

Division of Drinking and Ground Waters

**Report of Findings
Unsafe Water Supply Investigation
Wooster Township, Wayne County**



December 2006

*Bob Taft, Governor
Joseph P. Koncelik, Director*

Acknowledgments

The Ohio EPA expresses its thanks to all of the individuals who participated or supported this investigation. Special thanks must be given to the home owners who participated in the investigation, which would not have been possible without their assistance. While the Agency led the investigation, it could not have been completed without the assistance of the following organizations and individuals:

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EXECUTIVE SUMMARY

The results of Ohio Department of Health sampling in 2004 raised questions about the safety of the water supply for individual private wells in part of Wooster Township in Wayne County. Concerns related specifically to the source of elevated nitrate levels and pathogens in the local water supply. At the Wayne County Combined General Health District Board of Health's request, an investigation into unsanitary conditions was performed by Ohio EPA's Division of Surface Water of a common collector tile discharging effluent from several household sewage treatment systems. The results did not indicate a public health nuisance based on water quality criteria established for surface waters in the Ohio Administrative Code 3745-1-04. Subsequently the Board of Health renewed the public health nuisance declaration for the area and in January 2006 requested that Ohio EPA conduct an Unsafe Water Supply Investigation of the area per Ohio Revised Code (ORC) 6103.17.

After reviewing the available information and establishing the goals of the investigation, Ohio EPA's Division of Drinking and Ground Waters conducted a two phase investigation in the study area as detailed in *Unsafe Water Supply Investigation (ORC 6103.17) Sampling Plan for Scenic Heights/Batdorf Road Area, Wooster Township, Wayne County*.

The goal of the investigation conducted between April and June 2006 was to determine whether local ground water supplying private water systems has been impacted by the effluent from individual household sewage treatment systems within the subdivision or from other potential contaminant sources. Specifically, the investigation was designed to identify the probable source(s) of *E. coli* and nitrate contamination in the water well samples collected previously by the Wayne County Health Department and the Ohio Department of Health.

The investigation results indicate that the geologic setting within the study area is sensitive to ground water contamination from household sewage treatment systems. Due to the complex nature of fractured bedrock aquifers and multiple local sources of effluent from household sewage treatment systems (leach fields) in the study area, this study does not identify individual household sewage treatment systems as the cause for the unsafe water supply conditions. However, the results from the sampling and understanding of the hydrogeologic conditions in the study area suggest the household wastewater treatment systems installed into or just above bedrock are likely to have a greater impact on the water quality of the aquifer than those systems with more soil material to treat the effluent.

The geochemical and isotopic data give significant support to the conceptual model of a local ground water mixing, in varying proportions, with diluted effluent from household sewage treatment systems. The interpretation of the analytical results provide several lines of evidence which indicate that sewage effluent is a major source of nitrate and pathogen contamination of the local ground water. Historic nitrate and microbiological data indicate continuing input from a source related to human activities. Nitrate concentrations in excess of 10 mg/L indicate the source of the contaminants are from

sewage effluent. This conclusion is supported by analysis of the bromide and chloride data and the oxygen and nitrogen isotope data, both of which indicate a sewage-related source for the contaminants. Significant findings that support these conclusions are summarized as follows:

- The variability and persistence of microbiological indicators and nitrate contamination is documented by the previous sampling results and Phase 1 and 2 sampling for this study. Mapping the locations of these results demonstrates elevated nitrate concentrations and pathogen indicators are concentrated in the northern half of the study area, where glacial tills are thin to absent over a considerable area.
- Ground water sampling results for Phase 2 include unusually high dissolved oxygen concentrations, the presence of fecal *Bacteroidales* markers, and detections of *E. coli*, all of which provide strong evidence for rapid recharge. In addition, the fecal *Bacteroidales* markers and detections of *E. coli* support the presence of fecal-derived contamination.
- Chloride/bromide ratios document that sewage-type waste is present in the ground water. The direct correlation of nitrate concentration to dissolved oxygen and the association of elevated bromide with elevated nitrate suggest that a single process is the source of these indicators – most likely rapid recharge of sewage effluent to the ground water which is then captured by local wells.
- The nitrogen and oxygen isotope data provide strong evidence for the inclusion of sewage or manure derived waste in the ground water with a strong correlation to the household sewage treatment system composite sample.
- The observed nitrate isotope compositions, in addition to bromide, nitrate and chloride data, indicate that the water wells within the study area are drawing water which is composed, to varying degrees, of a mixture of local ground water and diluted sewage effluent. This mixture appears, in varying proportions, across the study area.
- The erratic presence of bacteria, nitrate, and other effluent indicators is to be expected, given the unpredictable nature of flow volume and direction in fractured bedrock, the differences in depth to the top of the bedrock surface across the area, the variable thickness of the soils and glacial tills (the barriers to contaminant transport) beneath leach fields, and the varied spatial relationships between each household's leach field and water well to those of its neighbors.

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INTRODUCTION

Beginning around the year 2000, residents in subdivision near Batdorf and Scenic Heights Roads, Wooster Township, Wayne County (Figure 1) expressed concerns about the safety of their water supply, septic odors and other nuisances. Several attempts were made to isolate and mitigate the source of these concerns, but none was completely successful. The results of Ohio Department of Health sampling in 2004 raised additional questions about the safety of the private water supplies supplied by individual wells; specifically nitrate and pathogens. A 2004 “unsanitary conditions” investigation of a common collector tile discharging from several household sewage treatment systems, performed by Ohio EPA's Division of Surface Water (DSW), did not indicate a public health nuisance based on water quality criteria established for surface waters in the Ohio Administrative Code 3745-1-04. Subsequently the Wayne County Combined General Health District's Board of Health renewed the public health nuisance declaration for the area; and in January 2006, requested Ohio EPA conduct an Unsafe Water Supply Investigation of the area per Ohio Revised Code 6103.17.

After reviewing the available information and establishing the goals of the investigation, Ohio EPA's Division of Drinking and Ground Waters (DDAGW) conducted a two phase investigation in the study area as detailed in *Unsafe Water Supply Investigation (ORC 6103.17) Sampling Plan for Scenic Heights/Batdorf Road Area, Wooster Township, Wayne County*. The goal of the investigation was to determine whether ground water resources used locally as the source for private water systems had been impacted by household sewage treatment systems within the subdivision or from other potential contaminant sources. Specifically, the investigation was to identify the probable source(s) of *Escherichia coli* (*E. coli*) and nitrate contamination in the water well samples collected previously by Wayne County Health Department and Ohio Department of Health.

The first phase of the investigation collected data on local ground water quality, identified potential sources of microbial and nitrate contamination, determined local ground water elevations and mapped ground water flow directions. The second phase focused on the collection and analysis of additional water quality indicators that can substantiate contamination from sewage or other sources.

Water quality sampling during phase one was a collaborative effort among Ohio EPA, ODH and Wayne County Health Department. Ohio EPA contracted with the United States Geologic Survey (USGS) Ohio Water Science Center to collect and analyze water quality samples for Phase 2 of the investigation.

This report documents the results and findings of the Unsafe Water Supply Investigation. Discussion includes an interpretation of the data and identification of the probable source(s) of the contaminants.

Physical Setting

The study area is located in Wooster Township, Wayne County, southeast of Wooster (Figure 1). The area is roughly bounded by Tolbert Road on the south, Batdorf Road on the east, Liahona Drive on the north and Mallard Way and Pheasant Run Drive on the west and covers approximately 140 acres (Figure 2). It occupies the southern end of a north-south trending ridge bounded on the east and south by Killbuck Creek and on the west by an unnamed tributary to Killbuck Creek. Ground surface elevations in the area range from 850 feet above mean sea level along Killbuck Creek to 1050 feet above mean sea level just north of Liahona Drive. The crest of the ridge falls to the south-southeast across the study area. Slopes are moderate to steep (6% or greater) throughout most of the study area, steepening east of Batdorf Road with more gentle

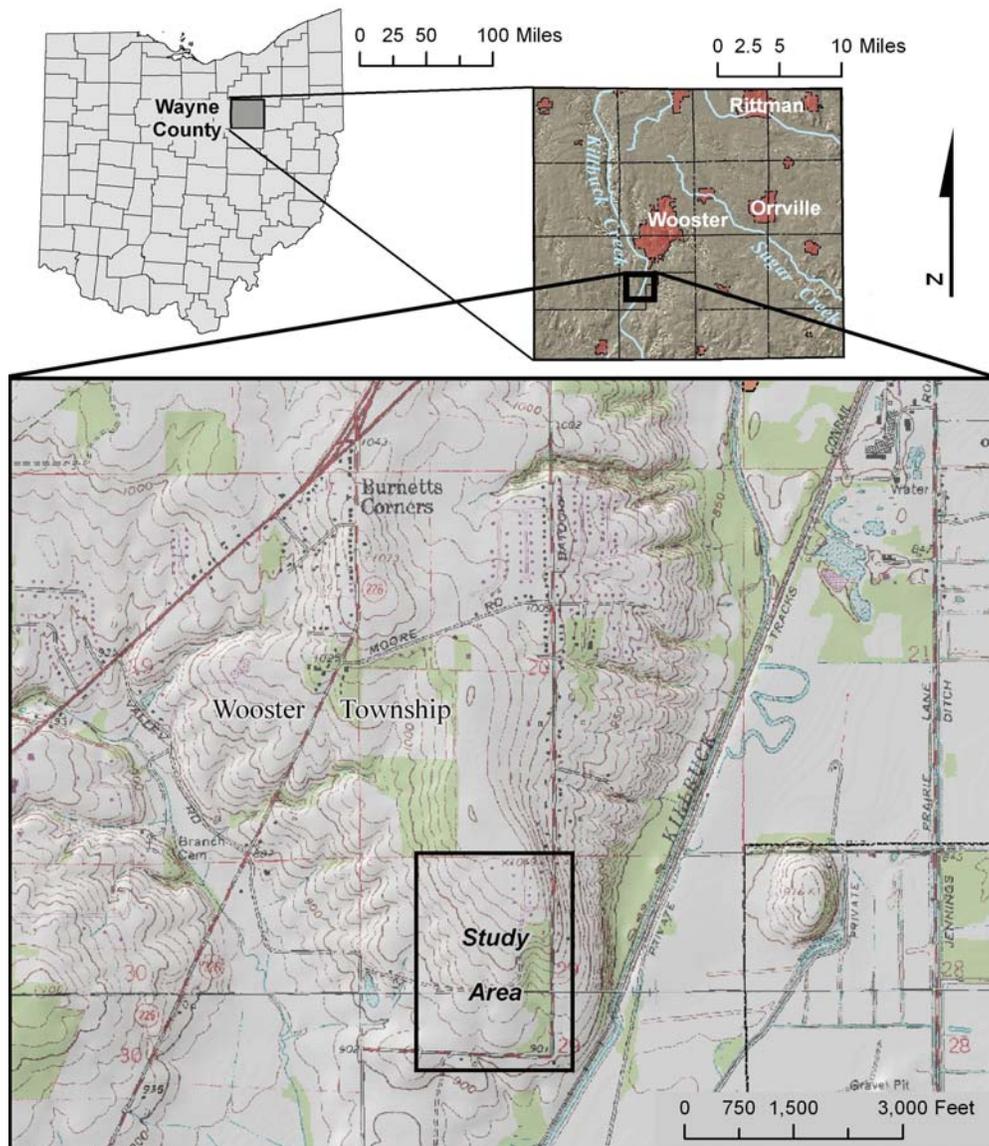


Figure 1. The location of the study area in southern Wooster Township, Wayne County, Ohio.

slopes on the southwest flank. North of the study area the ridge slopes more gently to the east. There is a pronounced north-south trending swale and scarp west of and parallel to Batdorf Road south of Scenic Heights Drive.

Land Use and Development History

Land use (Figure 3) within the study area is residential with some undeveloped lots. The lots along Batdorf Road and the small area south of Scenic Heights Drive are heavily wooded, the remainder are more open grassed lawns with mature ornamentals and trees at the older homes. The study area is surrounded by fields used for pasture and row crops. A small wooded area extends north of the properties at the upper end of Pheasant Run. Farmland is present north of development with crops such as strawberries and sweet corn for a farm market located on Batdorf Road. Railroad tracks and an aggregate mine are present east of the study area near Killbuck Creek.

Prior to the mid-1970s the study area was primarily farmland used for pasture and row crops broken by scattered wooded/forested areas. Several single family dwellings and farms existed along Batdorf Road. More intense development began along and near Lauraland Avenue in the late-1970s and continued into the 1980's. Beginning in the mid-1990s development began on the View at Hunter's Crossing. This development



Figure 2. Aerial photograph of the study area.

includes the westward extension of Scenic Heights Drive and the addition of Pheasant Run Drive and Mallard Way. Additional homes have also been built along Batdorf and Tolbert Roads and the easternmost portion of Scenic Heights. There are approximately 80 residences in the study area.

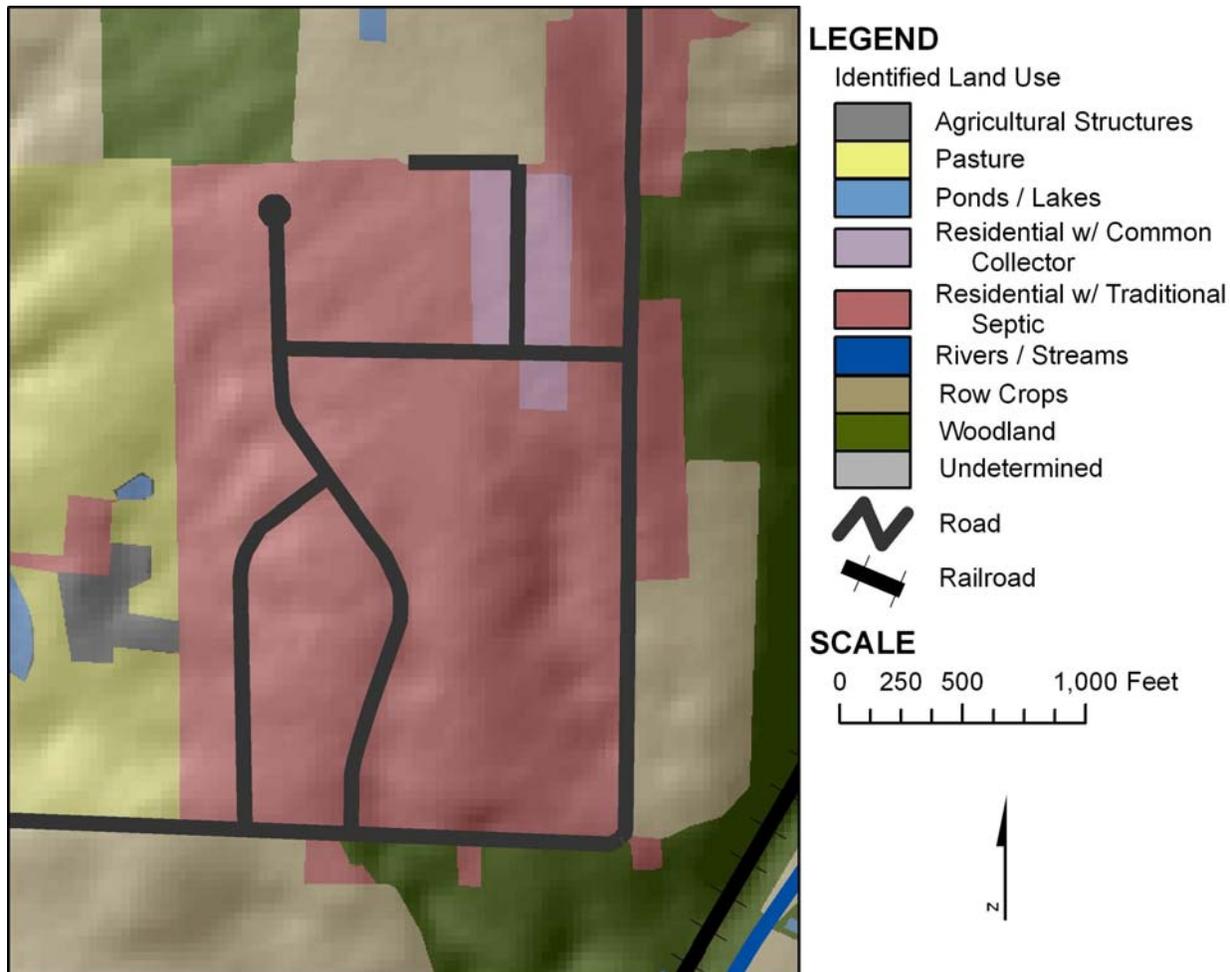


Figure 3. Land use in and around the study area. Adapted from 2004 aerial photographs.

