A STUDY OF SELECTED DRINKING WATER CONTAMINANTS AND THE RISK OF ADVERSE HEALTH OUTCOMES IN IOWA

Ammonia/Nitrite/Nitrate Groundwater Investigation

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Executive Summary:

The University Hygienic Laboratory (UHL) and the University of Iowa Center for Health Effects of Environmental Contamination (CHEEC), in collaboration with the Iowa Department of Natural Resources (DNR) Drinking Water Section, used existing databases and special monitoring efforts to conduct a statewide assessment of ground water quality and its relationship to certain public health outcomes. The study looked at the occurrence of ammonia, nitrite, nitrate and nitrifying bacteria in selected public water supplies. The study also included the linkage of analytical data maintained by the UHL and CHEEC, IDNR and health outcome data maintained by the Iowa Birth Defects Registry and the State Health Registry of Iowa. The study examined exposure to certain water contaminants and the incidence of various health outcomes at the community level.

The study goal was to provide the State of Iowa with an ecological assessment of the occurrence and concentration of ammonia, nitrite, and nitrate-nitrogen in community water supplies and the risk for adverse health outcomes including, low birthweight, certain birth defects and certain cancers.

Results of the investigation showed that systems with elevated ammonia concentrations in their source water had elevated nitrite and nitrate concentrations in their distributions systems. The results also indicated that bacterial growth, even with chlorination, was sufficient to lead to the reduction of ammonia and thus contribute to nitrite and nitrate concentrations in the distribution system. No adverse health outcomes, associated with the contaminants of concern, were identified in the study because of an insufficient study population.

The report make several recommendations related to monitoring for ammonia and nitrite, as well as control of scaling and biofilms in water supply distribution systems.

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Introduction:

Monitoring of ground water systems in recent years has indicated that certain shallow formations are susceptible to ammonia and nitrate contamination. Since ammonia, when reduced, will convert rapidly to nitrite and then to nitrate, with the latter being the stable form of nitrogen, past monitoring has focused on nitrate. Investigations of drinking water and ground water in the 1980's have shown wide spread nitrate contamination to be a problem in shallow ground water systems throughout the state. Monitoring conducted by the United States Geological Survey (USGS) and the Iowa Department of Natural Resources (IDNR) however, has detected the presence of ammonia in a number of ground water systems in Iowa. Although the source of the ammonia is probably naturally occurring, it is unclear how widespread the occurrence of ammonia and related compounds might be in Iowa's public drinking water supplies. In addition, public water supply monitoring has detected the presence of nitrite in the treatment process, at the entry point to the distribution system, and in the distribution systems of several supplies. Ammonia will convert to nitrite very rapidly under favorable circumstances. Nitrate can also be converted to nitrite in humans and both nitrate and nitrite may be tied to adverse health effects.

Certain adverse reproductive health outcomes, such as low birth weight (LBW), prematurity, intrauterine growth retardation (IUGR), and birth defects are known to be major determinants of health problems during the first year of life. A number of risk factors for these conditions have been identified, including socioeconomic factors, access to medical care, medical conditions related to pregnancy, behavior, and environmental conditions. Of the behavioral and environmental risks smoking, poor nutrition, alcohol and other substance abuse, and certain occupational exposures have been established.

Birth defects have emerged as the leading cause of infant death in the United States as infant deaths from other causes have declined. Many causes of birth defects such as genetic factors, maternal conditions including diabetes during pregnancy, medications (thalidomide and isotretinoin), poor nutrition, alcohol, smoking and infectious agents are known. The role of environmental contaminants as a cause of birth defects is less certain. The specific role of drinking water contaminants as risk factors for LBW, IUGR and birth defects has received some attention, mostly in the form of ecological studies of pesticides and herbicides. While

these studies suffer from the design flaws such as the inability to assign individual exposure levels of particular contaminants and the inability to account for potential confounders, they do provide an indication of possible problems for future study. The roles of nitrate, nitrite, and ammonia and the possible development of birth defects related to the presence of these contaminants have not been thoroughly evaluated.

Previous research studies have reported an association between nitrate in drinking water and the development of cancer (Isacson, et. al., Leclerc, et. al., Rademacher, et. al., Rogers, et. al., Ward, et. al. 1996 & 1998). A study of the risk of cancer in postmenopausal women (Iowa Women's Health Study) showed that an increased risk of bladder cancer related to nitrate in municipal drinking water supplies was dose-dependent (Weyer, et.al.). While the exposure variable (nitrate) was ecological (historic nitrate levels on a community basis), a wide variety of potential confounding factors necessitated adjustments in the data. In addition, non-Hodgkin's lymphoma was linked to nitrate levels in municipal water supplies in ecological studies in Iowa and Nebraska and in a case-control study in Nebraska (Weisenburger, et. al.).

Study Design:

The study was designed as a statistical examination of the occurrence of ammonia, nitrite and nitrate nitrogen in public drinking water supplies and the possible relationship between the exposure to these contaminants through municipal drinking water and the risk for certain adverse reproductive outcomes, bladder cancer and non-Hodgkin's lymphoma. The study was statewide in scope, and was conducted in two phases, over a two-year period. The University of Iowa Hygienic Laboratory (UHL) completed routine analyses of water samples.

Phase I (Year 1): A statewide monitoring effort of the groundwater used as source water by public drinking water supplies was undertaken. This investigation included a review of existing monitoring data and the sampling of 230 public water systems from across the state.

The water systems were selected at random from a list of public water systems in the state that met a pre-determined set of criteria. The source waters of selected systems were then compared to general demographics of the state to insure that the source water of the selected supplies represented the overall statewide use of those sources. The purpose of this approach was to ensure that all major groundwater sources of drinking water were represented by the selected public systems, and that the most heavily used sources were represented with the greatest frequency in the selected drinking water systems. Each public water supply was then contacted and asked if they would by willing to participate in the study.

Samples were collected directly from the wells, prior to treatment. Field pH, nitrite and temperature were taken at the time of sample collection. Samples were returned to the laboratory to be analyzed for the presence of nitrate, nitrite, ammonia and perchlorate. (The analysis of perchlorate was conducted independently of this study to determine the possibility of perchlorate contamination in Iowa.)

Data on the incidence of reproductive outcomes of interest was gathered from the Iowa Birth Defects Registry, and the data was linked electronically (by municipality name) to the water quality data described above to test for possible associations. Statistical evaluation of community incidence rates for the health outcomes of interest (adverse reproductive outcomes, cancers) and levels of chemical contaminants of interest were also performed.

Phase II (Year 2): The results of the first year's monitoring were assessed. Public water systems ammonia concentrations greater than one were ranked highest to lowest. This list was than reviewed by the staff at IDNR. IDNR staff identified 30 water systems from this group to be involved in the second year of the study. The selection these water systems was made to ensure that systems with the highest ammonia concentrations in their source water were represented, and that a variety of drinking water treatment processes were included in the study. Each public water supply was contacted to request assistance in conducting the study. In two cases the water system, based on the findings of the first year's work, had abandoned their source water. These systems were replaced with the next two systems on the list. Re-sampling included collection of water samples from each component of the treatment train (where access was available) and at various points within the distribution system to try and determine the effects of treatment and detention time on the ammonia, nitrate and nitrite concentrations. Samples were analyzed for the presence of nitrate, nitrite, ammonia nitrogen, dissolved oxygen, temperature, pH, heterotrophic plate counts (HPC) and nitrifying bacteria.

