylamine upon vitamin A status of sheep. J Anim Sci, 24:1136-1139, 1965.

Tollett JT, Becker DE, Jensen AH and Terrill SW: Effect of dietary nitrate on growth and reproductive performance of swine. J Anim Sci, 19:1297 (Abstract), 1960.

United States Department of Agriculture: Nitrate occurrence in U.S. Waters (and related questions). United States Department of Agriculture, Washington, D.C., pp. 1-35, 1991.

United States Department of Agriculture, Animal and Plant Health Inspection Service: National Swine Survey: data collection: 1990 [unnumbered technical report]. US Dept of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System, Fort Collins, CO., 1992.

United States Environmental Protection Agency: Proposed criteria for water quality. Volume I. Quality of water for livestock. Environ. Rep. Current Develop., 4:663-664, 1973.

van 't Klooster ATh, Malestein A, Kemp A, Geurink JH, and Schyt G: Nitrate intoxication in cattle. In Proc to the XIIth World Congress on Diseases of Cattle, pp.398-403. The Netherlands, September 7-10, 1982.

Vermunt J and Visser R: Nitrate toxicity in cattle. NZ Vet J, 35:136-137, 1987.

Vigil, J., Warburton, S., Haynes, W.S. and Kaiser, L.R., 1965. Nitrates in municipal water supply cause methemoglobinemia in infant. Pub. Health Rep., 80:1119-1121.

Walker R: Naturally occurring nitrate/nitrite in foods. J Sci Food Agric, 26:1735-1742, 1975.

Wallace JD, Raleigh RJ and Weswig PH: Performance and carotene conversion in Hereford heifers fed different levels of nitrate. J Anim Sci, 23:1042-1045, 1964.

Wang LC, Garcia-Rivera J and Burris RH: Metabolism of nitrate by cattle. Biochem J, 81:237-242, 1961.

Wanntorp H and Swahn O: The toxicity of whey for swine in conjunction with bacterial reduction of nitrate to nitrite. Proc of the 15th Int Vet Congress, Part 1:496-501, 1953.

Watts H, Webster M, Chappe A, and Leaver DD: Laboratory diagnosis of nitrite poisoning in sheep and cattle. Aust Vet J, 45:492, 1969.

Weichenthal BA, Embry LB, Emerick RJ and Whetzal FW: Influence of sodium nitrate, vitamin A and protein level on feedlot performance and vitamin A status of fattening cattle. J Anim Sci, 22:979-984, 1963.

Welsch C, Bloomfield RA, Garner GB, and Muhrer ME: Effect of dietary nitrate on thyroid and adrenal gland weight. J Anim Sci, 20:981 (Abstract), 1961.

Welsch CW, Bloomfield RA, Garner GB and Muhrer ME: Response of rats to low temperature and nitrate. J Anim Sci, 21:1032 (Abstract)1962.

Wendel WB: The control of methemoglobinemia with methylene blue. J Clin Invest, 18:179-185, 1939.

Whitehead JE: Potassium nitrate poisoning in a dog -- A case report. J Am Vet Med Assoc, 123:232-233, 1953.

Whitehead EI and Moxson AL: Nitrate poisoning. South Dakota Agricultural Experiment Station bulletin 424, 1952.

Williams MC: Accumulation of nitrate by annual goldeneye and showy goldeneye. *J Range Mgmt*, 42:196-198, 1989.

Wilson JK: Nitrate in foods and its relation to health. *Agron J*, 41:20-22, 1989.

Wilson JK: Nitrate in plants: Its relation to fertilizer injury, changes during silage making, and indirect toxicity to animals. *Agron J*, 35:279-290, 1943.

Winks WR, Sutherland AK, and Salisbury RM: Nitrite poisoning of pigs. *Queensland J Agric Sci*, 7:1-14, 1950.

Winter AJ: Studies on nitrate metabolism in cattle. *Am J Vet Res*, 23:500-505, 1962.

Winter AJ and Hokanson JF: Effects of long-term feeding of nitrate, nitrite, or hydroxylamine on pregnant dairy heifers. *Am J Vet Res*, 125:353-361, 1964.

Wood RD, Chaney CH, Waddill DG, and Garrison GW: Effect of adding nitrate or nitrite to drinking water on the utilization of carotene by growing swine. *J Anim Sci*, 26:510-513, 1967.

Wood PA: The molecular pathology of chronic nitrate intoxication in domestic animals: A hypothesis. *Vet Human Toxicol*, 22:26-27, 1980.

Woolley JL Jr, and Sigel CW: The role of dietary nitrate and nitrite in the reductive deamination of sulfadiazine by the rat, Guinea pig, and neonatal calf. *Life Sci*, 30:2229-2234, 1982.

Wright MJ and Davison KL: Nitrate accumulation in crops and nitrate poisoning in animals. *Adv Agron*, 16:197-247, 1964.

Würmli R, Wolffram S, and Scharrer E: Inhibition of chloride absorption from the sheep rumen by nitrate. *J Vet Med*, 34:476-479, 1987.

Wyngaarden JB, Wright BM, and Ways P: The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland. *Endocrinology*, 50:537-549, 1952.

Yadav KP, Garner GB, O'Dell BL, Flynn LM, Bloomfield RA, and Muhrer ME: Iodine and nitrate as factors in vitamin A storage. *J Anim Sci*, 21:1017 (Abstract), 1962.

Yong C, Brandow RA, and Howlett P: An unusual cause of nitrate poisoning in cattle. *Can Vet J*, 31:118, 1990.

Zemla B: A possible association between quality of drinking water and stomach cancer incidence among native and immigrant populations of a selected industrial city. *Neoplasma* 27: 55-61, 1980.

Zimmerman JE, Neumann AL, Durdle WM, and Smith GS: Nutritive value of corn silages as affected by soil fertilization, with further observations of impaired carotene utilization in silage-fed steers. *J Anim Sci*, 21:1018 (Abstract), 1962.