Private and Public Water Systems

Each of the individual homes in the study area is served by its own water supply well. The use of each well as a potable water supply was approved by the Wayne County Health Department. The majority of the water supply wells within the study area were installed and approved for use since the early 1990s. A discussion of the private water well water quality sampling is presented in the Previous Water Quality Investigations section of this report.

In addition to the homes in the study area, the Church of Jesus Christ of Latter Day Saints (LDS Church) occupies most of the south side of Liahona Drive. The LDS Church was first recognized as a public water system (PWSID # 8559812) in 1999 and was classified as a transient non-community system. Drinking water was supplied from an on-site well. Due to a history of unsafe bacteriological sample results, the LDS Church well was sealed and now hauls in its water from another public water system (DDAGW, 2006a).

North of the study area is Maurer Farms, a farm market on Batdorf Road. Maurer Farms was first recognized as a public water system (PWSID # 8560412) in 2001 and is classified as a transient non-community system. Maurer Farms is required to perform routine bacterial and nitrate monitoring of its well. These compliance samples meet water quality standards for nitrate and bacteria.

Potential Contaminant Sources

Potential contaminant sources within and surrounding the study area include residential and institutional sanitary waste, animal wastes, lawn and home care products, agricultural fertilizer and pest control products and application of road salt.

The majority of the homes in the area treat household waste through traditional household sewage treatment systems (septic tanks and leach fields). A common configuration for the leach field consists of 3 lines approximately 75 feet long with 8 feet of separation between the lines. In the northern portion of the study area, where depth to rock is very shallow, some septic tanks and their leach lines were installed into or just above the bedrock. Several older homes constructed prior to 1970 use cesspools (leaching pits) and at least one home uses "leach boxes" to disperse effluent. The homes on Lauraland plus one home on Scenic Heights Drive remove sewage effluent through a common collector system. Effluent from each household is treated by an aerator and chlorinator prior to discharge through a common outlet into the swale along Batdorf Road. The property receiving the discharge is undeveloped and the surrounding properties had not been developed at the time the system was installed. This discharge was, at the time, considered a temporary solution with the expectation that the local sanitary sewer system, which currently ends near 3000 Batdorf Road, would be extended into the study area. The newer homes' household sewage treatment system design consists of a septic tank, distribution box and leach lines.

Effluent from sanitary waste management systems may contain ammonia, nitrate, pathogens, sodium, chloride and personal and home care products. Water softeners discharging through these systems may introduce additional sodium and chloride into the environment.

A typical household sewage treatment system discharges approximately 40 pounds of nitrogen per year into the subsurface over an area of approximately 2,000 square feet (three 75 foot lines spaced 8 feet apart). Normally, effluent from a household sewage

treatment system discharges into the leach field and infiltrates slowly into the subsurface. During the infiltration, the primary source of nitrogen in sewage, ammonia, is converted to various forms of nitrogen including nitrate which is consumed by bacteria. However, if effluent infiltration is too rapid, not all of the nitrogen is consumed and if sufficient oxygen is present, nitrate concentrations can increase and be persistent in ground water. For the household sewage treatment systems with leach lines installed into or just above areas of shallow fractured bedrock, nitrogen loading rates can be excessive and result in localized ground water contamination.

In contrast, inorganic nitrogen fertilizer application rate for row crops can be as high as 120 to 150 pounds per acre. This rate would equal an upper limit of 7 pounds per 2000 square feet (the size of the typical leach field). The application rate is dependent on the type of crop, soil properties and the nutrient levels present in the soil. Fertilizer is often applied on or just below the ground surface several times during the growing season at fraction of the overall yearly application rate. The nitrogen in fertilizer also converts to nitrate during its infiltration with water recharging local ground water. Because fertilizer is applied at or very near the ground surface there is additional residence time in the soil. This makes the nutrients available for consumption by plants creating a longer infiltration pathway for nitrogen compounds prior to reaching the underlying aquifer when compared to the pathway for household sewage treatment system effluent.

Agriculture in the immediate vicinity of the study area is primarily row crops. During site visits in 2006 corn was observed east of Batdorf Road and south of Tolbert Road and crops for the Maurer Farm Market are grown in the fields north of Liahona. A small horse farm is located on the property immediately west of the study area. Potential contaminant sources associated with agriculture include storage and land application of manure and application of chemical fertilizers and pesticides used in farming practices. Runoff and infiltration of water from agricultural areas may introduce nitrates, pesticides and pathogens into the ground water.

Other sources of nitrate and pathogens include animal wastes. Residents in the study area keep pets; many of the homes have dogs. Deer and other wildlife frequent the wooded areas. Other potential sources of contaminants include brine injection, road salt and lawn care products. A brine injection well is located southeast and hydraulically down-gradient of the of study area; brine is injected into a confined formation 2,500 feet below ground surface. Commercial lawn care application was observed during visits to the study area. Road salt is applied during the winter.

Local Geology and Hydrogeology

The study area is characterized by rolling hills formed on bedrock covered with glacial till deposits. The underlying bedrock in the study area consists of siltstones, sandstones and shales belonging to the lower Logan and upper Cuyahoga Formations (Multer, 1967). Figure 4 is a geologic cross section, based on publicly available water well logs, running from the northwest to the southeast across the study area. The location and

depth of the wells used to construct the cross section are shown as heavy black (cased interval) and white (open borehole) lines. The water table is indicated by the blue line and triangles.

As seen in Figure 4, bedrock is covered by thin to moderately thick glacial till. In some areas along the crest of the ridge and on steep slopes the till is thin or absent (Bureau, Graham and Scherzinger, 1984). Consequently, bedrock was very near or exposed at the ground surface when some of the properties were developed. This is supported by reports from homeowners that installation of the basement for some of the homes in the study area required excavation of soft, rippable bedrock. Glacial tills are thickest on the southern and southwestern flank of the ridge. The till is primarily a silty clay till with few pebbles or boulders; thicker deposits containing gravel are present in portions of the study area. Some sandy pebbly till with cobbles and boulders is also present (White, 1967). Soils are predominantly silt loams, with small areas of loam and gravelly loam soil present in the southern edge of the study area. These soils are moderately well suited for crops and pasture and moderately well suited for leach fields, however fractures and macropores in thin unsaturated tills can rapidly transport water to the underlying bedrock, limiting the effectiveness of standard household sewage treatment systems (Bureau, Graham and Scherzinger, 1984).

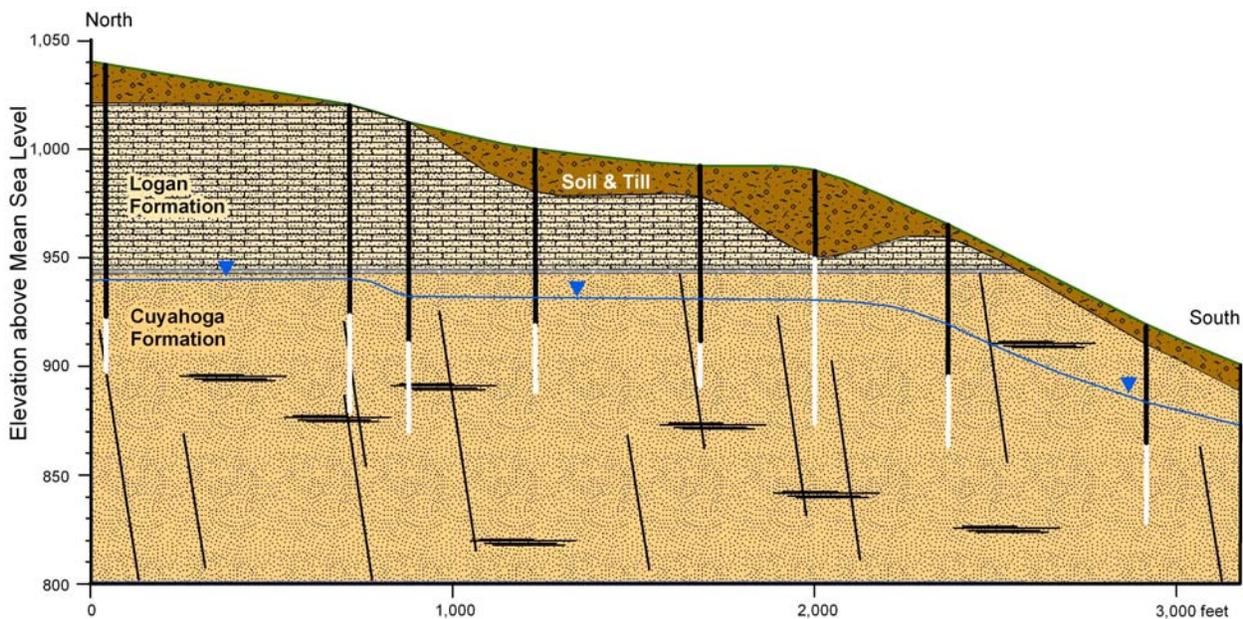


Figure 4. Cross section.

Static water levels for wells in the study area were obtained on May, 30, 2006; well casing elevations were surveyed on August 30, 2006. As illustrated in Figure 4 the depth of the water table ranges from less than 50 feet to over 100 feet. Static water levels were contoured to identify the water table as illustrated in Figure 5. Ground water flow is radial from the crest of the ridge perpendicular to the contours. All of the well logs

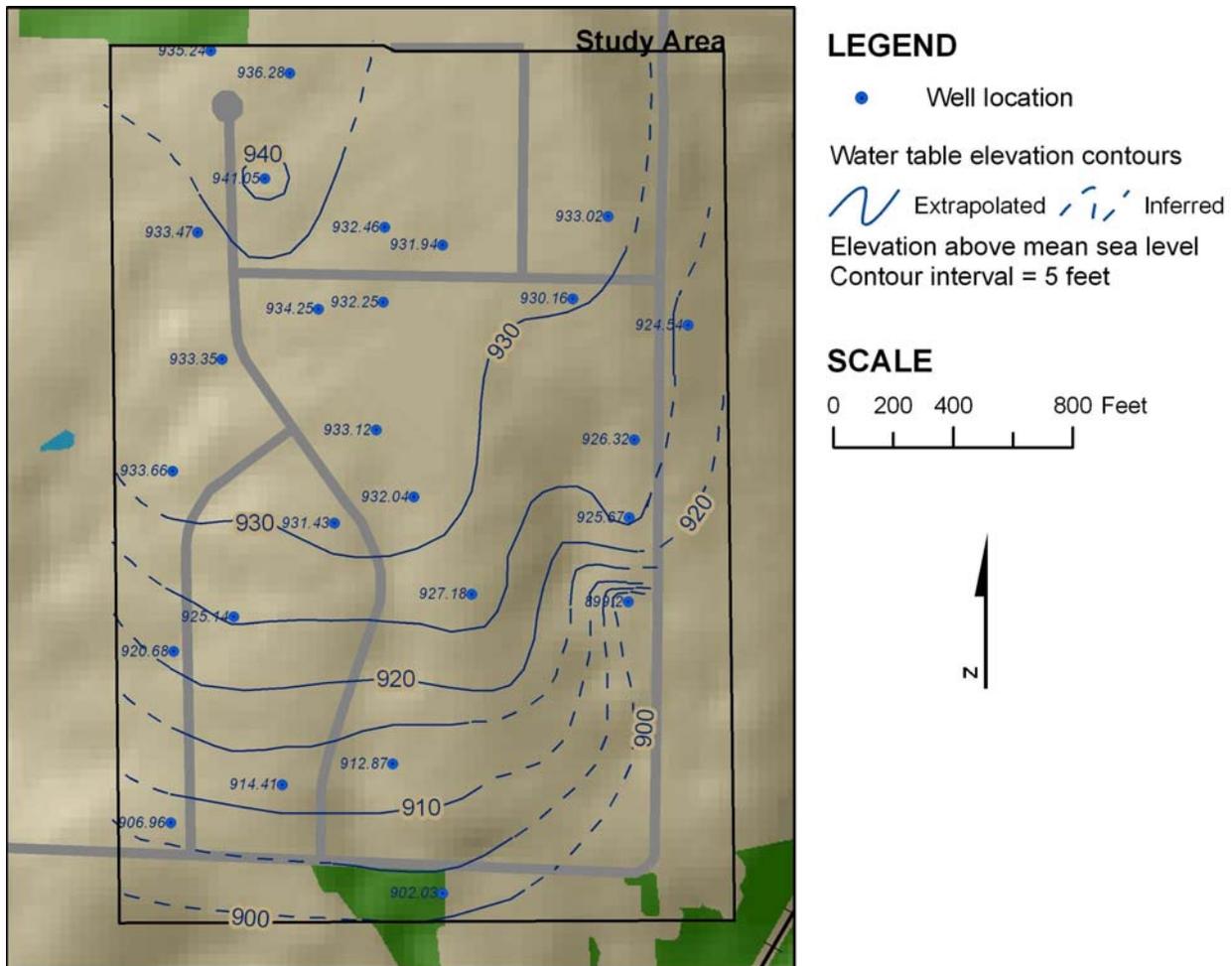


Figure 5. The water table as measured on May 30, 2006.

available for the area indicate they are cased at least to the bedrock surface; most wells are cased into the bedrock just below the water table. Well log information for the study area can be found in Appendix A. Based on water level data and review of the available well logs the primary aquifer is the upper Cuyahoga formation (Figure 4). The bedrock fractures are documented in a down-hole camera video taken by ODH in 2003 (Figure 6) and can be observed in bedrock exposed by streams cuts into the bedrock north of the study area and road cuts throughout the central Wayne County area. The fractures are nearly vertical (approximately 85° dip) and generally trend northeast and northwest. Ground water flow in the study area may be dominated locally by flow within these fractures during active pumping. Although the water table in the study area is generally deep, the presence of fractured bedrock below thin tills provide pathways for rapid recharge to ground water.

Well logs indicate the length of casing penetrating the water table ranges from -3 feet (the bottom of the casing is 3 feet above the water table) to 72 feet (the bottom of the casing is 72 feet below the water table) with an average penetration of 25 feet. During ground water elevation measurements, water was heard cascading into the well in

which the casing does not reach the water table, probably from the open borehole above the water table. The depth of casing penetration may play a significant factor in controlling the concentration of contaminants drawn into a well. Wells which have shorter lengths of casing below the water table are more likely to draw water closer to the surface of the water table. The shallower portions of the saturated aquifer are generally more likely to be contaminated with nitrate and pathogens, since the contaminant sources are related to surface and near surface land uses.

Previous Water Quality Investigations

Samples collected from private water systems in the area were positive for the presence of total coliform bacteria as early as 1991 and *Escherichia coli* (*E. coli*) as early as 2001. During fall 2003, the Wayne County Health Department (WCHD) conducted total coliform sampling on private water systems in the study area. Roughly 45 percent (26 of 58 systems) tested positive for total coliform at least once. Several systems tested positive for total coliform on more than one occasion or also tested positive for *E. coli*.

In May and November 2005, the Ohio Department of Health (ODH) sampled a total of 27 private water systems in and north of the study area for nitrate, ammonia, chloride, total coliform bacteria and *E. coli*. Twenty-five of these systems were tested during both events. During the May sampling 18 of the 26 (69 percent) systems tested were total coliform positive; eight of these also tested *E. coli* positive. Nitrate was detected above 5.0 milligrams/Liter (mg/L) in 16 systems and above 10.0 mg/L in nine of these systems. Four systems, all located on Mallard Way and Pheasant Run Drive, tested positive for total coliform and *E. coli* and had nitrate concentrations above 10.0 mg/L. Chloride ranged from 12.3 to 75.9 mg/L and ammonia ranged from <0.05 to 0.134 mg/L.