The goal of the study is to assess the distribution of ammonia, nitrate and nitrite in the source water of ground water systems in Iowa, the interaction of these compounds within the treatment systems and the possible implications on public health. The specific objectives of this study were:

- A review and evaluation of existing monitoring data and the possible identification of public water supply wells statewide for sampling.
- Review of state health registries for occurrence rates of certain adverse health outcomes.
- Assessment of existing water quality databases and develop data file linkages to health outcome data.
- Sampling and analysis for ammonia (NH_3-N), nitrite (NO_2), nitrate (NO_3-N) as well as necessary field measurements from public drinking water wells.
- Summarization of the results with recommendations to the Iowa Department of Natural Resources if findings were found to support changes in policy.

Sample Collection and Analytical Methods:

The staff of the University Hygienic Laboratory, following protocols defined by the UHL's Limnology section collected all samples. These are standard protocols followed in the collection of all samples collected by the UHL field staff.

All laboratory analyses were complete at the University Hygienic Laboratory's facility in Des Moines, with the exception of the bacteriological work, which was completed at the UHL laboratory in Iowa City. The laboratory is a certified laboratory and must meet specific performance criteria to maintain its certification. The US EPA as a condition of certification determines these certification criteria. Data generated outside the conditions of certification are not acceptable. Quality Assurance (QA) records are kept as part of routine analyses and are reported and reviewed in accordance with UHL's QA Documents.

Nitrate/Nitrite Nitrogen analyses were completed using EPA method 353.2, Cd Reduction, as defined in EPA Methods for Analysis of Water and Wastes. EPA-600/4-79-020, March 1983 and Method 353.2 Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. The analyses was completed on a Bran-Luebbe Auto Analyzer II.

Ammonia analyses were completed following EPA 350.1 as defined in, Nitrogen, Ammonia (Colormetric, Berthelot reaction) EPA Methods for Chemical Analysis Of Water And Wastes, Method 350.1. Analyses were completed using a Technicon TRAACS 800™, Industrial Method No. 780-86T. Ammonia analyses included analysis for ionized ammonia and calculation for un-ionized ammonia using a standardized worksheet.

Field values for temperature, pH and dissolved oxygen were collected using an Orion model 2304 meter. Field values for nitrite and nitrate were determined using Hach Chemical's Aquachek Nitrate/Nitrite test strips (Cat 27454-25) with a range of 0 – 3 mg/l.

Bacterial analyses were performed following the appropriate EPA methods. Hetrotrophic Plate Counts were completed using pour plate method SM 9215B, as defined by Standard Methods for the Examination of Water and Wastewater, 18th edition. Analysis for nitrifying bacti was conducted using HACH's N-BART media and method.

Discussion and Analyses:

Ammonia in the source water of a public water supply can impact the supply's ability to provide high quality water to its customers. When ammonia is present in the source water it will often react with chlorine. As many water supplies use breakpoint chloronation to achieve greater effectiveness against bacteria in the distribution system, high ammonia in the source water can result in a greater chlorine demand to achieve free chlorine in the system.

The presence of ammonia in the source water can also promote the growth of nitrifying bacteria, such as Nitrosomonas and Nitrobacter which oxidize the ammonia to nitrite and then nitrate. Nitrite and nitrate are regulated contaminates under the Safe Drinking Water Act (SDWA). The maximum contaminant levels (MCL) for nitrite and nitrate are 1 and 10 mg/l, respectively.

Groundwater is used as a source of drinking water for approximately 75% of the public water systems in the state. Since the presence of ammonia in drinking water can result in

increased chlorine demand, increases in heterotrophic plate count (HPC) and total coliform bacteria counts and impact a system's ability to meet the requirements of the SDWA, more attention has been focused on ammonia concentrations in Iowa's groundwater in recent years. Researchers at the US Geological Survey, Geological Survey Bureau of the Iowa Department of Natural Resources and the Geological Sciences Department at Iowa State University are currently, or have in the recent past, looked at the distribution and source of ammonia in Iowa's groundwater. The focus of this investigation was on the conversion of ammonia to nitrite and nitrate within the water treatment and distribution systems of public water supplies, and the possible implications to the health of the public.

A total of 289 Iowa municipal water supplies were initially eligible for this study based on the following criteria:

- Historical raw water data indicated that ammonia existed in the municipal supply's source water and could linked to a specific well
- Water supplies had a single source of water, which is defined as receiving ≥ 90% of their water from the same aquifer and receiving the same treatment
- Their 1980 population was greater than 400.

From 289 municipalities, 230 were selected for the study. Municipalities selected for the study included all towns with populations greater than 1,000 (n=134), and 96 municipalities selected at random from towns with populations less than 1,000. Raw water from a total of 230 municipal supplies were sampled in the fall of 1999 and analyzed for ammonia, nitrite and nitrate. During the course of the first years monitoring, three municipal systems elected to connect to regional or rural water systems and were dropped from the data set. Of the remaining 227 municipal systems a total of 124 municipal supplies were found to have detectable levels of ammonia in their raw water source, the remaining 103 supplies had no detectable ammonia. Ammonia values ranged from 0.0 mg/L and 7.4 mg/L with the median concentration of 0.8 mg/L (Table 1). Maximum values for nitrite and nitrate were 1.3 mg/L and 16 mg/L, respectively.

The relationship between the field test parameters and analytical values provided by the laboratory was fairly good. There were few positive nitrite samples to compare. Only one percent of the samples had a detectable concentration of nitrite. The field nitrite kits were higher than the matched laboratory sample as often as they were low. The field nitrate tests were higher than the matching laboratory analyses only slightly more frequently than they were lower. On average, higher field tests were 2.1 mg/l greater than the matching lab results. The lower field nitrate tests averaged 2.1 mg/l lower than their matching laboratory test.

Table 1

Summary statistics for the first year of sampling.
(Concentrations are measured in mg/L)

	N	N > MDL	% Detections	Minimum	Maximum	Mean	Median

Nitrite (NO2)	234	2	1%	0	1.3	0.006	0
NO2-Field	230	3	1%	0	1	0.006	0
Nitrate (NO3-N)	233	99	42%	0	16	2.26	0
NO3-Field	230	101	44%	0	15	1.93	0
Ammonia	233	120	52%	0	7.4	0.68	.8

Quartile Data						
(positive detections only)		**Ammonia**	**Nitrite** (NO2)	**NO2-Field**	**Nitrate** (NO3N)	**NO3N + NO2-Field**
		N = 120	N = 2	N = 3	N = 99	N = 101
	Minimum	0.1	0.1	0.2	0.1	0.5
	25%	0.3	0.4	0.2	1.9	2
	50%	0.8	0.7	0.2	4.5	4
	75%	1.625	1	0.6	8	5

Fifty two percent of the well samples had detectable concentrations of ammonia. The ammonia, nitrite and nitrate detections were separated by aquifer (Table2). Ninety percent of the samples taken from Ordovician and 74% of the samples taken from drift wells contained measurable concentrations of ammonia. The mean concentration of ammonia in samples taken from Ordovician and drift wells was 1.09mg/l and 1.7mg/l, respectively. Although 100% of the Pennsylvanian wells were positive for ammonia and the mean concentration was the highest at 2.3 mg/l, only two wells from this aquifer were sampled and it is somewhat doubtful that they are representative of the aquifer. A similar pattern was evident in nitrate concentrations. A complete listing of the parameters and associated analytical summaries by aquifer can be found in Appendix 2.