During the November 2005 sampling, 12 of the 26 systems (46 percent) tested total coliform positive and four of these also tested *E. coli* positive. Nitrate was detected

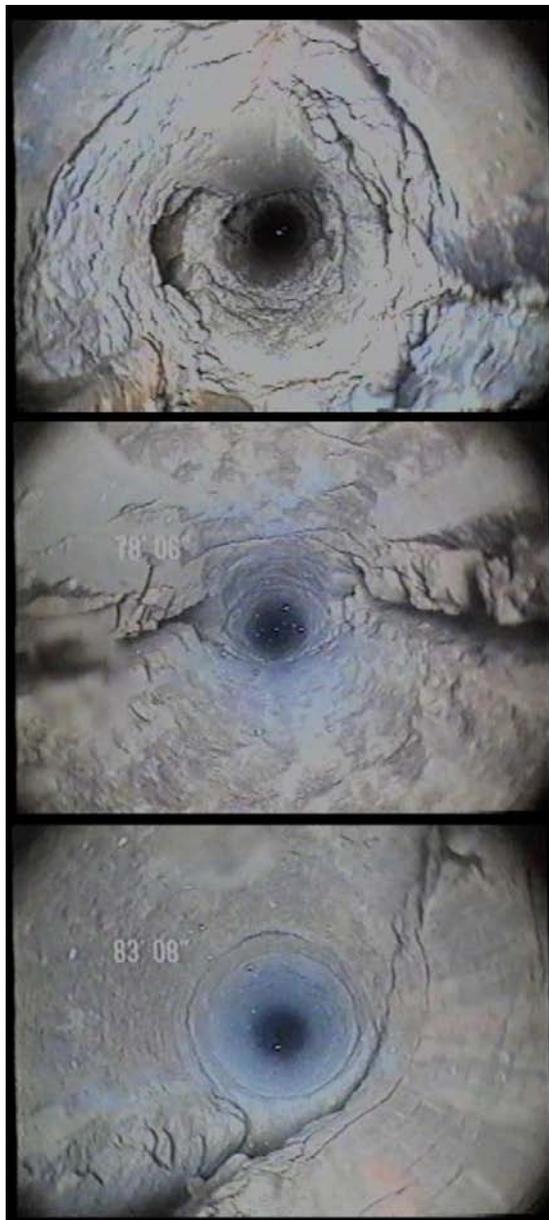


Figure 6. Bedrock fractures observed in a well within the study area.

above 5.0 mg/L in 13 systems and above 10.0 mg/L in four of these systems. Only one system, located on Pheasant Run Drive, tested total coliform and *E. coli* positive and contained nitrate above 10.0 mg/L. Chloride ranged from 14.9 to 76.2 mg/L and ammonia ranged from <0.05 to 0.62 mg/L.

Of the 25 private water systems sampled during both events, nitrate above 5.0 mg/L was detected during both events at 13 locations and above 10.0 mg/L during both events at three locations. Ten private water systems tested total coliform positive during both events and three tested *E. coli* positive both times. Three private water systems, all located west of the intersection of Mallard Way and Pheasant Run Drive, tested both total coliform and *E. coli* positive and had nitrate concentrations above 5.0 mg/L during both events. The complete data sets are included in Appendix B.

While regulated as a transient non-community public water system, the LDS Church was required to conduct routine monitoring for unsafe conditions. A review of this information indicates that nitrate was detected in all sampling events, with concentrations ranging from 7.3 to 9.16 mg/L. In addition water at the LDS Church also tested total coliform positive on multiple occasions and *E. coli* positive once (in 2003). These results only cover the period the LDS Church used it's on-site well as the source of drinking water. The LDS Church converted to a hauled water system prior to March 2004 and had the well sealed at that time (DDAGW, 2006a). Routine sampling at the Maurer Farms public water system have shown safe bacterial conditions and nitrate concentrations have ranged from 0.83 to 4.7 mg/L (DDAGW, 2006b).

2006 UNSAFE WATER SUPPLY INVESTIGATION

The geologic setting and previous water quality investigations in the study area suggest the wells are sensitive to rapid recharge which may include effluent from home sewage treatment systems. Specific concerns relate to the presence of nitrate and pathogens, which are present in sewage effluent. Excessive levels of nitrate in drinking water can cause methemoglobinemia, also known as Blue Baby syndrome. Although methemoglobinemia is rare among adults, cases have been reported among infants when nitrate-contaminated water was used to prepare formula and other baby foods. Six human pathogens, including *E. coli*, account for more than 90% of food and waterborne diseases in humans. Potential health effects include gastrointestinal illness with diarrhea, abdominal discomfort, nausea, vomiting and other symptoms. The effects of water-borne disease are usually acute and may result from a single exposure. Most gastrointestinal illnesses are of short duration and result in mild symptoms, but some can result in severe illness and even death.

The goal of the Ohio EPA's Unsafe Water Supply Investigation was to determine whether ground water resources used locally as private water supplies have been impacted by the operation of individual household sewage treatment systems within the subdivision or from other potential contaminant sources. Specially, the investigation was to identify the probable source(s) of *E. coli* and nitrate contamination in the water well samples collected previously by Wayne County Health Department and Ohio Department of Health.

Sampling Design

After reviewing the available information and establishing the goals of the investigation, Ohio EPA's Division of Drinking and Ground Waters (DDAGW) conducted a two phase investigation in the study area as detailed in *Unsafe Water Supply Investigation (ORC 6103.17) Sampling Plan for Scenic Heights/Batdorf Road Area, Wooster Township, Wayne County*. The first phase of the investigation collected data on local ground water quality, identified potential sources of microbial and nitrate contamination, determined local ground water elevations and mapped ground water flow directions. The second phase focused on the collection and analysis of additional water quality indicators that can substantiate contamination from sewage or septic sources.

During Phase 1, an initial sampling of residential wells in and around the investigation area was conducted to obtain current information about the presence and distribution of total coliform bacteria, *E. coli* and nitrate in ground water. In addition to these data DDAGW collected other ground water quality parameters. DDAGW conducted this sampling on April 27, 2006 with the assistance of staff from ODH and WCHD. Ground water levels and flow directions were also determined with the assistance of staff from WCHD and the Ohio Department of Natural Resources Division of Water.

Sampling locations selected for Phase 1 were based on the results of prior sampling events. Locations were selected to confirm past results and to extend sampling of conventional wastewater-related parameters (total coliform bacteria, *E. coli* and nitrate) to additional households where little or no data was available. The sampling locations fell into the following categories:

- Private water systems in the study area with minimal or no reported impacts (total coliform negative or low nitrate concentrations) or which had not been sampled in previous studies. This was a total of 17 locations.
- Private water systems where prior sampling showed the system to be total coliform positive with a nitrate concentration greater than 5.0 mg/L, the well tested *E. coli* positive or the well had a nitrate concentration greater than 10.0 mg/L. This was a total of 14 locations.
- Background wells north of the study area (along Batdorf and Moore Roads) and to the west of the subdivision. This included an irrigation well just north of the LDS Church and totaled five locations.
- Samples were also collected from the outfall for the Lauraland common collector system.

Based on the results of the Phase 1 sampling, Ohio EPA staff collaborated and contracted with USGS Ohio Water Science Center to select locations and perform sample collection and analysis for 12 wells during Phase 2 sampling. The wells included 11 residential wells which were deemed to give the best chance of detecting wastewater indicators and 1 well outside the study area to be used for background sampling. Sample analysis at all locations included total coliform, *E. coli*, bromide, chloride, nitrate, ammonia and oxygen and nitrogen isotopes. Sample analysis for bacteriological DNA maker samples were collected at eight locations, wastewater and home and personal care products compound samples were collected at five locations and optical brightener samples were collected at six locations. In addition to ground water samples, five household sewage treatment system samples were collected and composited into one baseline sample for *Bacteroides* DNA makers and oxygen/nitrogen isotope analysis. Phase 2 sampling was conducted during June 19 through June 21, 2006. Households selected for sampling were contacted prior to the investigation.

Water Quality Sampling Results

A listing and summary of analytical results for Phase 1 are included in Appendix C and for samples analyzed by Ohio EPA during Phase 2 in Appendix D. Individual sample results for Phase 1 and Phase 2 samples were mailed to each household participating in the study after each sampling event. Analytical results for the samples collected by USGS during Phase 2 were finalized in October 2006. The USGS report is included in Appendix E.

Dissolved oxygen, nitrate, microbiological parameters (total coliform bacteria and *E. coli*) and chloride are useful in describing the quality of ground water and the extent and magnitude of contamination. Bromide/chloride relationships, oxygen/nitrogen isotopes, wastewater compounds and specific microbiological parameters (*Enterococci* markers and *Bacteroidales*) provide indicators of the source of the contaminants.

Water quality results are presented in various geographic and graphic formats to aid in interpretation of results. The variability of results at any single location reflects the dynamic nature of the ground water system and source(s) of contamination. This complex interaction, compounded by the difficulty in predicting fracture flow, makes identifying the exact location of the contamination source for a particular well extremely difficult. The general contamination source within the study area can, however, be identified as effluent from household sewage treatment systems. Evaluating the similarities and differences between the 2004 and 2006 sampling events confirms the water quality impacts. While all of the data is presented and summarized in the appendices, those data which characterize the water quality impacts and identify the likely source of the contamination are presented in the Detailed Interpretation of Results.

Summary of Significant Findings

The investigation results indicate that the geologic setting within the study area is sensitive to ground water contamination from household sewage treatment systems. Due to the complex nature of fractured bedrock aquifers and multiple local sources of effluent from household sewage treatment systems (leach fields) in the study area, this study does not identify individual household sewage treatment systems as the cause for the unsafe water supply conditions. However, the results from the sampling and understanding of the hydrogeologic conditions in the study area suggest the household wastewater treatment systems installed into or just above bedrock are likely to have a greater impact on the water quality of the aquifer than those systems with more soil material to treat the effluent.

The geochemical and isotopic data give significant support to the conceptual model of a local ground water mixing, in varying proportions, with diluted effluent from household sewage treatment systems. The interpretation of the analytical results provide several lines of evidence which indicate that sewage effluent is a major source of nitrate and pathogen contamination of the local ground water. Historic nitrate and microbiological data indicate continuing input from a source related to human activities. Nitrate concentration in excess of 10 mg/L indicate the source of the contaminants are from sewage effluent. This conclusion is supported by analysis of the bromide and chloride data and the oxygen and nitrogen isotope data, both of which indicate a sewage-related source for the contaminants. Significant findings that support these conclusions are summarized as follows with references to the appropriate section of the Detailed Interpretation of Results:

- The variability and persistence of microbiological indicators and nitrate contamination is documented by the previous sampling results and Phase 1 and 2 sampling for this study. Mapping the locations of these results demonstrates elevated nitrate concentrations and pathogen indicators are concentrated in the in the northern half of the study area, where glacial tills are thin to absent over a considerable area (see Impact of Local Geology on Ground Water Quality and Nitrate and *E. coli*)
- Ground water sampling results for Phase 2 include unusually high dissolved oxygen concentrations, the presence of fecal *Bacteroidales* markers, and detections of *E. coli*, all of which provide strong evidence for rapid recharge. In addition, the fecal *Bacteroidales* markers and detections of *E. coli* support the presence of fecal-derived contamination. (See Nitrate and *E. coli* and Microbiological Markers)
- Chloride/bromide ratios document that sewage-type waste is present in the ground water. The direct correlation of nitrate concentration to dissolved oxygen and the association of elevated bromide with elevated nitrate suggest that a single process is the source of these indicators – most likely rapid recharge of sewage effluent to the ground water which is then captured by local wells. (See Nitrate and *E. coli* and Bromide).
- The nitrogen and oxygen isotope data provide strong evidence for the inclusion of sewage or manure derived waste in the ground water with a strong correlation to the household sewage treatment system composite sample. (See Nitrogen and Oxygen Isotopes of Nitrate)
- The observed nitrate isotope compositions, in addition to bromide, nitrate and chloride data, indicate that the water wells within the study area are drawing water which is composed, to varying degrees, of a mixture of local ground water and diluted sewage effluent. This mixture appears, in varying proportions, across the study area.
- The erratic presence of bacteria, nitrate, and other effluent indicators is to be expected, given the unpredictable nature of flow volume and direction in fractured bedrock, the differences in depth to the top of the bedrock surface across the area, the variable thickness of the of the soils and glacial tills (the barriers to contaminant transport) beneath leach fields, and the varied spatial relationships between a each household's leach field and water well to those of it's neighbors.

DETAILED INTERPRETATION OF RESULTS

Impact of Local Geology on Ground Water Quality

A review of well logs available for the study area indicates several areas where tills and soils were thin or absent when the properties were developed (Figure 7). Locally, bedrock is very near (or exposed at) the ground surface. Bureau, Graham and Scherzinger (1984) report soils within the study area are moderately well suited for leach fields, but some areas have insufficient soil thickness above bedrock to support standard leach fields. Consequently effluent discharge from leach fields can be released into fractured bedrock allowing rapid recharge to and potential contamination of the underlying ground water. Figure 7 also shows the approximate leach field locations derived from a review of Wayne County Health Department files and soil information from Bureau, Graham and Scherzinger (1984).

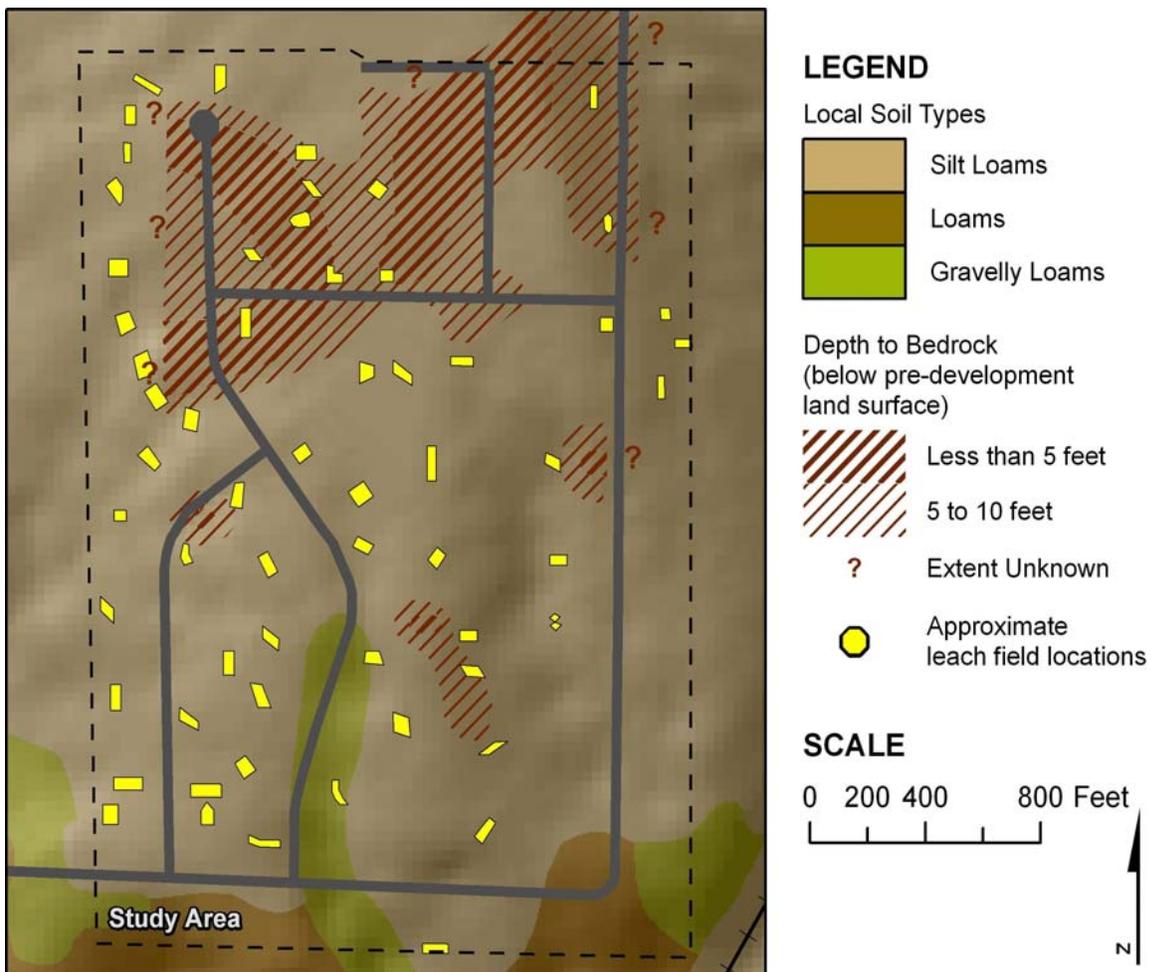


Figure 7. Local soils, pre-development depth to bedrock and approximate leach field locations.