Table 2

Ammonia, nitrate and nitrite detections by aquifer
(Concentrations are measured in mg/L)

	AQUIFER	SAMPLES	N DETECTS	% Detection	MEAN	MINI-MUM	25%	MEDIAN	75%	MAX-IMUM
Ammonia (NH3)	**ALLUVIUM**	74	22	30%	0.22	0	0	0	0.2	2.8
	CAMBRIAN	4	2	50%	0.1	0	0	0	0.1	0.3
	CAMBRIAN/ ORDOVICIAN	14	9	64%	0.66	0	0	0.7	1.2	1.5
	DAKOTA	23	14	61%	0.9	0	0	0.3	1.4	3.6
	DRIFT	31	23	74%	1.73	0	0	1	2.7	7.4
	MISSISSIPPIAN	17	10	59%	0.54	0	0	0.3	0.7	2.2
	ORDOVICIAN	10	9	90%	1.09	0	0.3	0.7	1	5.1
	PENNSYLVANIAN	2	2	100%	2.3	1.5	1.5	1.5	3.1	3.1
	SILURIAN/ DEVONIAN	58	29	50%	0.56	0	0	0	0.5	4.7
	Total all data	**233**	**120**	**52%**	**0.68**	**0**	**0**	**0.1**	**0.8**	**7.4**

	ALLUVIUM	74	2	3%	0.02	0	0	0		1
Nitrite	**CAMBRIAN**	4	0	0%	0	0	0	0	0	0
(NO2)	**CAMBRIAN/ ORDOVICIAN**	14	0	0%	0	0	0	0	0	0
	DAKOTA	23	0	0%	0	0	0	0	0	0.2
	DRIFT	32	0	0%	0	0	0	0	0	0
	MISSISSIPPIAN	17	0	0%	0	0	0	0	0	0
	ORDOVICIAN	10	0	0%	0	0	0	0	0	0
	PENNSYLVANIAN	2	0	0%	0	0	0	0	0	0
	SILURIAN/ DEVONIAN	58	0	0%	0	0	0	0	0	0
	Total all data	**234**	**2**	**1%**	**0.006**	**0**	**0**	**0**	**0**	**1**

	ALLUVIUM	74	53	72%	4.09	0	0	2.2	7.5	14
Nitrate	**CAMBRIAN**	4	0	0%	0	0	0	0	0	0
(NO3)	**CAMBRIAN/ ORDOVICIAN**	14	3	21%	0.22	0	0	0	0	2
	DAKOTA	23	9	39%	1.84	0	0	0	1.3	16
	DRIFT	31	4	13%	0.35	0	0	0	0	4.4
	MISSISSIPPIAN	17	7	41%	2.4	0	0	0	4.7	11
	ORDOVICIAN	10	1	10%	0.48	0	0	0	0	4.8
	PENNSYLVANIAN	2	0	0%	0	0	0	0	0	0
	SILURIAN/ DEVONIAN	58	22	38%	2.14	0	0	0	3.4	12
	Total all data	**233**	**99**	**42%**	**2.26**	**0**	**0**	**0**	**3.4**	**16**

Although there were no adverse health outcomes associated with the populations using the public water supplies involved in this study. It is clear from the data in this study that populations using water provided by systems with high ammonia concentrations have a greater potential for exposure to elevated nitrite and nitrate concentrations than in systems using source water that is free of ammonia nitrogen. The health outcomes report is discussed in greater detail later in this report.

Since the effort of the second year of monitoring was to focus on changes within the treatment and distribution system only supplies that could be expected to have ammonia concentrations in the source water were monitored in the second phase of the study. In the second phase of the study, 30 municipal systems with initial ammonia concentrations of greater than 1 mg/l were selected for further study. Analyses were conducted for ammonia, nitrite and nitrate. The results are compared to the results from the initial 1999 sampling in table 3.

Table 3

Summary statistics from raw water samples taken in 1999 and 2000

	Fall 1999 (n=227)			*Summer 2000 (n=30)*		
Analyte	**% Detection**	**Mean***	**Median***	**% Detection**	**Mean***	**Median***
Ammonia	52	0.68	0.0	87	2.67	2.35
Nitrite	1	0.006	0.0	13	0.12	0.0
Nitrate	42	2.26	0.0	20	0.92	0.0

*(*mg/l)*

In the second year of the study, the Hygienic Laboratory field staff worked with public water supply officials to identify locations within the treatment systems where samples could be collected. Plant operators generally walked through the treatment system with the field staff and assisted in collecting samples after major points of treatment whenever possible. However, the ability to collect samples in many cases was restricted by plant design. Many newer plants have closed systems with water moving from one treatment process to the next within closed piping. Often there are no sampling taps available.

The analytical results of the second year of monitoring suggest that the ammonia in the source water is being oxidized to nitrite and nitrate. Nitrifying bacteria were identified in 12 of the 30 water supplies (40%) in the second phase of this study. Heterotrophic plate counts ranged from 0 to 5400 cfu/ml (colony forming units per milliliter). The highest concentrations generally occurred in the distribution systems of the supplies studied.

Ammonia, nitrite and nitrate concentrations ranged from 0 to 13, 0 to 1.6, and 0 to 12 mg/l, respectively (Table 4).

Table 4

Summary statistics of second phase of sampling.

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Parameter	*Field Temp*	*Field DO*	*Field*	*Ammonia*	*Nitrite*	*Nitrate*	*Heterotropic Plate Count*	*Nitrifying Bacti*
Units	*C*	*Mg/L*	*pH*	*Mg/L*	*mg/L*	*mg/L*	*CFU*/mL*	*CFU*/mL*
Minimum	0	0	0	0	0	0	0	0
Maximum	34.0	10.5	10.7	13.0	1.6	12.0	5400	~100,000
Mean	14.7	5.7	7.6	2.67	0.12	.92	266.1	

* Colony Forming Units

Ammonia was detected in the source water of 26 of the 30 (87 %) supplies in phase two of the study. Nitrite was detected in the raw water of 4 of these supplies and nitrate in 5 supplies. Three of the four supplies with nitrite detected in the raw water had detectable concentrations of nitrite in the their distribution systems. Nitrite was detected in the treatment and/or distribution systems of the 23 of the 30 supplies (77 %) in the study. Thirteen of the 30 supplies (43 %) had detectable concentrations of nitrite at the end of their distribution systems.

The water treatments used by the public water supply systems involved in this study varied. Samples were generally collected behind individual treatments at each plant. However, the inability to collect samples in many plants (the systems were often closed systems) and the variety of treatment technologies in use at these plants resulted in few

samples being collected in association with any one particular treatment technique. Variations in ammonia, nitrite and nitrate concentrations did not appear to be associated with any particular treatment technique or series of treatments. It should be noted however, that samples were collected directly behind the cholination feeds of 12 of the public water supplies and in the distribution systems (well down stream of the cholination points) of all the supplies. In 13 of the 31 distribution samples (42 %) and 7 of the 12 post chlorination samples (58 %) bacteria growth was detected. Although the authors did not measure chlorine residuals at the time of samples all the supplies involved in this study conduct routine bacterial monitoring under the Safe Drinking Water Act. To the authors' knowledge, none of the water supplies were experiencing or had a history of bacterial contamination. In general, field staff indicated that water systems were well run and the operators knowledgeable.

Ammonia in Drinking Water and Health Outcomes:

For the purposes of evaluating possible relationships between exposure to ammonia in drinking water and chronic health effects (cancer, adverse reproductive outcomes), the 227 towns sampled in the first phase of the study were stratified into three exposure categories:

- No exposure: No detectable ammonia from the fall 1999 analyses of raw water samples (n=103 municipal supplies)
- Low exposure: Mean ammonia level (historical and recent analyses) ≤ 1 mg/L (n=60 municipal supplies)
- High exposure: Mean ammonia level (historical and recent analyses) > 1 mg/L (n=64 municipal supplies)

Table 5 presents population changes by decade for the three exposure categories. Iowa has been experiencing a general decline in population since 1980. This is important to consider when interpreting general trends in the incidence of cancers and other chronic health outcomes.

Table 5

Population by decade and % change for Iowa municipalities by ammonia exposure group

Exposure group	1970 pop	1980 pop, % change	1990 pop, % change
No exposure (n = 103 towns)	397,003	429,534 (+ 8.2%)	419,933 (- 2.2%)
Low exposure (n = 60 towns)	93,604	100,820 (+ 7.7%)	96,276 (- 4.5%)
High exposure (n = 64 towns)	107,454	117,962 (+ 9.8%)	112,755 (- 4.4%)

Caveats: Several caveats must be added to this analysis of nitrogen compounds in Iowa municipal water supplies and chronic health outcomes:

- The analysis is ecological: the exposure is based on community exposure, there are no data on personal water consumption, nor are there any data on contaminant levels at the tap
- There are no personal data on other potential risk factors for specific cancers or birth defects
- The analytical data are sparse: some municipalities may have only 2-3 analyses for a particular contaminant
- Interpretation of results must consider that Iowa's population is generally declining, that the population is aging, and that cancer, in particular, is a disease of the elderly

Chronic Health Effects: Cancer

Nitrate from drinking water sources is a potential health concern because it is a precursor compound in the endogenous formation of *N*-nitroso compounds (NOC), which are potent animal carcinogens (Mirvish, 1995). Nitrate is the final oxidation product of organic nitrogen compounds and ammonia; therefore, ammonia levels in raw water are of interest in evaluating potential variation in nitrate levels within a specific drinking water system. The research literature contains no reports of chronic health effects from exposure to ammonia via drinking water. However, levels of ammonia in source water are of interest (due to the oxidation of ammonia $\rightarrow$ nitrate $\rightarrow$ nitrite $\rightarrow$ NOC), as they may give an indication of the potential for nitrate and nitrite levels in finished drinking water. Nitrate levels at the tap may vary depending on municipal treatment process (standard chlorination vs. chloramation). In epidemiological studies to date, nitrate data from a utility (usually a town) was used to characterize exposure for all residents of the town(s) served by the utility. We use this ecological exposure classification was also used for this analysis.

N-nitroso compounds caused tumors in every animal species tested and at many organ sites. Long-term exposure to low levels of NOC via oral ingestion has been shown to be the most important pattern of exposure for carcinogenesis (Lijinsky and Taylor, 1977). An estimated 20% of ingested nitrate is reduced within the body to nitrite, which reacts readily with amines and amides in the acid environment of the stomach to form NOCs. The nitrosation reaction can also occur in the bladder and the large intestine (Mirvish, 1995). Dietary intakes (primarily from vegetables) and contaminated drinking water are the major sources of nitrate intake for most individuals (Nat Acad Sci, 1981). Nitrosation can be prevented by consumption of vitamin C, E, or other antioxidants within one-two hours of nitrate ingestion (Mirvish et al., 1995). Inhibition of nitrosation by micronutrients found in vegetables provides a plausible explanation for the inverse association sometimes observed between dietary intake and cancer risk (Ward 1996; Mirvish, 1995). In contrast, drinking water nitrate is often consumed without concurrent intake of micronutrients, and is likely to be an important source of nitrate for nitrosation reactions.

The maximum contaminant level for nitrate in drinking water is based on the prevention of methemoglobinemia (blue baby syndrome) in infants and not on cancer risk. Until recently, the studies evaluating nitrate and cancer risk were primarily ecological studies of stomach

cancer and a few other sites, and they lacked comprehensive data on historical exposures (Cantor, 1997). Nitrate exposure from drinking water is an important focus of an ongoing study of non- Hodgkin's lymphoma (NHL) in Iowa, because the risk of NHL was associated with increasing nitrate levels in public water supplies in Nebraska in a population-based case-control study (Ward et al., 1996). Exposure to higher nitrate levels in public drinking water over forty years (average >4mg/l) was associated with a risk of NHL that is twice as great as exposures for the same period of time to concentrations of less than the average. A cohort study of older women in Iowa evaluated historical exposure to drinking water nitrate for ten or more years and risk of NHL, bladder, ovarian, colon, and other gastrointestinal cancers (Weyer, 1998). Average exposures in public water ranged from <1.0 mg/l (lowest quartile) to >6 mg/l nitrate (highest quartile). The nitrate level was not associated with risk of NHL, colon, or other GI tract cancers; however, risks of bladder and ovarian cancer increased with increasing exposure. Thus, recent well-designed studies have shown elevated cancer risk at drinking water nitrate levels below the MCL.

Based on biologic plausibility and/or previous research on exposure to nitrate in drinking water and risk of cancer, cancer sites included in our analysis of ammonia, nitrite and nitrate exposures via drinking water are stomach, bladder, colon, rectum, NHL, and ovary. A brief background is given on known risk factors for each of these cancers (Schottenfeld, 1996) and a discussion of preliminary findings on incidence in the Iowa exposure groups follows.

Stomach: Stomach (gastric) cancer is associated with lower socioeconomic status. While it is believed that occupation does not play a major role in the development of gastric cancer, some studies have found associations with employment in coal mining, asbestos work, chemical, rubber, oil refinery, and metal products industries and other industries involving mineral dust exposures. Known lifestyle risk factors for gastric cancer include cigarette smoking, diets high in starch, low in fat, low in protein, low in intake of fruits and fresh leafy vegetables and high in salt content, and high alcohol intake. Beta carotene may be protective against gastric cancer. Another potential factor in gastric carcinogenesis is *Helicobacter pylori*. Environmental risk factors include nitrate and related compounds, and ionizing radiation.

In our study population, a general decline is seen in stomach cancer incidence across ammonia exposure strata and across decades (Table 6). This decline mirrors the general trend in the U.S. of decreasing stomach cancer incidence since the 1950s. These preliminary data do not indicate a need for further analysis of ammonia levels in water and stomach cancer.

Table 6

Frequency of stomach cancers for ammonia exposure groups by decade of diagnosis (Dx), and % change from previous decade

Exposure group	1969-78 Dx	1979-88 Dx	1989-98 Dx
No exposure	432	422 (-2.4%)	381 (-9.7%)
Low exposure	167	165 (-1.2%)	113 (-31.5%)

High exposure	191	147 (-23%)	130 (-11.6%)

Bladder: Bladder cancer is most common in white males (twice the incidence of black males) and is 3-4 times more prevalent in males than in females. While studies have reported over 40 occupations that may pose increased risks for bladder cancer, many of these associations have not been consistently found. Occupations that may increase the risk for bladder cancer include dyestuffs workers and dye users, aromatic amines manufacturing workers, rubber workers, leather workers, painters, truck drivers, aluminum workers, and machinists. Lifestyle factors that have been associated with bladder cancer include cigarette smoking, coffee drinking, high intake of cholesterol and total fat; vitamin A appears to be protective. Environmental risk factors include ionizing radiation, drinking water disinfection by-products, and use of hair dyes. A familial predisposition to bladder cancer has been reported in clinical studies.