Dissolved Oxygen

Field data collected during the Phase 2 (June 2006) sampling (Table 1) contain very high dissolved oxygen values. All values were measured by USGS using a flow through sampling device after well stabilization. The values are more typical of surface water than ground water and are consistent with fractured bedrock overlain by little or no glacial till. Ground water in areas of silty soils commonly contains less than 0.1 mg/L dissolved oxygen (Freeze and Cherry, 1979). The observed dissolved oxygen levels suggest very rapid transport of water to the water table. This rapid recharge may include the transport of surface and shallow subsurface contaminants.

Table 1. Dissolved oxygen concentrations observed during Phase 2 sampling.

Site ID	Collection Date	Dissolved Oxygen	
		mg/L	Percent Saturation
WSI-02	06/21/06	6.77	61.8
WSI-03	06/19/06	4.95	42.9
WSI-05	06/19/06	6.51	63.3
WSI-06	06/20/06	2.25	21.6
WSI-07	06/21/06	1.26	11.8
WSI-12	06/20/06	4.04	38.3
WSI-20	06/20/06	7.65	72.7
WSI-21	06/21/06	9.95	89.9
WSI-22	06/20/06	6.73	64.1
WSI-23	06/19/06	4.96	46.9
WSI-26	06/19/06	7.15	67.5
WSI-30	06/19/06	8.56	81.6

The amount of nitrate in ground water depends on a number of factors, including the presence of dissolved oxygen in the aquifer (Speiran and others, 1998). Where dissolved oxygen is abundant, nitrate is the principal form of nitrogen in ground water. The presence of high levels of dissolved oxygen in local ground water retards denitrification and allows nitrate concentrations to remain stable over time. Figure 8 illustrates a strong association between nitrate and dissolved oxygen concentrations.

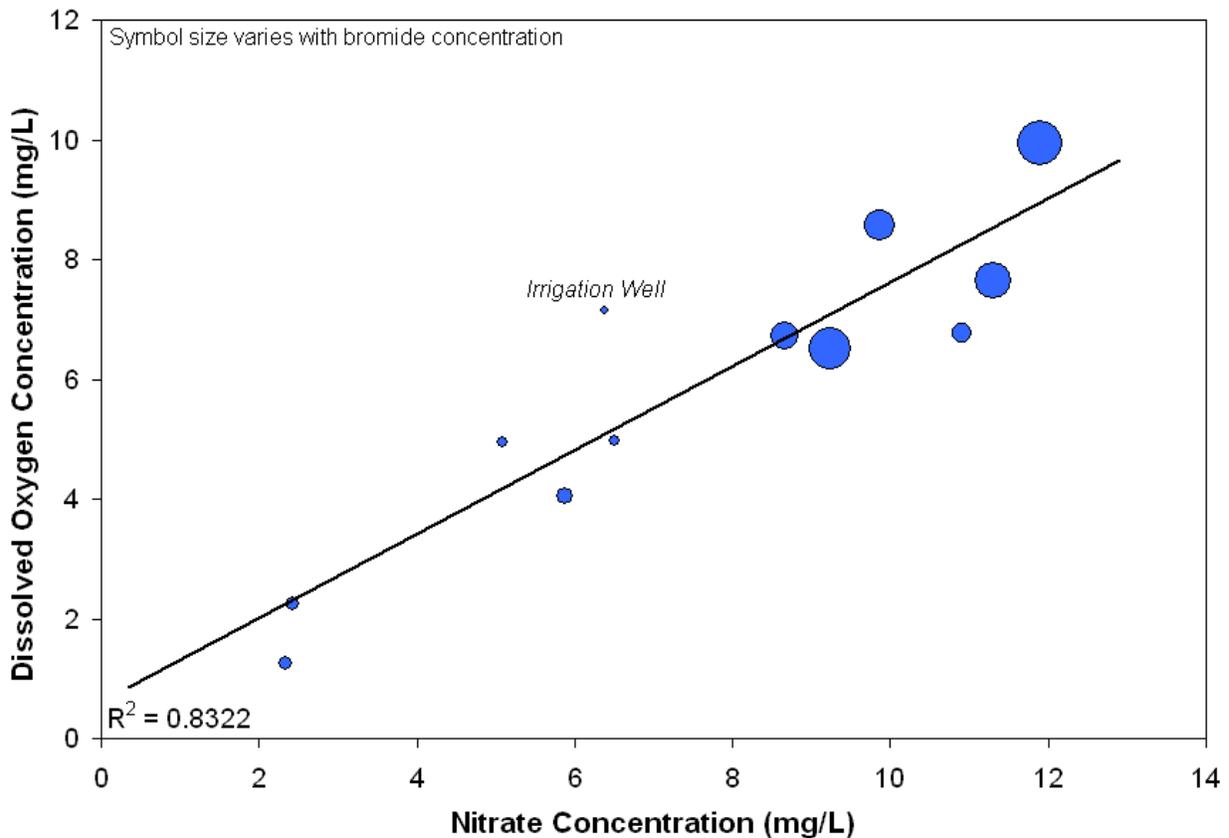


Figure 8. The relationship between nitrate and dissolved oxygen concentrations.

Nitrate and *E. coli*

Four rounds of sampling have been completed in the study area; two by ODH in 2004 and two by DDAGW in 2006. Figure 9 shows the distribution of nitrate concentrations in each of these studies. The distribution of nitrate displays fluctuations in concentrations over time but two areas – the area of the Pheasant Run cul-de-sac (north of Scenic Heights and east of Pheasant Run) and just north of Scenic Heights along Batdorf Road – had nitrate concentrations above 10 mg/L in each event. Figure 9 also shows the distribution of *E. coli* detections in each of these studies. Three events (May and November 2004 and June 2006) found *E. coli* present within an area from the east side of the cul-de-sac south to the bend in Mallard Way. Three events (May 2004 and April and June 2006) found *E. coli* present in the vicinity of the Mallard Way - Pheasant Run intersection from north of the bend in Mallard Way eastward beyond Pheasant Run. Over four sampling events, 64 percent of samples have tested total coliform positive and 25 percent of samples have tested *E. coli* positive (Table 2). The distribution of these two contaminants indicate a persistent source of both nitrate and *E. coli*. Communication with residents during the April sampling indicates that wells are occasionally shock chlorinated to remove pathogens. This may influence the *E. coli* distribution observed during any given event.



LEGEND

- Nitrate concentration lines
- Extrapolated Inferred
- Contour interval = 2 mg/L
- Areal extent of *E. coli* detections



SCALE

0 500 1,000 2,000 Feet

Figure 9. Nitrate and *E. coli* distributions for the 2004 and 2006 sampling events.

Table 2. Summary of total coliform and *E. coli* results for the 2004 and 2006 sampling events.

Agency	Date	Sample Locations	Number of Samples	Total coliform positive		<i>E. coli</i> Positive	
				Number	Percent	Number	Percent
ODH	May 2004	Study Area	23	18	78%	8	35%
ODH	November 2004	Study Area	23	12	52%	4	17%
Ohio EPA	April 2006	Study Area	31	15	48%	3	10%
Ohio EPA	June 2006	Study Area (Selected Wells)	11	11	100%	7	64%
ODH	May 2004	Background	3	0	0%	0	0%
ODH	November 2004	Background	3	0	0%	0	0%
Ohio EPA	April 2006	Background	5	0	0%	0	0%
Ohio EPA	June 2006	Background	1	0	0%	0	0%

Nitrate concentrations during the four events range from <0.01 to greater than 16 mg/L (Table 3). Baker and others (1989) analyzed more than 16,000 ground-water samples from wells in Ohio for nitrate; of these samples, 2.9 percent had nitrate concentrations in excess of 10 mg/L, and 68.2 percent had concentrations less than 0.3 mg/L; the average concentration was 1.32 mg/L. These authors defined background nitrate levels in Ohio as 0.3 mg/L or less. Data from the Maurer Farms public water system indicate the nitrate concentrations from agricultural sources ranges from 2 to 5 mg/L with some seasonal variability, with the lowest concentrations during the winter and the highest during the early growing season.

Table 3. Summary of nitrate results for the 2004 and 2006 sampling events.

Agency	Date	Sampling Locations (Study Area)	Range (mg/L)	Nitrate >3.1 mg/L		Nitrate >10 mg/L	
				Number	Percent	Number	Percent
ODH	May 2004	23	<0.1 - 15.6	17	73.9	9	39.1
ODH	November 2004	23	<0.1 - 16.9	14	68.1	4	17.4
Ohio EPA	April 2006	31	<0.1 - 12.8	23	74.2	4	12.9
Ohio EPA	June 2006	11	2.34 - 11.9	9	81.8	3	27.3

Work by Dumouchelle and Stoeckel (2005) show nitrate concentrations between 3.1 and 10 mg/L may indicate effects of human activity; nitrate concentrations in excess of 10 mg/L are most likely due to impacts from sewage-related waste. Work by Panno and others (2006a) show nitrate concentrations between 2.1 and 15 mg/L indicate effects of human activity; nitrate concentrations in excess of 15 mg/L are indicative of significant

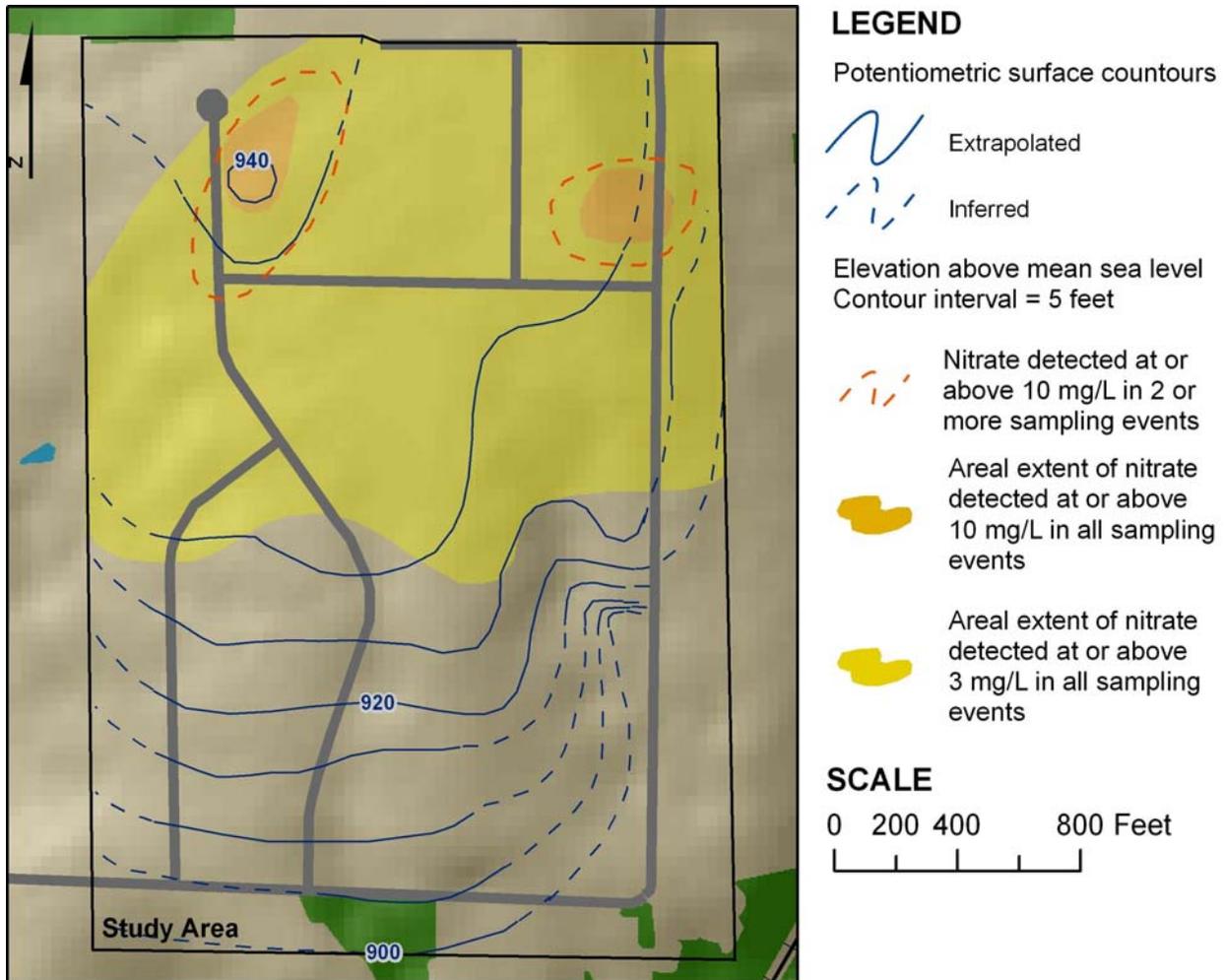


Figure 10. Extent of persistent nitrate impacts in the study area.

impacts from sewage effluent or concentrated animal feeding operations. Figure 10 integrates the results obtained by ODH and DDAGW to indicate ground water is impacted by human activities in the northern portion of the study area.

Chloride

Chloride is a conservative ion; that is, chloride in ground water is not involved in many chemical reactions. Household sewage treatment systems tend to have higher chloride concentrations than natural waters because sewage effluent may include residual chemicals from water softeners or household products containing chloride. The highest concentrations were found in samples collected in the vicinity of the Mallard Way - Pheasant Run intersection.

Expected background chloride levels can be derived from other data sources (Table 4). Chloride concentrations for samples collected between 1997 and 2004 for wells at the City of Orrville in eastern Wayne County using upper Cuyahoga sandstones ranged from 12.6 to 20 mg/L. (DDAGW, 2006c). The statewide range of chloride concentrations for sandstone is < 1.0 to 530 mg/L (DDAGW, 2006d) but values above 100 mg/L are unusual and suggest anthropogenic input. The chloride concentrations observed for the study area fall within the range for chloride statewide. Many of the values obtained for both background and study area sites are above values obtained from the Orrville well producing from an aquifer of the same age east of the study area and which is expected to be representative of the chloride concentration in the region.

Table 4. Chloride concentrations in Ohio sandstone aquifers and in Phase 1 samples.

Concentrations are reported in mg/L. Data for chloride concentrations in sandstone from DDAGW (2006c, 2006d)

Aquifer Media	Data source	Max.	Min.	Mean	Median	Total Samples	Number Below Reporting Limit
Sandstone	Ambient	530	< 1.0	39.4	22.0	733	65
	Orrville	20	12.6	15.2	15	16	0
Interbedded sandstone, siltstone and shale	Study Area (Phase 1)	70.2	6	34.9	35.2	31	0
	Study Background (Phase 1)	66.4	32.1	44.9	39.9	5	0

Potential sources of excess chloride to groundwater include road and sidewalk salts, fertilizers, discharge from water softeners, natural evaporite deposits, and sewage effluent. The most common salt is sodium chloride (NaCl), also known as halite. Sodium chloride is the dominant mineral of most road, table, and water softener salts. Although potassium chloride is also a common fertilizer salt, there is no indication that this salt contributes significantly to the groundwater chemistry in the study area. Halite is also common in natural geologic deposits called evaporites, which in certain areas contributes considerable amounts of chloride and sodium to groundwater. However, natural salt deposits have been excluded as a possible source of chloride within the study area based on the evaluation of well logs and other geologic information. Because sodium and chloride are such common ions within groundwater, it is often difficult to determine their origin with any certainty. Instead, the relationship between sodium chloride's two components (sodium and chloride ions) can be evaluated to determine their relative abundances. This is done for two separate cases: local ground water and a modeled solution. A model solution is one in which the mineral sodium chloride is the only contributor of sodium and chloride ions to the model. In other words, it is a hypothetical solution based on real data.

In order to test these relationships, the sodium concentrations are plotted against chloride concentrations for the groundwater well samples (Figure 11). A linear regression is computed on the data, which expresses the position and strength of this relationship (data points and upper line in Figure 11). For the sample data, the regression indicates a significant relationship ($r^2 = 0.91$) with a slope of 3.4. The model solution, indicated by the lower line in Figure 11, plots a line of slope 1.5, and is based on the combining weights of sodium and chloride (milligrams per liter). In other words, when sodium and chloride ions in solution are derived *exclusively* from the dissolution of sodium chloride, samples from that solution will form a line with slope equal to 1.5, as this is the ratio of their formula weights, and represents the ratio at which they combine (and dissolve) in nature.

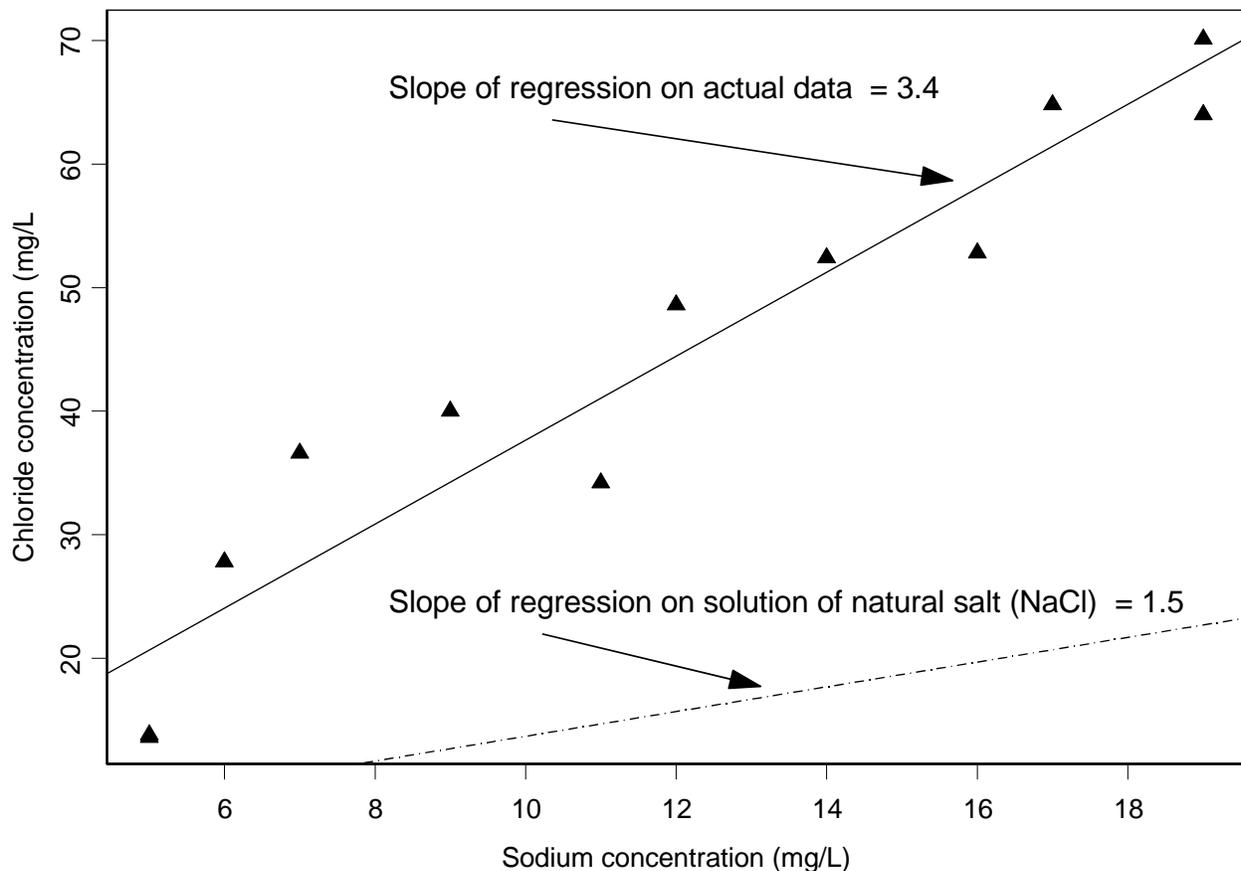


Figure 11. Sodium and chloride concentrations in Phase 1 samples.

Comparison of the slopes between the Phase 1 data and the model solution on Figure 11 clearly indicate chloride concentrations in excess of those expected by the dissolution of sodium chloride are present within the study area. These excess values

are most likely associated with household sewage treatment systems, which would include elevated chloride output from human waste, cleaners and cleansers, water softener discharge and other common household sources.

The degree to which sewage effluent affects the chloride concentrations in the study area can be seen by comparing chloride concentrations and distribution of positive *E. coli* results for the April 2006 sampling (Figure 12). Because of chloride's conservative nature, it can be a more reliable long term indicator of sewage effluent influence than *E. coli*, which tends to die off rapidly in highly oxygenated environments. The overlapping occurrence of chloride and *E. coli* in the vicinity of the Mallard Way - Pheasant Run indicates discharge of effluent and rapid recharge to the water table. Water softener discharges to household sewage treatment systems may help explain the higher chloride concentrations. The presence of concentric contours around individual wells suggests that some wells are drawing in a higher proportion of effluent including water softener discharge than adjacent wells, which is consistent with the complex nature of flow in fractured bedrock aquifers.

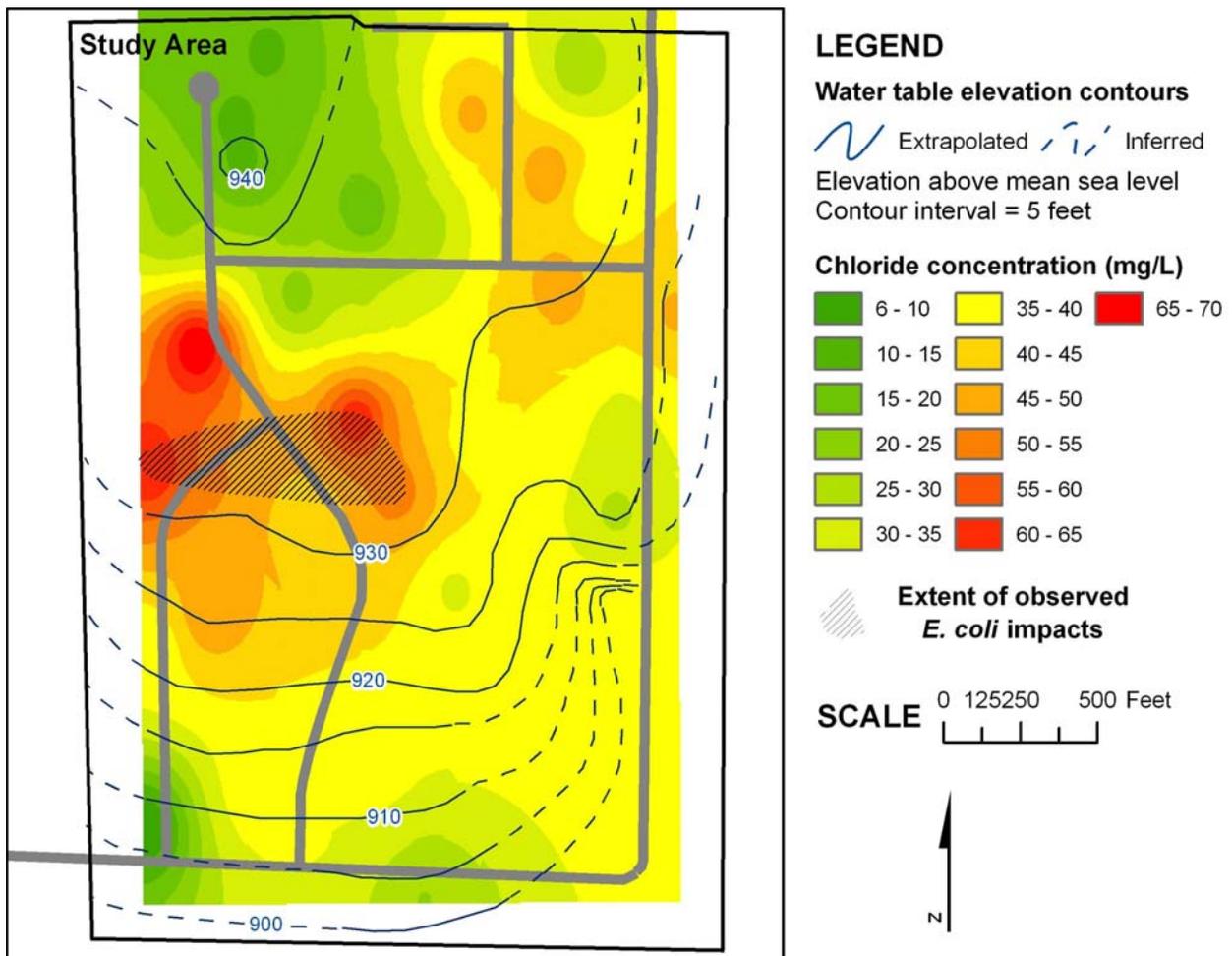


Figure 12. Chloride concentrations in ground water, Phase 1 sampling.

Bromide

In natural waters, bromide like chloride is chemically conservative, meaning that it does not enter into significant subsurface reactions. Because of this predictable behavior, and because they move freely within the subsurface, chloride and bromide ions are used extensively to study the movement of ground water and to describe the effects of anthropogenic sources on water quality. Bromide concentrations elevated above background are associated with a variety of anthropogenic sources, including natural and synthetic salts and brines, road salt, sewage effluent and animal waste.

For un-impacted ground water, precipitation is the primary source of both chloride and bromide ions in ground water. Chloride is as much as 8000 times more abundant than bromide in un-impacted water, and consequently, small changes in the total bromide mass will lead to large variations when expressed as a chloride/bromide ratio, provided the chloride mass remains relatively constant (Davis and others, 1998). Different chloride/bromide ratios can indicate whether the chloride and bromide are derived, at least in part, from sources other than precipitation. The chloride/bromide ratio in domestic sewage has been shown to range from about 400 to about 1000 (Davis and others, 1998).

In areas where halite (rock salt) does not occur, ground waters have a lower chloride/bromide ratio than waters affected by anthropogenic sources. This is the case for the glacial sediments and bedrock within the study area and ground water in this area would be expected to have chloride/bromide ratios which indicate little or no impact from salt or brines. For example, the great majority of 251 potable water samples which contain less than 200 mg/L chloride have chloride/bromide ratios of less than 200 (Davis and others, 1998); these waters are considered to be unaffected by contaminants (Table 4). The influence of road salt on chloride/bromide ratios may have to be evaluated in areas where salts are applied.

The chloride/bromide ratio of ground water contaminated with effluent from a septic or sewage source represent mixing of the effluent and local ground water. This means that a background ground water, when mixed with increasing proportions of an effluent, will produce a mixing line between two end-members – local ground water with low chloride and bromide concentration (chloride/bromide ratios typically between 90 and 300) and sewage effluent with moderate chloride and moderate to high bromide concentrations (chloride/bromide ratios typically between 400 and 1,000). Similarly, other end members such as natural salts and brine can be considered. Figure 12 shows three hypothetical mixing lines based on mixing brine, effluent, and natural salts with ground water. These lines produce two main ranges of values – ground water and sewage (GW + sewage, above the middle line) and ground water and brine (GW + brine, below the middle line). Data for ground water samples with mixed sources of chloride and bromide will fall into an area based on the source of the excess chloride and bromide. The plotted lines represent the mixture of a background groundwater (the black dot at the lower left) with natural salts (the upper line, sewage effluent (the middle line) and brine (the lower line).

These regions are built from empirical data, and are modified from their presentation in Francey et al., 2004.

Plotted on Figure 13 are the data for samples collected during Phase 2. The chloride concentrations for the Phase 2 samples range from 12.6 and 61 mg/L and bromide values range from 0.04 to 0.19 mg/L (Table 5). The chloride/bromide ratios for the wells in the study ranged from 79 to 890 with a mean of 432. The chloride/bromide ratio for the irrigation well was 1330.

Table 5. Comparison chloride and bromide data from Phase 2 to data for potential contaminant sources, precipitation and ground water. The shaded rows in Table 4 provide data from Panno and others (2006b) for comparison.

Sample	n	Chloride (mg/L)			Bromide (mg/L)			Cl ⁻ /Br ⁻ Ratio		
		Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
Study Area (Phase 2)	11	12.6 - 61.4	38.5	39.7	0.05 - 0.19	0.108	0.09	78.75 - 890	431.5	422.9
Background (Phase 2)	1	53.2	n/a	n/a	0.04	n/a	n/a	1330	n/a	n/a
Precipitation (Midwest US)	49	0.04 - 0.19	0.1	0.1	ND	ND	ND	n/a	n/a	n/a
Precipitation (Midwest US)	4	0.1 - 0.3	0.2	0.2	0.0032 - 0.0053	.00425	.0045	20 - 56	42.6	47
Soil water	2	0.7 - 1.6	1.15	1.15	0.0103 - 0.0147	0.0125	0.0125	68 - 112	90	90
Sand & gravel aquifer	12	0.8 - 12	5.05	5.37	0.0138 - 0.167	0.031	0.0551	23 - 521	106	156
Sewage effluent (private)	29	21 - 5620	334	91	<0.0 - 0.104	0.172	0.09	65 - 5404	1164	769
Animal waste (hog & horse)	4	440 - 1980	1028	847	0.3 - 1.59	0.758	0.572	1245 - 1654	1422	1395
Sewage effluent-affected ground water (Illinois)	3	40 - 116	69	51	0.078 - 0.288	0.154	0.095	403 - 533	482	509
Animal waste-affected ground water (Illinois)	11	33 - 280	88.9	57.0	0.084 - 0.515	0.149	0.140	247 - 791	455	436

For comparison Figure 13 also shows the ranges of chloride and bromide concentrations and chloride/bromide ratios from other studies. The comparison sources include the range of values from sewage sources (green box) and United States ground water (black box) (Davis and others, 1998); the red line represents the highest chloride/bromide ratio expected for un-impacted ground water in an urban/residential setting (chloride/bromide ratio of 400 from Thomas (2000)). The point in the lower left corner of Figure 13 is an estimate for hypothetical background ground water from the area, and is the probable input from precipitation, after slight evaporation before recharge occurs. The background ground water point (lower left) is the estimated starting point for mixing local ground water with non-precipitation derived chloride and bromide. This starting point is estimated, in part, from values consistent with chloride (0.5 mg/L) and bromide

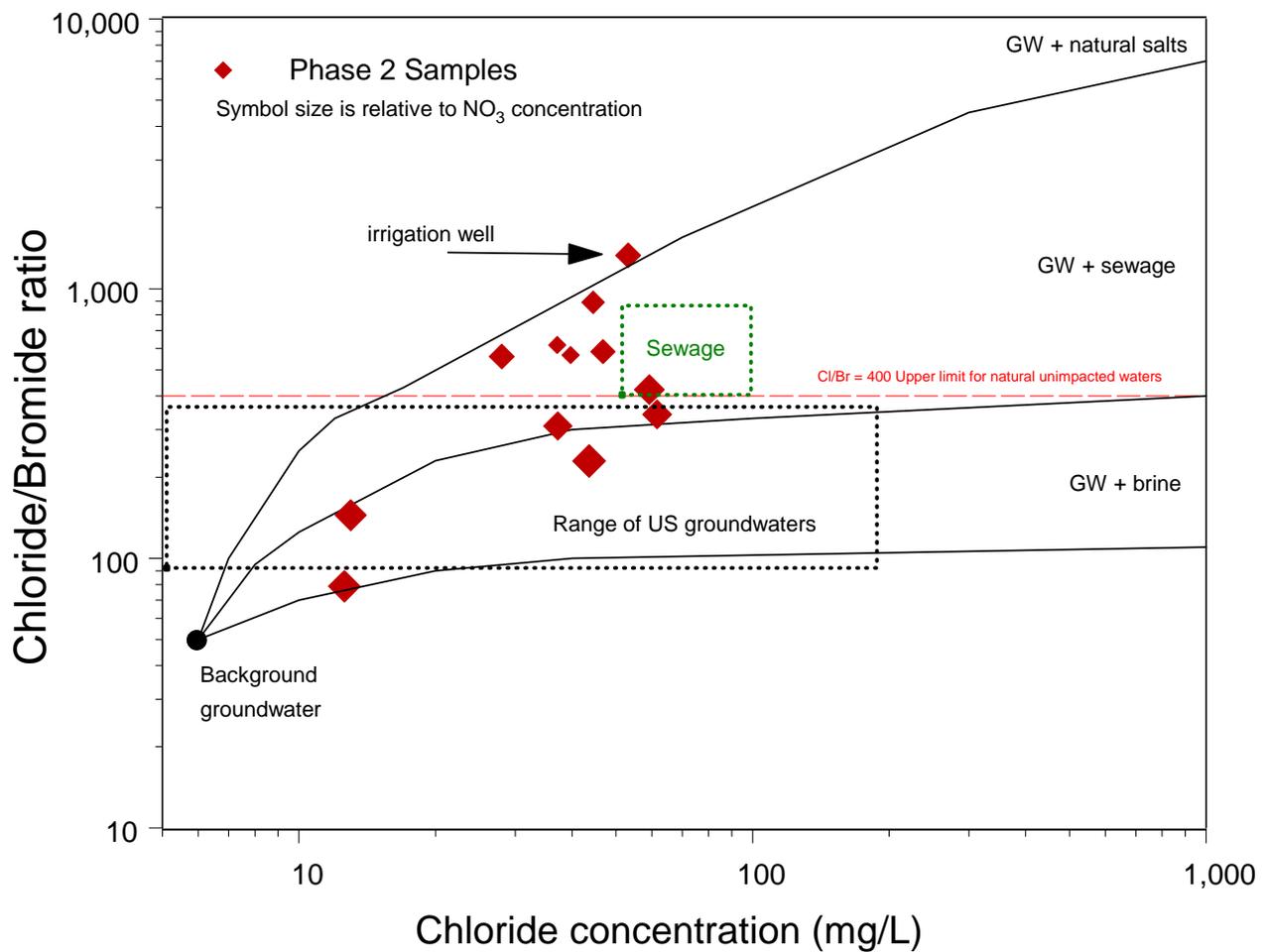


Figure 13. Chloride concentration compared to chloride/bromide ratio for Phase 2 samples. (Figure modified from Francey, et. al., 2004).