Table 7 shows a considerable increase in bladder cancer incidence across decades for our study population, but this increase is fairly comparable across exposure groups: an approximate 30% increase in bladder cancer incidence between 1969-78 an 1989-98, regardless of whether any ammonia was present in drinking water supplies. The Iowa data mirror the general trend in the U.S. for bladder cancer incidence, which has been increasing since 1973. Exposure to nitrogen compounds in drinking water and development of bladder cancer is an ongoing research interest of CHEEC.

Table 7

Frequency of bladder cancers (invasive + in situ) for ammonia exposure groups by decade of diagnosis (Dx), and % change from previous decade

Exposure group	1969-78 Dx	1979-88 Dx	1989-98 Dx
No exposure	939	1,194 (+27.2%)	1,244 (+4.2%)
Low exposure	297	338 (+13.8%)	387 (+14.5%)
High exposure	326	384 (+17.8%)	434 (+13%)

Colon / Rectum: Colorectal cancer incidence is consistently higher in urban residents compared to rural residents, and is positively correlated with higher socioeconomic status. Colorectal cancer has not been related to many occupational risk factors, although a weak association has been reported for asbestos exposures. Genetic predisposition to colorectal cancer has been found (familial adenomatous polyposis, and Gardner syndrome). Lifestyle factors include high calorie intake and high fat intake, while fiber, fruits and vegetables appear to be protective. Alcohol consumption may increase the risk for rectal cancer, while cigarette smoking increases the risk of developing adenomatous polyps (pre-cancerous lesions) in the colon and rectum. Non-steroidal anti-inflammatory drugs (such as ibuprofen, aspirin, and acetaminophen) may be protective against colorectal cancer.

Table 8 shows a marked increase in colon cancer in all exposure groups during 1979-

88, which may be reflective of better screening techniques and programs. This incline flattens out in 1989-98, with a slight decline seen in the high exposure group. These preliminary data do not indicate a need for further analysis of ammonia levels in water and colon cancer.

Table 8

Frequency of colon cancers for ammonia exposure groups by decade of diagnosis (Dx), and % change from previous decade

Exposure group	1969-78 Dx	1979-88 Dx	1989-98 Dx
No exposure	2,264	2,964 (+30.9%)	3,105 (+4.8%)
Low exposure	700	1,017 (+45.3%)	1,067 (+4.9%)
High exposure	811	1,078 (+32.9%)	1,058 (-1.9%)

Table 9 presents a steady increase in rectal cancer in the high exposure group that is not consistently seen in the low exposure group. Additional analyses on rectal cancer may be warranted.

Table 9

Frequency of rectal cancers for ammonia exposure groups by decade of diagnosis (Dx), and % change from previous decade

Exposure group	1969-78 Dx	1979-88 Dx	1989-98 Dx
No exposure	952	1,079 (+13.3%)	1,076 (-0.3%)
Low exposure	328	348 (+6.1%)	325 (-6.6%)
High exposure	356	373 (+4.8%)	391 (+4.8%)

Non-Hodgkin's Lymphoma: Non-Hodgkin's lymphoma (NHL) may be correlated with higher socioeconomic status, and familial aggregations have been reported in case studies. Persons with congenital immunodeficiency are at higher risk of developing NHL by the time they become adults. Also, persons who have AIDS or who have undergone organ or bone marrow transplants are at higher risk of developing NHL. Some viruses (HTLV-1, EBV) are risk factors for NHL, and persons receiving chemotherapy or blood transfusions appear to be at increased risk. Occupational exposures increasing the risk of NHL include the styrene-butadiene rubber industry, meat workers, woodworkers, and metal workers. Use of hair dyes is a risk factor, and an association has been found between cigarette smoking and NHL. While dietary risk factors have not been confirmed, an association between nitrate in drinking water and NHL has been reported.

Table 10 shows striking increases in NHL incidence across decades for all exposure groups, with the greatest % increase across decades in the high exposure group more than double the incidence in 1969-78. General U.S. trends are similar. As the etiology of NHL is not well understood, particularly with respect to possible environmental factors, further study

of water contaminants as causative agents appears warranted.

Table 10

Frequency of non-Hodgkin's lymphomas for ammonia exposure groups by decade of diagnosis (Dx), and % change by decade

Exposure group	1969-78 Dx	1979-88 Dx	1989-98 Dx
No exposure	590	819 (+38.8%)	1035 (+26.4%)
Low exposure	198	277 (+39.9%)	358 (+29.2%)
High exposure	169	304 (+79.9%)	392 (+28.9%)

Ovary: A familial predisposition exists for ovarian cancer, and a number or reproductive factors appear to be related to an increased risk (early age at menarche, late age at menopause, never having been pregnant, and late age (>35) for first full term pregnancy). Obesity is a risk factor for postmenopausal women, as is a diet with high fat and calorie content. While alcohol consumption has been reported to be associated, the findings are inconsistent. Estrogen replacement therapy is positively correlated with ovarian cancer, and occupational exposure to asbestos appears to increase risk. No other environmental risk factors have been reported.

Table 11 shows a fairly steady increase in ovarian cancer incidence across all exposure groups by decade, but no gradient exists between those groups. To date, no environmental risk factors have been linked to the risk for ovarian cancer. There is no biologic plausibility between exposure to nitrate, nitrite or ammonia and the development of ovarian cancer. Therefore, further gross analyses of water quality data and the incidence of ovarian cancer does not appear to be warranted.

Table 11

Frequency of ovarian cancers for ammonia exposure groups by decade of diagnosis (Dx), and % difference change by decade

Exposure group	*1969-78 Dx*	*1979-88 Dx*	*1989-98 Dx*
No exposure	474	490 (+3.9%)	580 (+18.9%)
Low exposure	142	186 (+31%)	189 (+1.6%)
High exposure	155	177 (+14.2%)	185 (+4.5%)

Nitrite in Drinking Water and Health Outcomes:

Of the 227 municipal wells sampled for nitrogen compounds in the fall of 1999, only two had detectable levels of nitrite. While historical data on nitrite was a criterion for inclusion in the study, many municipal supplies had only one analytical result, and many were from finished water supplies (Table 12). For comparison, Iowa Safe Drinking Water Act data (1993-

1999) have very few detections of nitrite in public water supplies. For towns >400 population, 1,341 finished water samples were analyzed for nitrite; 118 of these had detectable levels (~9%). Sixty-six raw water samples were analyzed for nitrite, only 5 (~8%) had detectable levels). The SDWA regulations require only a one-time analysis of the finished drinking water as it enters the distribution system. However, violations of nitrite standard and values in exceedance of the standard have been observed in this study from samples collected in the distribution system. It appears that the incidence of nitrite present at the taps of consumers may have been underestimated.

Table 12

Iowa municipal water supplies with historical (not SDWA) nitrite data by type of water sample, and % of total sampling frame

Historical nitrite data	Supplies with single samples	Supplies with multiple samples
Raw water	43 (19%)	18 (8%)
Finished water	138 (61%)	81 (36%)

Analyses of cancers and nitrate exposures via drinking water were also presented in that report. Since that time, Weyer et al. (2001) reported an association between elevated nitrate levels in municipal water supplies and the risk for bladder cancer and ovarian cancer in a cohort of elderly Iowa women who had been consuming that water for longer than 10 years. In addition, a study by Ward et al. (2000) reported an increased risk of nasopharyngeal cancers in Taiwanese who consumed a high nitrite diet during childhood; the researchers did not look at nitrate in drinking water.

In the second phase of the study the focus of health outcomes was on ammonia, nitrite and nitrate exposures via drinking water and the risk for adverse reproductive outcomes/developmental defects.

Chronic Health Effects: Adverse Reproductive Outcomes / Birth Defects

Ammonia exposure via drinking water: The human health effects from short-term exposure (< 14 days) to drinking water containing specific levels of ammonia are not known, while long-term exposure (>14 days) is associated with minimal risk based on animal studies (ATSDR, 1990). In a case study in Johnston, New York, a community health concern was expressed over the presence of ammonia detected at about 2200 μg/l in a private drinking water well near a landfill. A public health assessment of the site cited laboratory animal studies reporting liver and kidney damage in animals and humans exposed to much higher levels than those found in the well (New York Dept. Public Health, 1995). Other toxicological data focus on respiratory effects from acute exposures via the inhalation pathway, and do not cite any studies on reproductive effects related to parental ingestion of ammonia in water (EPA, 1991).

Nitrite exposure via drinking water: The primary health concern related to nitrite

exposure is the risk of methemoglobinemia, or "blue-baby syndrome". Blood hemoglobin is oxidized to methemoglobin in the presence of nitrite; methemoglobin has a reduced capacity to transport oxygen. While nitrite is rarely detected in drinking water, nitrate is commonly found, and can be reduced to nitrite in the human body via nitrate-reducing bacteria in the gut. Very young infants (< 6 months old) are susceptible to developing methemoglobinemia due to their high gut content of nitrate-reducing bacteria and their low enzymatic capacity to reduce methemoglobin to hemoglobin (EPA, 1997). With respect to reproductive or developmental effects related to ingestion of nitrite, a number of animal studies have found no conclusive evidence of adverse effects. Hugot et al. (1980) found no reproductive effects in rats administered $NaNO_2$ at doses of 90 or 160 mg nitrite-nitrogen/kg/day. Druckrey et al. (1963) reported no teratogenic or adverse reproductive effects in rats fed water with 100 mg/kg/day $NaNO_2$ for three generations. Til et al. (1988) fed rats drinking water with up 3000 mg/L KNO_2 (equivalent to 50-mg nitrite-nitrogen/kg/day) for 13 weeks, with no adverse health effects.

An additional concern related to nitrite ingestion via food or water relates to the potential reduction of nitrate to nitrite to nitrosamines, which are known carcinogens and teratogens (ATSDR, 1989). A number of nitrosamines have been shown to induce central nervous system defects in animal studies (Koyama et al., 1970; Pfaffenroth et al., 1974; Ivankovic, 1979). Other reported nitrite toxicity in humans involves impacts on blood flow and hormone synthesis, but these are not related to ingestion via the diet or drinking water (Panesar and Chan, 2000; Gladwin et al., 2000). Chronic human health impacts of exposure to nitrite in drinking water have not been reported in the scientific literature.

Of the 227 municipal wells sampled for nitrogen compounds in the fall of 1999, only two had detectable levels of nitrite. While historical data on nitrite was a criterion for inclusion in the study, many municipal supplies had only one analytical result, and many were from finished water supplies. Iowa Safe Drinking Water Act data (1993-1999) have very few detections of nitrite in public water supplies. For towns >400 population, 1,341 finished water samples were analyzed for nitrite; 118 of these had detectable levels (~9%). Sixty-six raw water samples were analyzed for nitrite, only 5 (~8%) had detectable levels). Nitrate detections in the re-sampling group were few (4/30 samples had detections, mean nitrite level = 0.12 mg/L, median nitrite level = 0.0 mg/L).

Nitrate exposure via drinking water: The maximum contaminant level (MCL) of 10 mg/L nitrate in drinking water is based on concerns related to methemoglobinemia in infants. Nitrate toxicity is due mainly to its conversion to nitrite, which oxidizes the Fe (+2) form of iron in hemoglobin to the Fe (+3) form (methemoglobin). Studies of human exposure to nitrate in drinking water and adverse health effects other than methemoglobinemia are few and have mixed findings. Dorsch et al. (1984) reported a statistically significant increase in the risk of birth defects in children of women who consumed well water containing 5-15 mg/L nitrate compared to women consuming well water with <5 mg/L nitrate. Arbuckle et al. (1988) found a slight increase (not significant) in the risk for birth defects in children of women exposed to well water with 26 mg/L nitrate, but noted a decreased risk for exposure to nitrate in spring water (at 17 mg/L) or in public water (at 26 mg/L). Laboratory animal studies are also

inconclusive. Sleight and Attalah (1968) found no reproductive or developmental defects in guinea pigs exposed to drinking water with nitrate-nitrogen doses at 0, 12, 102, 507 or 1130 mg/kg/day.

Knox (1972) first reported an association between nitrate ingestion and neural tube defects in an ecologic study in the United Kingdom. Croen et al. (2001) found an increased risk for anencephaly in offspring of women consuming groundwater with elevated nitrate levels, but this finding was not apparent in offspring of women drinking water from mixed sources. About 20 cases of anencephaly are reported in Iowa annually.

Incidence data from the Iowa Birth Defects Registry combined by community grouping (ammonia exposure category) are presented in Table 13. A number of large communities (>10,000 pop.) are included in the "no exposure" group, while the vast majority of communities in the low and high exposure groups were small communities (<10,000 pop.) Population figures were derived from the 2000 Census. While all birth defect categories are included in the 1983-90 diagnosis period, only "major" defect categories are included in the 1991-99 diagnosis period. In addition, reporting between 1983-85 is incomplete.

Table 13

Frequency of birth defects by community grouping (ammonia exposure category) and date of diagnosis (Dx), 1983-1999

Community group	***1983-1990 Dx***	***1991-1999 Dx***
No exposure	2809	2053
Low exposure (≤ 1 mg/L)	800	484
High exposure (> 1 mg/L)	872	525

No exposure towns (n = 107; total population = 476,338)
Low exposure towns (n = 57; total population = 94,308)
High exposure towns (n = 63; total population = 95,787)

This frequency distribution shows there is a small difference in the raw number of cases diagnosed in communities with varying levels of ammonia in their drinking water. Looking at the average annual number of cases per 100,000 population in each exposure category shows no trend across categories (Table 14).

Table 14

Average annual number of cases of birth defects per 100,000 population, by community grouping (ammonia exposure category) and date of diagnosis (DX), 1983-99

Community group	**1983-90 Dx**	**1991-99 Dx**
No exposure	74	48

Low exposure	106	57
High exposure	101	61

Due to the lack of information on known risk factors for various birth defects in this population, plus the fact that any individual category of defect would have very small numbers, we did not pursue a detailed analysis of birth defects incidence in this population. Lack of any scientific evidence on low level exposure to ammonia via drinking water and the risk for birth defects supports this decision. However, the Iowa Birth Defects Registry will continue to track incidence of various birth defects in these communities, and will conduct additional statistical studies if deemed appropriate.