(0.01 mg/L) concentrations in precipitation, which then evolve toward increasing chloride concentration due to evaporation before recharge. The mean and median chloride values reported for sand and gravel aquifers (Table 4) is considered to be background for un-impacted ground water. All of the data in Figure 13 are represented in logarithmic form to compress the values so the relationships between the data and expected values are more clear.

As seen in Figure 13, the majority of the Phase 2 samples fall in or very near the ground water and sewage (GW + sewage) suggesting that the chloride/bromide ratio and associated chloride concentrations are consistent with values deemed effluent-impacted from other investigations. The two samples containing the lowest chloride concentrations (diamonds in lower left corner of Figure 13) have concentrations similar to the minimum background chloride concentration observed at the Orrville well. This suggests these wells are not impacted by chloride, but the elevated nitrate and bromide are strong evidence for water quality impacts associated with household sewage treatment systems. The most obvious explanation for the low chloride these two wells is that the wells are not impacted by elevated chloride concentrations associated with water softener discharge. This results in low chloride concentrations and relatively low chloride -bromide ratios for these wells. The irrigation well, just north of the study area, identified in Figure 13 by the black arrow, has the lowest bromide concentration (0.04 mg/L), but the chloride concentration is elevated (53.2 mg/L) above background levels, resulting in the highest chloride/bromide ratio of the Phase 2 samples. The elevated chloride may be associated with natural salts in the aquifer since the well is significantly deeper than the residential wells. An alternative explanation is the elevated chloride may be associated with fertilizer application and leaching of chloride applied as potassium chloride.

A key interpretation of bromide data in shallow ground water is understanding that household sewage treatment system effluent carries bromide, sodium and chloride in excess of local ground water concentrations. This excess reveals itself in bromide's positive association with nitrate (Figure 8); nitrate and bromide are positively correlated further supporting sewage effluent as the source of the contamination.

Nitrogen and Oxygen Isotopes of Nitrate

Analysis of the stable isotopes of nitrogen and oxygen in nitrate was performed on the Phase 2 samples to help identify the source of the nitrate found in the ground water. Isotopes are variations in elements which have the same number of protons, but a different numbers of neutrons, in the nucleus; a stable isotope is one that does not undergo radioactive decay. Typically one isotope is most common, and one (or more) is less abundant. Standard notation for isotope identification is to place the sum of the number of protons and neutrons in the upper left corner of the symbol used for the element. An example is nitrogen, represented by the symbol N; the ^{15}N isotope contains 15 protons and neutrons while the ^{14}N isotope contains 14 protons and neutrons. The lighter isotope, ^{14}N , is 273 times the more abundant than ^{15}N , which is heavier, rarer, and will preferentially accumulate in the residual product of a chemical reaction. Biological process chemically prefer to use the lighter isotope. Information about the physical system is can be determined by analyzing the slight mass differences between the isotopes, which can create large, systematic differences in their behavior.

Isotope pairs, such as ^{15}N and ^{14}N , are always presented with the heavier (less abundant) isotope in the numerator. Standard “delta” notation is used for nitrogen and other isotopes:

$$\delta^{15}\text{N} = \left\{ \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{air}}} \right] - 1 \right\} \times 1000$$

The δ -value is expressed as a parts per thousand, or per mil (‰) difference from a standard. For example, a $\delta^{15}\text{N}$ value of +15 per mil indicates that the sample has 15 parts per thousand (one and one half percent) more ^{15}N than the standard. A positive δ -value is said to be “enriched” or “heavy” and while a negative δ -value is said to be “depleted” or “light”. The reference standard for the stable isotopes of nitrogen ($^{15}\text{N}/^{14}\text{N}$) is atmospheric nitrogen (Clark and Fritz, 1997).

Oxygen isotopes are reported in the same manner. The heavier, less abundant isotope is ^{18}O , and its lighter, more common isotope is ^{16}O , and the ratio is calculates in the same way as the nitrogen ratio. The for oxygen is Vienna Standard Mean Ocean Water (VSMOW). The lighter isotope, ^{16}O , is 500 times the more abundant than ^{18}O .

A number of steps in the nitrogen cycle can modify the stable isotope composition of a nitrogen bearing compound such as nitrate. These changes, called fractionation, occur due to physical and chemical changes acting upon the differences in mass of each isotope. Generally, these changes tend to cause the heavier isotope to remain in the starting material of the chemical reaction, leaving the source of the nitrogen compounds enriched in heavier isotopes, and the products depleted. One of the main modifiers of nitrate isotope composition is the process of nitrification.

Nitrification is the multi-step process of converting, through microbial oxidation, the nitrogen source, in this case urea expressed as ammonia (NH_4^+), into an intermediate

form, nitrite (NO_2^-) and finally into nitrate (NO_3^{2-}). These steps are accomplished through the microbial action of two main bacteria; oxidation to nitrite by *Nitrosomonas*, and oxidation to nitrate by *Nitrobacter*. Our bodies are slightly enriched in ^{15}N relative to our diets; this occurs due to the removal of slightly depleted urine in the waste stream. The effect of these transformations is a conversion to nitrate which leaves the residual waste material, feces, highly enriched in ^{15}N , with a typical range for $\delta^{15}\text{N}$ of +10 to +25 ‰ from an initial value of about +5 ‰. Volatilization of ammonia in the household sewage treatment system can further enhance this process. The final “product” of this nitrification is a sewage effluent rich in nitrate which has characteristic $\delta^{15}\text{N}$ values of from +10 to +25 ‰. Typical $\delta^{15}\text{N}$ values for common nitrogen sources are given in Table 5.

Nitrate is an ionic compound made up of one nitrogen and three oxygen atoms, which carries a negative two (-2) charge (NO_3^{2-}). For stable isotope analysis, the task is to determine the nitrogen composition of the ground water nitrate (see Table 5 for typical values expected). Because the $\delta^{15}\text{N}$ values in ground water nitrate can overlap each other (Table 6), a “dual isotope” approach is used – that is, to also determine the oxygen isotope composition of the same nitrate molecule, which then allows some separation between $\delta^{15}\text{N}$ values when they are plotted against $\delta^{18}\text{O}$ values for nitrate.

Table 6. δ -nitrogen values for common sources of nitrogen compounds which may impact to ground water. From Seiler, 1996.

Nitrogen source	$\delta^{15}\text{N}$ (‰)
Precipitation	-3
Commercial Fertilizer	-5 to +4
Organic Nitrogen in soil	+4 to +8
Animal or Human waste	+8 to +25

Plotted in Figure 14 are $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values from the Phase 2 sampling effort. The x-axis records the $\delta^{15}\text{N}$ values and the $\delta^{18}\text{O}$ values are plotted on the y-axis. The various boxes drawn on this graph indicate typical fields into which samples with a particular nitrogen source would fall. Note that the chemical fertilizer field (upper left) coincides with the value $\delta^{18}\text{O}$ of +23.5 ‰ (orange line), indicating that the source of oxygen in nitrate derived from chemical fertilizer is the atmosphere. This is in stark contrast to the results of the Phase 2 samples (blue triangles). In an effort to clarify whether sewage effluent is a potential nitrate source to the study area ground water, an isotopic analysis was also performed on local household sewage treatment system effluent. This sample was a composite of five different effluent samples, and is indicated in Figure 14 by a red triangle. The final nitrate isotopic composition of the composite sample is influenced most strongly by nitrification just outside the septic zone, where microbes convert ammonium to nitrate where sufficient oxygen is present. The initial nitrate formed is lighter (has a lower $\delta^{15}\text{N}$ value) than the ammonium being left behind and as organisms transform all the NH_4^+ , the $\delta^{15}\text{N}$ values of the product nitrate become increasingly enriched in $\delta^{15}\text{N}$. The nitrogen isotope composition of the irrigation well north the study is noted in Figure 14 for reference to the study area samples.

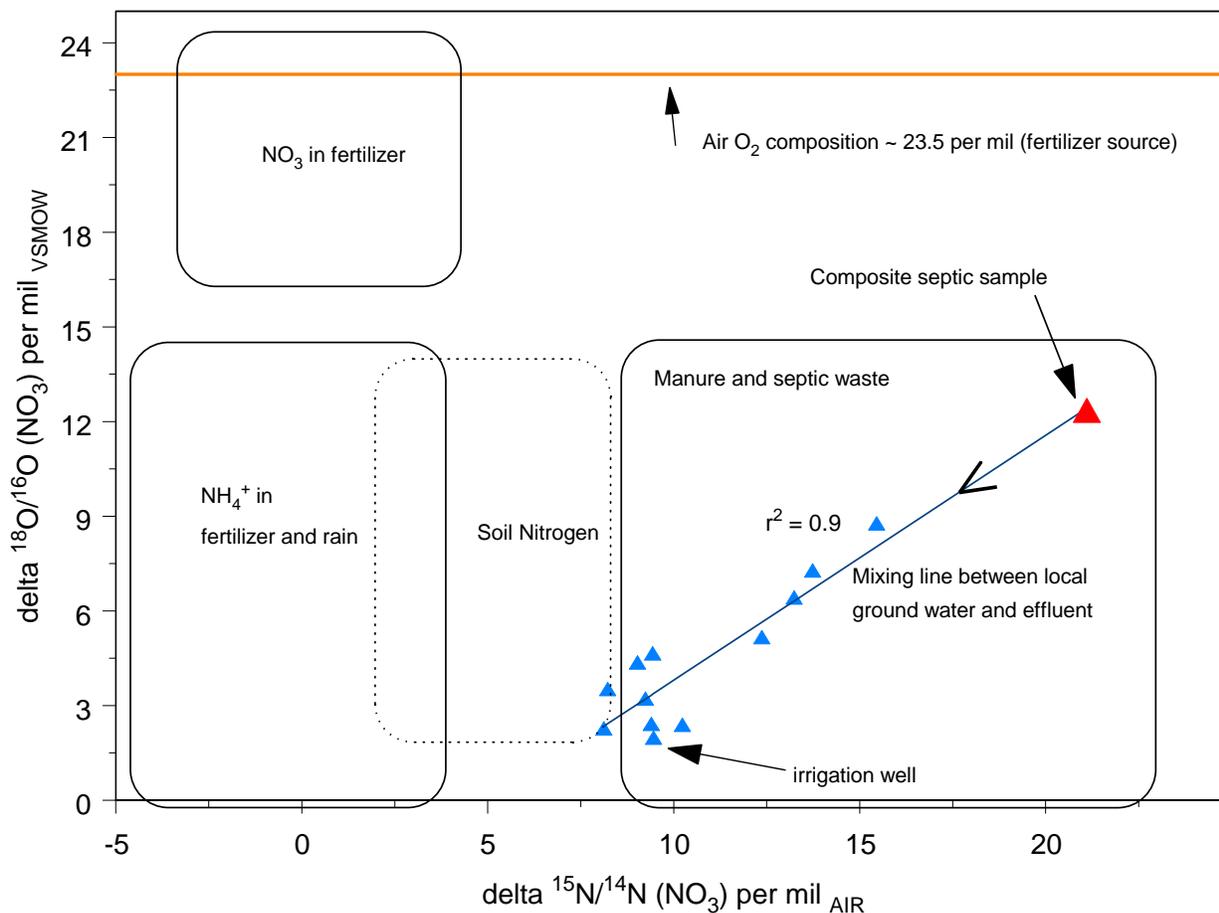


Figure 14. Oxygen and nitrogen isotope ratios.

The Phase 2 samples plot along a mixing line (lower right in Figure 14) and extend from an isotopic composition which clearly implicates the effluent sample as the source of $\delta^{15}\text{N}$ values for the study area samples. In addition, the very strong correlation ($r^2 = 0.9$) between the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of these samples is further evidence that the relationship is not coincidental. The correlation described in Figure 14 is evidence of a two-member mixing curve. The end-members of this mixing model are the nitrate composition of sewage effluent ($\delta^{15}\text{N}$ of +21.1‰ and $\delta^{18}\text{O}$ of +12.2‰) and that of the effluent-influenced local ground water ($\delta^{15}\text{N}$ of +8‰ and $\delta^{18}\text{O}$ of +3‰). The local ground water has a nitrate isotopic composition consistent with a soil-nitrogen source.

Wastewater Compounds

Wastewater compounds are chemicals in products used daily which can be released to the environment through discharges from industrial facilities, animal feed lots, wastewater treatment plants, individual household sewage treatment systems, or through runoff from land applications in agricultural and urban areas. Compounds include antioxidants, detergent metabolites, disinfectants, fire retardants, fragrances/flavors, insect repellants, prescription and nonprescription drugs, pesticides, plasticizers, solvents and steroids. A complete list can be found in Appendix E.

Samples for wastewater compound analysis were collected from 5 of the wells within the study area that have a higher probability of detecting sewage influenced ground water based on past sample results. Of the 69 compounds analyzed, only two compounds, metolachlor and caffeine, were detected in the 5 samples. The detected compounds were reported as estimated concentrations, which means that the compound was present but the concentration was too low to quantify accurately. Complete analytical results, including detection limits, for the samples are presented in the USGS report in Appendix E

Metolachlor was detected in all 5 well samples in concentrations below the laboratory reporting limit of 0.5 micrograms per liter ($\mu\text{g/L}$). The estimated concentrations of metolachlor ranged from 0.0102 to 0.1160 $\mu\text{g/L}$. Metolachlor is a herbicide and general use pesticide and has a high potential to contaminate ground water since it is relatively mobile and persistent. The local source of metolachlor may be agricultural or commercial lawn and garden care application but this cannot be determined with certainty.

Caffeine is a component of beverages, food products, and medications used specifically for human consumption. Caffeine was detected in only one sample (WSI-05) at an estimated level of 0.120 $\mu\text{g/L}$, below lab reporting limit of 0.5 $\mu\text{g/L}$. The presence of caffeine in ground water in the study area may suggest an impact from a wastewater-related source. While the caffeine concentration was estimated, the analytical results for many other compounds for this well suggest a wastewater impact. The nitrate concentrations are in excess of 9.0 mg/L, *E. coli* and fecal *Streptococci* were present, chloride results ranged between 60 - 70 mg/L and the bromide concentration, 0.18 mg/L, was the second highest value of all samples.