Conclusions and Recommendations

Monitoring for nitrite at the point of entry to the distribution system may underestimate both the presence and exposure to nitrite in public water supply systems in Iowa. The data suggest that as long as there is ammonia in the raw water there is a high probability that it will be converted to nitrite and nitrate before it reaches the consumer. Although ammonia can be converted to nitrite and nitrate without the presence of bacteria, these ideal conditions are not believed to be common in most public water supplies. It is the presence of bacteria that is generally responsible for the conversion. This study suggests that the conditions are often favorable for the necessary bacterial growth to occur. It is evident that bacterial colonies survive routine chlorination even in well-run public water supplies. The presence of scale and biofilms within the distribution systems provide the necessary protection these organisms need for survival. With the presence of a viable bacterial colony the addition of ammonia allows for increases in nitrite and nitrate concentrations in the water served to consumers.

Although all of the supplies selected for inclusion in phase two of the study had detectable concentrations of ammonia in their water in phase one of the study, ammonia was only detected in 26 of the 30 water supplies (87 %) in phase two of the study. The data suggest that there may be some degree of variability in the occurrence and concentration of ammonia in ground water. Thus infrequent monitoring of the source water may lead to artificially low estimates of the occurrence of ammonia in ground water systems.

There is no evidence that measurable adverse health effects are occurring as a result of the increased exposure to elevated ammonia, nitrite and nitrate values in the drinking water of the supplies involved in this study. However, it should be noted that the collective population served by these supplies is too small for a valid epidemiological study. There is sufficient scientific evidence in the literature to suggest that the contaminants of concern in this study have the potential to pose an adverse risk to human health.

The State of Iowa should seek a better understanding of the occurrence of ammonia in ground water, as well as the impact ammonia may be having on the quality of the drinking water delivered to the public. A better understanding of the relationship between ammonia and water supply operations would benefit both the State and public water supply systems.

Public officials should reconsider the appropriateness of current monitoring

requirements for ammonia and nitrite in public drinking water supplies. Since ammonia and nitrite both, contribute to nitrate concentrations and pose a potential for adverse health impacts, increased monitoring may be appropriate.

Controlling bacterial growth in public water supplies is both difficult and expensive. Controlling bacterial growth involves changes in water chemistry, disinfecting chemical feeds, distribution system repair and maintenance programs. Increased attention and support should be given to improving our knowledge on how to control and eliminating scale and biofilms in the distribution systems of the state's public water supply systems.

References

Arbuckle TE, Sherman GJ, Corey PN, Walters D, Lo B. 1988. Water nitrates and CNS birth defects: A population-based case-control study. *Arch Environ Health*. 43(2):162-167.

Agency for Toxic Substances and Disease Registry (ATSDR). 1989. Public Health Statement: N-nitrosodi-n-propylamume. U.S. Centers for Disease Control & Prevention.

Agency for Toxic Substances and Disease Registry (ATSDR) 1990. Public Health Statement: Ammonia. U.S. Centers for Disease Control & Prevention.

Buckmiller, Robert. USGS. *Personal correspondance, April, 1999*.

Cantor KP. Drinking water and cancer. *Cancer Causes Control* 8:292-308;1997.

Croen LA, Todoroff K, Shaw GM. 2001. Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. *Am J Epidemiol*. 153:325-331.

Dorsch MM, Scragg RKR, McMichael AJ, Baghurst PA, Dyer KF. 1984. Congenital malformations and maternal drinking water in rural South Australia: A case-control study. *J Epidemiol*. 119(4):473-486.

Druckrey H, Steinhoff D, Beuthner H, Schneider H, Klarner P. 1963. Screening of nitrite for chronic toxicity I rats. *Arzneim Forsch*. 13:320-323.

EPA. Integrated Risk Information System.1991. Nitrate: CASRN 14797-55-8.

EPA. Integrated Risk Information System.1997. Nitrite: CASRN 14797-65-0.

Gladwin MT, Shelhamer JH, Schechter AN, Pease-Fye ME, Waclawiw MA, Panza JA, Ognibene FP, Cannon RO. 2000. *Proceedings of the National Academy of Sciences of the United States of America*. 97(21):11482-11487.

Hugot D, Causeret J, Richir C. 1980. The influence of large amounts of sodium nitrite on the reproductive performance in female rats. *Ann Nutr Alim*. 34:1115-1124.

Ivankovic S. 1979. Teratogenic and carcinogenic effects of some chemicals during perinatal life in rats, Syrian golden hamsters, and Minipigs. *Natl Cancer Inst Monogr*. 51:103-115.

Knox EG. 1972. Anencephalus and dietary intake. *Br J Prev Soc Med*. 26:219-223.

Koyama T, Handa J, Handa H, et al. 1970. Methylnitrosourea-induced malformations of brain in SD-JCL rat. *Arch Neurol*. 22:342-347.

Lijinsky W, Taylor HW. Nitrosamines and their precursors in food. In: HH Hiatt, JD Watson , JA Winsten (Eds), *Origins of Human Cancer* , pp. 1579-1590, New York:Cold Spring Harbor, 1977.

Mirvish SS, Grandjean AC, Reimers KJ, Connelly BJ, Chen S-C, Gallagher J, Rosinsky S, Nie G, Tuatoo H, Payne S, Hinman C, Ruby EI. Dosing time with ascorbic acid and nitrate, gum and tobacco chewing, fasting, and other factors affecting *N*-Nitrosoproline formation in healthy subjects taking proline with a standard meal. *Cancer Epidemiol Biomarker Prev* 4:775-82; 1995.

Mirvish SS. Role of *N*-Nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 93:17-48;1995.

National Academy of Sciences. *The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds*. Washington DC:National Academy Press, 1981.

New York State Department of Health. 1995. Public Health Assessment: Johnston City Landfill, Johnston, Fulton County, New York. CERCLIS No. NYD980506927. Under cooperative agreement with the Agency for Toxic Substances and Disease Registry.

Panesar NS, Chan KW. 2000. Decreased steroid hormone synthesis from inorganic nitrite and nitrate: studies in vitro and in vivo. *Toxicology & Applied Pharmacology*. 169(3):222-230.

Pfaffenroth MJ, Das GD, McAllister JP. 1974. Teratologic effects of ethylnitrosourea on brain development in rats. *Teratology*. 9:305-315.

Schilling, K., Geological Survey Bureau, Iowa Iowa Department of Natural Resources. *Personal correspondance, May, 2000*.

Schottenfeld D, Fraumeni, Jr. J. *Cancer Epidemiology and Prevention (2nd Edition)*, New York, Oxford University Press, 1996.

Simpkin, William, Associate Professor. Geological Sciences Department, Iowa State University. *Personal correspondance, February, 1999*.

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

Til HP, Falke HE, Kuper CF, Willems MI. 1988. Evaluation of the oral toxicity of potassium nitrite in a 13-week drinking-water study in rats. *Food Chem Toxicol*. 26(10):851-859.

Ward MH, Mark SD, Cantor KP, Correa A, Weisenburger DD, Zahm SH. Drinking water nitrate and the risk of non-Hodgkin's lymphoma. *Epidemiology* 7:465-471;1996).

Ward MH, Pan WH, Cheng YJ, Li FH, Brinton LA, Chen CJ, Hsu MM, Chen IH, Levine PH, Yang CS, Hildesheim A. 2000. Dietary exposure to nitrite and nitrosamines and risk for nasopharyngeal carcinoma in Taiwan. *Intl J Cancer*. 86(5):603-609.