Microbiological Markers

Several microbial source tracking methods were used during Phase 2 sampling, including the presence of bacterial from the order *Bacteroidales* and DNA-markers associated with *Bacteroidales*. A complete description is provided in Appendix E. Water samples were tested for the presence of general fecal, human-associated and ruminant-associated *Bacteroidales* markers; only the fecal *Bacteroidales* marker was detected. This indicates contamination from a fecal-related source, the absence of human-

associated and ruminant-associated markers may indicate, among other possibilities, that the amount of contamination for these host specific species is below detection limits. Fecal bacteria of the order *Bacteroidales* are anaerobic bacteria and are expected to survive for relatively short periods in oxygen-rich waters. At 14°C, slightly higher than the observed temperature range of 11.3°C to 13.8°C *Bacteroidales* DNA lasts only 4 to 5 days; the presence of the general fecal *Bacteroidales* marker in ground water samples from the study area is highly indicative of recently recharged ground water (Dumouchelle, 2006).

Optical Brighteners

Optical Brighteners are fluorescent white dyes added to almost all laundry soaps and detergents. Cotton absorbs blue rays that are present in sunlight. When optical brighteners are applied to a cotton fabric, the fabric absorbs ultraviolet light from sunlight and releases blue light to give the fabric the appearance of being “whiter than white.” Because the main commercial use of these dyes is in laundry detergents and textile finishing, optical brightener dyes are generally found in domestic wastewater that has a component of laundry effluent. Optical brighteners can therefore enter the subsurface environment as a result of ineffective sewage treatment (Fay, Spong, and Alexander, 1995). Using an optical brightener analysis to indicate a wastewater impact in ground water has not been extensively utilized, but was attempted in this study in hopes to find a low cost tool to indicate wastewater impacts on ground water. Sampling for the presence of optical brighteners is most frequently used in surface water studies.

Samples collected from private water systems during Phase 1 and Phase 2 were negative for their presence of fluorescence; the sample collected from the common collector outfall was positive. This indicates optical brighteners are present in household sewage treatment system effluent but may not have been detected in ground water due sorption to waste solids or soils, dilution by native ground water or the limited sensitivity of the method.

Vertical Extent of Contamination

Figures 15 and 16 illustrate the relationship between the depth the well casing penetrates the saturated portion of the aquifer and the concentrations of two sewage-related contaminants, nitrate (Figure 15) and chloride (Figure 16). Its logical to assume that wells with casing that penetrates farther into the saturated portion of the aquifer draw in lower levels of contaminants. In a gross sense this appears to be true for nitrate; nitrate was not detected where the casing extends more than 40 feet into the aquifer. The elevated chloride concentrations, however, document the impact of sewage effluent in several wells with out detectable concentrations of nitrate. In the deeper portions of the aquifer the water has lower dissolved oxygen concentrations and nitrate is not stable while chloride, being a conservative ion, is present. This documents the impact of surface and near surface sources of contamination to a considerable depth into the saturated portion of the aquifer.

The scattered nature of both the nitrate and the chloride results in the upper 40 feet of the aquifer suggests the sources of contamination and transport pathways carrying contamination are variable. This is exactly what is expected in a relatively small area with individual household sewage treatment systems discharging into fractured bedrock and pumping wells drawing contaminated water along irregular fractures, allowing contamination to be drawn into private water system wells.

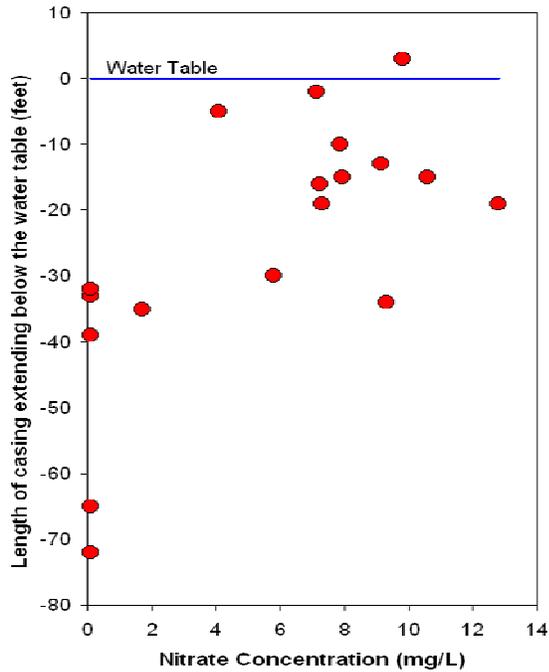


Figure 15. Nitrate concentrations and casing penetration into the local aquifer.

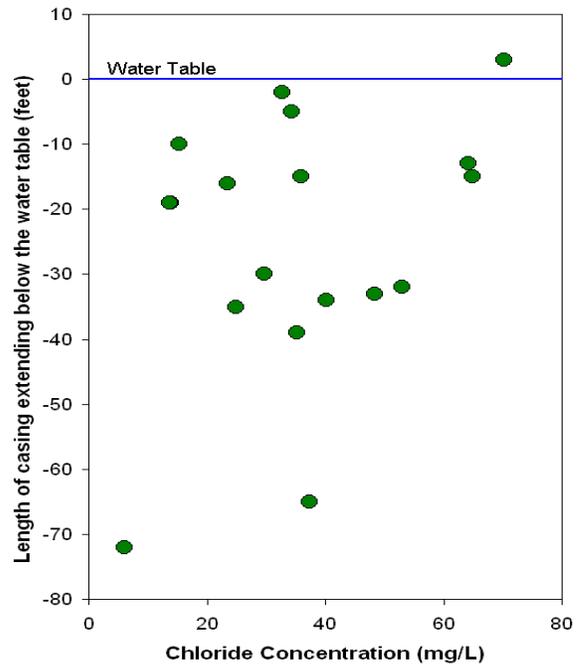


Figure 16. Chloride concentrations and casing penetration into the local aquifer.

Other Inorganic Compounds

Samples collected during Phase 1 were analyzed for several other inorganic compounds which provided information on either the general quality of local ground water or could indicate the presence of household sewage treatment effluent in ground water. These included nitrite, ammonia, total Kjeldahl nitrogen, total organic carbon, phosphorous, calcium, zinc and arsenic. The results of these analyses were not significant in determining the source of elevated nitrate or *E. coli* in ground water in the study area and are not discussed in detail in this report. The data for these compounds can be found in Appendixes C and D.

SUMMARY AND CONCLUSIONS

The geologic setting within the study area appears to be sensitive to ground water contamination due to the presence of thin or no glacial material and soil overlying a fractured bedrock aquifer resulting in rapid recharge of ground water. Phase 1 sampling duplicates the results of previous sampling and documents the continuing presence of water quality impacts within the study area. The variability and persistence of microbiological indicators and nitrate contamination is documented by the previous results and Phase 1 and 2 sampling for this study. Mapping the locations of these results demonstrates elevated nitrate concentrations and pathogen indicators are concentrated in the northern half of the study area, where the areas where glacial tills are thin or absent are most extensive.

It was suspected that rapid recharge impacted by household sewage treatment system effluent was a significant source of the water quality impacts, so Phase 2 sampling was designed to look for indicators of rapid recharge and sewage effluent. Ground water sampling results included unusually high dissolved oxygen concentrations, elevated nitrate concentrations, the presence of fecal *Bacteroidales* markers and detections of *E. coli*, all of which provide strong evidence for rapid recharge. In addition, the fecal *Bacteroidales* markers and detections of *E. coli* support the presence of fecal contamination.

Chloride/bromide ratios document that sewage waste is present in the ground water as illustrated in Figure 11. The direct correlation of nitrate concentration to dissolved oxygen and the association of elevated bromide with elevated nitrate as demonstrated in Figure 8 suggest these elevated concentrations result from a single process – most likely rapid recharge of sewage-related waste to the ground water which is then captured by local wells. The nitrogen and oxygen isotope data also provide convincing evidence for the inclusion of sewage or manure derived nitrate in the ground water with a strong correlation to the household sewage treatment system composite sample as shown on Figure 14.

The observed nitrate isotope compositions, in addition to bromide, nitrate and chloride data, indicate that the water wells within the study area are drawing water which is composed of, to varying degrees, a mixture of local ground water and a diluted sewage effluent. This mixture appears, in varying proportions, across the study area.

The erratic presence of bacteria, nitrate, and other effluent indicators is to be expected, given the unpredictable nature of fracture flow volume, direction of ground water flow, the timing of contaminant discharges, the differences in depth to the top of the bedrock surface across the area, the variable thickness of the of the soils and glacial tills (the barrier to contaminant transport), the varied spatial relationships between each home's leach field and water well and to those of it's neighbors and the timing of recharge events. All of these factors complicate the extent to which one household sewage treatment system might influence the capture zone of any single well water well.

These results provide several lines of evidence which indicate that sewage effluent is a major source of nitrate and pathogen contamination of the local ground water. Historic nitrate and microbiological data indicate continuing input from a source related to human activities. Nitrate concentration in excess of 10 mg/L indicate the source of the contaminants are from sewage effluent. This conclusion is supported by analysis of the bromide and chloride data and the oxygen and nitrogen isotope data, both of which indicate a sewage-related source for the contaminants. The presence of caffeine, even at extremely low levels, can only be attributed to impacts from a sewage source. These lines of evidence are summarized in Table 7; orange-shaded boxes indicate evidence of human impacts and red-shaded boxes indicate sewage-related impacts.

Table 7. Chemical and microbiological contaminant source indicators for locations sampled during Phases 1 and 2.

Site ID	Biological Indicators							Chemical Indicators				
	<i>E. coli</i>		Fecal coliform	Fecal Strep.	<i>Bacteroidales</i>			Nitrate		Wastewater Compounds*	Bromide/ Chloride	Isotopes
	Apr.	Jun.			Fecal	Human	Ruminant	Apr.	Jun.			
WSI-02	-	(+)	-	-	n/a	n/a	n/a	>10	>10	n/a	(+)	(+)
WSI-03	-	-	-	-	-	n/a	n/a	3 - 10	3 - 10	n/a	(+)	(+)
WSI-05	-	(+)	-	(+)	(+)	-	-	3 - 10	3 - 10	M, C	(+)	(+)
WSI-06	-	-	-	-	n/a	n/a	n/a	3 - 10	<3	n/a	(+)	(+)
WSI-07	-	-	-	-	-	n/a	n/a	3 - 10	<3	n/a	(+)	(+)
WSI-12	(+)	(+)	(+)	-	(+)	-	-	3 - 10	3 - 10	M	(+)	(+)
WSI-20	-	(+)	-	-	-	n/a	n/a	>10	>10	M	(+)	(+)
WSI-21	-	(+)	-	-	n/a	n/a	n/a	>10	>10	n/a	(+)	(+)
WSI-22	(+)	(+)	-	-	(+)	-	-	3 - 10	3 - 10	M	(+)	(+)
WSI-23	-	-	-	-	n/a	n/a	n/a	3 - 10	3 - 10	n/a	(+)	(+)
WSI-26	-	-	-	-	n/a	n/a	n/a	3 - 10	3 - 10	n/a	(+)	(+)
WSI-30	(+)	(+)	(+)	-	(+)	-	-	3 - 10	3 - 10	M	(+)	(+)

* M = metolachlor; C = caffeine.
n/a = analyses not performed.

Due to the complex nature of fractured bedrock aquifers and the multiple local sources of household sewage treatment system effluent in the study area, the results do not point to specific household sewage treatment systems as the source of contamination. Even with these hydrologic and geologic complications, the geochemical and isotopic data give significant support to the conceptual model of a local ground water mixing, in varying proportions, with diluted effluent from household sewage treatment systems. Therefore, this study does not identify individual household sewage treatment systems as the cause for the unsafe water supply. However, the results from the sampling and understanding of the hydrogeologic conditions in the study area suggest the household wastewater treatment systems installed into or just above bedrock result in a greater impact on the water quality of the aquifer than those systems with thicker soil material, which provides more treatment of the effluent.

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Base Maps

United States Geologic Survey, 1994, Holmesville 7.5 minute quadrangle topographic map

United States Geologic Survey, 1994, Wooster 7.5 minute quadrangle topographic map

United States Department of Agriculture Farm Service Agency Aerial Photography Field Office, 2004, National Aerial Imagery Program photo

Appendix A

Summary of Study Area Well Logs

Table A-1. Summaries of well logs for the study area.

Well Log Number	Year Completed	Surface Elevation	Static Water Level		Total Depth	Depth to Bedrock	Aquifer Material	Casing		Installation Method
			Depth (below surface)	Elevation				Length	Material	
739226	1991	910	16	894	120	50	Unknown	51	Unknown	Unknown
776984	1994	985	55	930	116	3	Sandstone	52	Steel	Cable
796675	1994	1025	86	939	140	31	Sandstone	106	PVC	Rotary
802907	1994	1030	102	928	150	0	Shale	120	PVC	Rotary
805625	1996	987	57	930	135	18	Shale	79	Unknown	Unknown
814823	1995	1020	78	942	110	4	Sandstone	98	PVC	Rotary
829133	1996	1020	88	932	150	6	Shale	119	Unknown	Unknown
839560	1996	1000	81	919	105	5	Sandstone	84	Unknown	Unknown
839585	1996	992	66	926	100	14	Shale	79	Unknown	Unknown
847371	1998	935	30	905	125	32	Sandstone	103	Steel	Cable
852343	1997	947	32	915	85	19	Sandstone	66	PVC	Rotary
887029	1998	985	60	925	118	2	Sandstone	66	PVC	Rotary
896144	1999	962	37	925	140	7	Shale	103	PVC	Cable
898332	1999	980	55	925	105	18	Shale	88	PVC	Rotary
913679	2000	939	20	919	90	55	Unknown	60	Steel	Cable
934811	2001	1030	96	934	157	6	Shale	107	PVC	Rotary
938219	2001	952	38	914	90	19	Sandstone	71	Unknown	Unknown
955325	2003	1039	100	939	140	18	Siltstone	116	Unknown	Unknown

Appendix B

**Analytical Results
Ohio Department of Health Investigation
May and November 2004**

Table B-1. Sampling results for May 2004.

Sample ID	Total coliform	<i>E. coli</i>	Nitrate (mg/L)	Ammonia (mg/L)	Chloride (mg/L)
ODH-01	Negative	Negative	0.71	< 0.050	54.9
ODH-02	Negative	Negative	3.98	< 0.050	51.8
ODH-03	Positive	Negative	13.2	< 0.050	12.3
ODH-04	Positive	Negative	13.2	< 0.050	18.7
ODH-05	Negative	Negative	2.77	< 0.050	33.6
ODH-06	Positive	Negative	11.2	< 0.050	33.1
ODH-07	Positive	Negative	1.75	< 0.050	19.5
ODH-08	Positive	Negative	13.2	< 0.050	12.9
ODH-09	Positive	Positive	15.6	< 0.050	12.5
ODH-10	Positive	Negative	7.46	< 0.050	22.2
ODH-12	Positive	Positive	13.2	< 0.050	75.9
ODH-13	Positive	Positive	11.5	< 0.050	68.8
ODH-14	Positive	Negative	8.5	0.078	20.1
ODH-15	Positive	Negative	10.9	< 0.050	21.6
ODH-16	Negative	Negative	< 0.10	0.078	25.9
ODH-17	Positive	Positive	7.46	< 0.050	38.6
ODH-18	Positive	Positive	8.85	< 0.050	42.7
ODH-19	Positive	Positive	11	< 0.050	66.6
ODH-20	Positive	Positive	9.09	< 0.050	51.4
ODH-21	Positive	Positive	5.61	< 0.050	42.8
ODH-22	Positive	Negative	0.8	0.055	38.2
ODH-23	Negative	Negative	6.93	< 0.050	47.4
ODH-24	Positive	Negative	< 0.10	0.052	45.1
ODH-25	Negative	Negative	0.12	0.051	37.6
ODH-26	Negative	Negative	0.1	0.05	38.8
ODH-27	Negative	Negative	< 0.10	0.134	31.7

Table B-2. Sampling results for November 2004.