Weyer PJ. Municipal drinking water nitrate level and risk of non-Hodgkin's lymphoma, colon cancer, and GI tract cancers: The Iowa Women's Health Study. Ph.D. dissertation, University of Iowa, Department of Preventive Medicine and Environmental Health; 1998.

Weyer PJ, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, Jones MP, Zheng W, Lynch CF. 2001. Municipal drinking water nitrate level and cancer risk in older women: The Iowa Women's Health Study. *Epidemiology*. 11(3): 327-338.

Appendix 2. 1999 Data by Aquifer

	AQUIFER	SAMPLES	N DETECTS	%	MEAN	MINIMUM	25%	MEDIAN	75%	MAX
Ammonia	ALLUVIUM	74	22	30%	0.22	0	0	0	0.2	2.8
	CAMBRIAN	4	2	50%	0.1	0	0	0.05	0.1	0.3
	CAMBRIAN / ORDOVICIAN	14	9	64%	0.66	0	0	0.75	1.2	1.5
	DAKOTA	23	14	61%	0.9	0	0	0.3	1.25	3.6
	DRIFT	31	23	74%	1.73	0	0	1	2.5	7.4
	MISSISSIPPIAN	17	10	59%	0.54	0	0	0.3	0.7	2.2
	ORDOVICIAN	10	9	90%	1.09	0	0.3	0.75	0.97	5.1
	PENNSYLVANIAN	2	2	100%	2.3	1.5	1.5	1.5	3.1	3.1
	SILURIAN / DEVONIAN	58	29	50%	0.56	0	0	0.05	0.5	4.7
	Total all data	**233**	**120**	**52%**	**0.68**	**0**	**0**	**0.1**	**0.8**	**7.4**
Nitrite (NO2)	ALLUVIUM	74	2	3%	0.02	0	0	0	0	1.3
	CAMBRIAN	4	0	0%	0	0	0	0	0	0
	CAMBRIAN / ORDOVICIAN	14	0	0%	0	0	0	0	0	0
	DAKOTA	23	0	0%	0	0	0	0	0	0
	DRIFT	32	0	0%	0	0	0	0	0	0
	MISSISSIPPIAN	17	0	0%	0	0	0	0	0	0
	ORDOVICIAN	10	0	0%	0	0	0	0	0	0
	PENNSYLVANIAN	2	0	0%	0	0	0	0	0	0
	SILURIAN / DEVONIAN	58	0	0%	0	0	0	0	0	0
	Total all data	**234**	**2**	**1%**	**0.006**	**0**	**0**	**0**	**0**	**1.3**
Nitrite-Field	ALLUVIUM	74	2	3%	0.02	0	0	0	0	1
	CAMBRIAN	4	0	0%	0	0	0	0	0	0
	CAMBRIAN / ORDOVICIAN	14	0	0%	0	0	0	0	0	0
	DAKOTA	22	1	5%	0.01	0	0	0	0	0.2
	DRIFT	29	0	0%	0	0	0	0	0	0
	MISSISSIPPIAN	17	0	0%	0	0	0	0	0	0
	ORDOVICIAN	10	0	0%	0	0	0	0	0	0
	PENNSYLVANIAN	2	0	0%	0	0	0	0	0	0
	SILURIAN / DEVONIAN	58	0	0%	0	0	0	0	0	0
	Total all data	**230**	**3**	**1%**	**0.006**	**0**	**0**	**0**	**0**	**1**
Nitrate NO3N	ALLUVIUM	74	53	72%	4.09	0	0	2.2	7.4	14
	CAMBRIAN	4	0	0%	0	0	0	0	0	0
	CAMBRIAN / ORDOVICIAN	14	3	21%	0.22	0	0	0	0	2
	DAKOTA	23	9	39%	1.84	0	0	0	1.05	16
	DRIFT	31	4	13%	0.35	0	0	0	0	4.4
	MISSISSIPPIAN	17	7	41%	2.4	0	0	0	4.7	11
	ORDOVICIAN	10	1	10%	0.48	0	0	0	0	4.8
	PENNSYLVANIAN	2	0	0%	0	0	0	0	0	0
	SILURIAN / DEVONIAN	58	22	38%	2.14	0	0	0	3.4	12
	Total all data	**233**	**99**	**42%**	**2.26**	**0**	**0**	**0**	**3.4**	**16**
Nitrate-Field	ALLUVIUM	74	52	70%	3.55	0	0	2	5	15

(NO3N + NO2)	CAMBRIAN	4	1	25%	0.13	0	0	0	0	0.5
	CAMBRIAN / ORDOVICIAN	14	2	14%	0.18	0	0	0	0	1.5
	DAKOTA	22	8	36%	1.27	0	0	0	2	10
	DRIFT	29	8	28%	0.57	0	0	0	1	4
	MISSISSIPPIAN	17	8	47%	2.24	0	0	0	2	10
	ORDOVICIAN	10	1	10%	0.35	0	0	0	0	3.5
	PENNSYLVANIAN	2	1	50%	2.5	0	0	0	5	5
	SILURIAN / DEVONIAN	58	20	34%	1.51	0	0	0	3	10
	Total all data	**230**	**101**	**44%**	**1.93**	**0**	**0**	**0**	**3**	**15**
pH	ALLUVIUM	74	74	100%	7.28	6.3	7.1	7.2	7.4	11.4
	CAMBRIAN	4	4	100%	7.7	7.4	7.4	7.5	7.6	8.3
	CAMBRIAN / ORDOVICIAN	14	14	100%	7.57	7.1	7.4	7.5	7.8	8
	DAKOTA	22	22	100%	7.18	6.7	7.1	7.2	7.3	7.5
	DRIFT	29	29	100%	7.43	7	7.2	7.4	7.6	8
	MISSISSIPPIAN	17	17	100%	7.38	7	7.2	7.4	7.5	7.8
	ORDOVICIAN	10	10	100%	7.41	7.2	7.3	7.4	7.5	7.6
	PENNSYLVANIAN	2	2	100%	7.25	7.2	7.2	7.2	7.3	7.3
	SILURIAN / DEVONIAN	58	58	100%	7.54	7.1	7.4	7.5	7.7	8
	Total all data	**230**			**7.395**	**6.3**	**7.2**	**7.4**	**7.5**	**11.4**
Temperature	ALLUVIUM	74	74	100%	12.26	10.1	11.1	12	12.8	17
	CAMBRIAN	4	4	100%	13.35	10	10	10.5	16.4	16.5
	CAMBRIAN / ORDOVICIAN	14	14	100%	13.64	9	10.5	12.5	14.5	22
	DAKOTA	22	22	100%	12.58	9.3	10.8	11.5	12	26.8
	DRIFT	29	29	100%	11.48	7.8	10	11.7	12.5	18
	MISSISSIPPIAN	17	17	100%	11.79	10	10.8	11.1	11.5	23
	ORDOVICIAN	10	10	100%	9.67	8.5	9	9.5	10	13
	PENNSYLVANIAN	2	2	100%	11.85	11.7	11.7	11.7	12	12
	SILURIAN / DEVONIAN	58	58	100%	11.07	8.5	10	11	12	18
	Total all data	**230**			**11.84**	**7.8**	**10.5**	**11.5**	**12.5**	**26.8**