Sample ID	Total coliform	<i>E. coli</i>	Nitrate mg/L	Chloride mg/L	Ammonia mg/L
ODH-01	Negative	Negative	0.1	50	< 0.050
ODH-02	Negative	Negative	< 0.10	58.4	< 0.050
ODH-03	Negative	Negative	9.21	14.9	< 0.050
ODH-04	Negative	Negative	9.94	33.3	< 0.050
ODH-05	Negative	Negative	2.23	33.4	< 0.050
ODH-06	Positive	Negative	6.29	35	< 0.050
ODH-07	Positive	Negative	1.97	21.6	< 0.050
ODH-08	Positive	Positive	16.2	17.4	< 0.050
ODH-09	Positive	Negative	16.4	17.2	< 0.050
ODH-10	Positive	Negative	8.34	27.3	< 0.050
ODH-11	Positive	Negative	16.9	31.7	< 0.050
ODH-12	Positive	Positive	9.92	58.8	< 0.050
ODH-13	Positive	Positive	9.02	51.8	< 0.050
ODH-14	Negative	Negative	7.94	31.1	< 0.050
ODH-15	Negative	Negative	8.58	76.2	< 0.050
ODH-16	Negative	Negative	< 0.10	25.4	0.062
ODH-17	Positive	Negative	1.36	30.8	< 0.050
ODH-18	Negative	Negative	9.12	60.6	< 0.050
ODH-19	Positive	Positive	8.05	50.3	< 0.050
ODH-20	Negative	Negative	3.08	30	< 0.050
ODH-22	Negative	Negative	< 0.10	32.4	< 0.050
ODH-23	Positive	Negative	10.4	43.7	< 0.050
ODH-23	Negative	Negative	< 0.10	34	< 0.050
ODH-24	Negative	Negative	< 0.10	42	< 0.050
ODH-26	Positive	Negative	< 0.10	38.2	< 0.050
ODH-27	Negative	Negative	< 0.10	31.2	0.052

Appendix C

Phase 1 Investigation Summary and Results April 2006

Phase 1 Sampling

Phase 1 sampling was conducted by five teams from DDAGW, ODH and WCHD; each team included at least one DDAGW staff member. Samples from 36 private water systems and the outfall for the common collector system were collected. The basic sampling pattern was to purge, decontaminate the sample tap, re-purge, record field measurements, and then collect samples. Field parameters were collected using calibrated meters and included temperature, pH, conductivity, oxidation/reduction potential, dissolved oxygen and total dissolved solids. Colorimetric nitrate and chlorine test strips were used at each private water system. These procedures were based on standard ODH and DDAGW procedures. Samples were collected from outside taps where possible; inside taps included a kitchen sink, treatment bypasses and pressure tanks were also used. Figure C-1 shows the locations of the private water systems and the common collector outfall sampled during Phase 1.

At each site four one quart samples were collected in disposable cubitainers – two un-preserved samples, one preserved with nitric acid and one preserved with sulfuric acid. A 100 mL sample was collected in a clear plastic container with sodium thiosulfate (to counteract residual chlorine) and a 120 mL sample were collected for microbiological analysis. One un-preserved sample was placed in a box and out of the sunlight for use in determining the presence of optical brighteners. The remaining samples were placed on ice in a cooler. One field blank sample and four sets of duplicate samples were collected during Phase 1 sampling for quality control (QA/QC).

All samples were analyzed using standard US EPA methods. Optical brightener samples were screened using a long wave (365 nanometer) 4-watt ultraviolet light.

Summary of Results

The following sections summarize some of the results of Phase 1 sampling and note the importance of some of these compounds not discussed in the Unsafe Water Supply Investigation Report. Complete field and analytical data can be found in Tables C-3 through C-8.

Nutrients

During Phase 1 a total of 37 samples from were analyzed for nitrate, nitrite, ammonia, total Kjeldahl nitrogen (a measure of total ammonia and organic nitrogen). Thirty-one samples were collected within the study area, five were collected outside the study area as background samples and one was collected from the common collector tile outfall. Nitrate was detected in 28 samples at concentrations ranging from <0.1 to 12.8 mg/L. Four samples exceeded the MCL of 10 mg/L. Nitrite was not reported for any sample. Ammonia concentrations ranged from <0.05 to 0.489 mg/L and was detected in 5 samples. Total Kjeldahl nitrogen concentrations ranged from <0.2 to 1.1 mg/L and was detected in 21 samples. Phosphorous concentrations ranged from <0.01 to 0.047 mg/L and was detected in four samples. The sample collected at the outfall from the common

collector tile contained nitrate at 8.74 mg/L, ammonia at 6.3 mg/L, total Kjeldahl nitrogen at 10 mg/L, and phosphorus at 8.93 mg/L.

Microbiological Indicators

In Phase 1, DDAGW collected 36 samples for total coliform bacteria, *E. coli*, fecal coliform bacteria and fecal streptococci plate counts. Testing for total coliforms and *E. coli* only provide information on presence or absence of the bacteria; samples from 16 locations (52 percent) within the study area were positive for the presence of total coliform bacteria and three were positive for the presence of *E. coli*. Thirty-six samples were collected for quantification of fecal coliforms and fecal streptococci; one was lost in transit so only 35 were analyzed. Fecal coliforms were present in two samples (at 30 and 10 colony forming units per 100 mL [cfu/100 mL]); fecal streptococci at were present at one location at 260 cfu/100 mL. Total coliforms, *E. coli*, fecal coliforms and fecal streptococci were not detected in any of the background samples. The samples collected at the collector outfall were positive for the presence of total coliforms and *E. coli*; fecal coliforms were present at 270 cfu/100 mL and fecal streptococci at were present at 1,700 cfu/100 mL.

Total Organic Carbon

Total organic carbon measures the amount of carbon available in organic forms and is a general

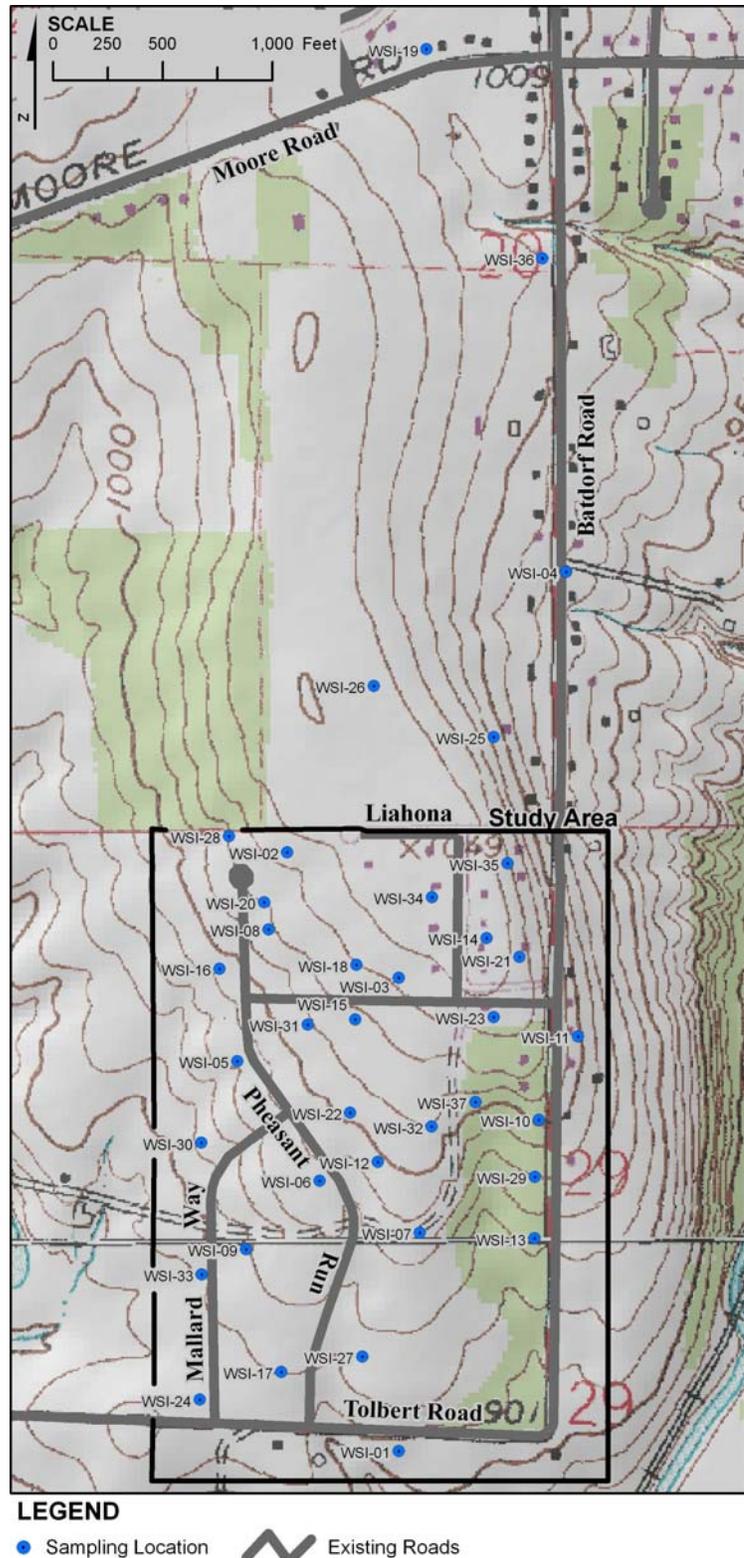


Figure C-1. Phase 1 sampling locations.

measure of water quality. Total organic carbon was not reported for any sample collected from the background or study area wells, including the QA/QC samples. Total organic carbon was detected at 18 mg/L in the effluent from the common collector tile.

Chloride

Chloride is present in all natural waters, but concentrations are generally low; concentrations in precipitation inland from an ocean are generally less than 1 mg/L (Hem, 1989), concentrations in recently recharged ground water are around 5 mg/L. A Secondary MCL of 250 mg/L has been established for chloride; a secondary MCL is established only as a guideline to assist public water systems in managing their drinking water for aesthetic considerations, such as taste, color and odor. Compounds with a Secondary MCL are not considered to present a risk to human health at those concentrations.

Arsenic

Arsenic is a common, naturally occurring element in the earth's crust. Most arsenic found in Ohio's ground water is naturally occurring. The current MCL for arsenic for public water systems is 0.010 mg/L or 10 micrograms per liter ($\mu\text{g/L}$). DDAGW did not expect to find arsenic present near the MCL; samples were analyzed for arsenic as a part of the standard suite of analyses for ground water. Of the 31 study and five background samples collected arsenic was present in three samples collected within the study area; at 2.0, 3.0 and 3.9 $\mu\text{g/L}$. Arsenic was not detected at the collector tile outfall. The results for the duplicate samples were within appropriate ranges.

Zinc

Zinc is a common metal used in galvanized steel, in alloys such as brass, as a pigment in watercolors or paints and in many commercial and industrial applications Zinc is also used as a sun block (zinc oxide), in calamine lotion and is an essential dietary nutrient. The presence of zinc in ground water may indicate impact from human activities. Background zinc levels may be derived from other sources. Zinc levels were obtained from ambient wells (DDAGW, 2006) ranged from <10.0 to 1600 $\mu\text{g/L}$ (Table C-1). In this study zinc was only observed to occur with nitrate; not all samples with nitrate contain zinc but all zinc occurrences coincide with nitrate above 5 mg/L (Table C-2). The degree to which sewage effluent affects zinc concentration cannot be directly determined from this data. For example the sampling point for WSI-26 was a relatively new brass fitting, which may be the source of zinc in this sample. However the presence of zinc with nitrate indicates the potential for a common source.

Table C-1. Zinc concentrations in µg/L for Ohio sandstone aquifers and in Phase 1 samples. Data for zinc concentrations in sandstone from DDAGW (2006)

Aquifer Media	Data source	Max.	Min.	Mean	Median	Total Samples	Number Below Reporting Limit
Sandstone	Ambient	1600	< 10.0	31.4	12.0	636	288
Interbedded sandstone, siltstone and shale	Study Area (Phase 1)	51	< 10.0	12.4	16	31	25
	Study Background (Phase 1)	14	< 10.0	10.8	10	5	4

Table C-2. Zinc and nitrate concentrations for Phase 1 samples where zinc is present.

Area	Rpt ID	Zinc (ug/L)	Nitrate (mg/L)
Study Area	WSI-11	14	7.19
	WSI-12	16	9.13
	WSI-20	11	11.2
	WSI-22	51	9.3
	WSI-32	26	9.78
	WSI-35	17	8.69
	WSI-37	27	8.74
Background	WSI-26	14	5.75

References Cited

DDAGW, 2006d, 2006 305(b) Report - Ohio's Ground Water Quality, Ohio Environmental Protection Agency, 93 p.

Hem, J.D., 1989, *Study and Interpretation of the Chemical Characteristics of Natural Water* (3rd ed.): U.S. Geological Survey Water-Supply Paper 2254, 263 p.

Table C-3. Sampling results for the study area - April 2006.

Site ID#	Total coliform	<i>E. coli</i>	Fecal coliform	Fecal Strep.	Ammonia	Nitrate	TKN	Total Phosphorous	TDS	TOC	Arsenic	Calcium	Sodium	Zinc	Chloride
Laboratory Reporting Limit	n/a	n/a	10 cfu/100 mL		0.05 mg/L	0.1 mg/L	0.2 mg/L	0.01 mg/L	10 mg/L	2 mg/L	2 ug/L	2 mg/L	5 mg/L	10 ug/L	5 mg/L
WSI-01	-	-	<10	<10	<0.05	1.72	<0.2	<0.01	222	<2	<2	46	5	<10	24.9
WSI-02	(+)	-	<10	<10	<0.05	10.6	<0.2	<0.01	186	<2	<2	34	<5	<10	13.9
WSI-03	(+)	-	<10	<10	<0.05	7.94	<0.2	<0.01	234	<2	<2	45	6	<10	27.9
WSI-05	(+)	-	<10	260	<0.05	9.82	0.47	<0.01	306	<2	<2	41	19	<10	70.2
WSI-06	(+)	-	<10	<10	<0.05	8.34	1.1	0.047	268	<2	<2	45	9	<10	40.1
WSI-07	(+)	-	<10	<10	<0.05	4.1	0.2	0.012	190	<2	<2	35	11	<10	34.3
WSI-08	(+)	-	<10	<10	<0.05	12.8	0.25	0.024	140	<2	<2	25	<5	<10	13.7
WSI-09	-	-	10	<10	<0.05	<0.1	0.33	0.014	298	<2	3.9	62	14	<10	48.3
WSI-10	-	-	<10	<10	<0.05	7.12	0.51	<0.01	194	<2	<2	28	10	<10	32.8
WSI-11	(+)	-	<10	<10	<0.05	7.19	0.23	<0.01	326	<2	<2	62	10	14	45.7
WSI-12	(+)	(+)	30	<10	<0.05	9.13	0.56	<0.01	240	<2	<2	30	16	16	52.9
WSI-13	(+)	-	n/a	n/a	<0.05	<0.1	<0.2	<0.01	298	<2	<2	<2	65	<10	37.4
WSI-14	-	-	<10	<10	<0.05	7.07	0.27	<0.01	346	<2	<2	69	11	<10	48.7
WSI-15	(+)	-	<10	<10	<0.05	5.78	0.32	<0.01	216	<2	<2	46	5	<10	29.6
WSI-16	-	-	<10	<10	<0.05	5	<0.2	<0.01	172	<2	<2	31	<5	<10	24
WSI-17	-	-	<10	<10	0.079	<0.1	0.28	<0.01	304	<2	2.7	59	12	<10	38.3
WSI-18	-	-	<10	<10	<0.05	7.87	<0.2	<0.01	208	<2	<2	34	<5	<10	15.2
WSI-20	(+)	-	<10	<10	<0.05	11.2	<0.2	<0.01	180	<2	<2	27	<5	11	13.7
WSI-21	(+)	-	<10	<10	<0.05	12.6	0.3	<0.01	272	<2	<2	42	7	<10	36.7
WSI-22	(+)	(+)	<10	<10	<0.05	9.3	0.5	<0.01	288	<2	<2	37	19	51	64.1
WSI-23	-	-	<10	<10	<0.05	5.26	0.81	<0.01	340	<2	<2	71	12	<10	48.7
WSI-24	-	-	<10	<10	0.371	<0.1	0.36	0.025	200	<2	<2	26	32	<10	6
WSI-27	-	-	<10	<10	0.07	<0.1	0.21	<0.01	308	<2	3	59	12	<10	35.9
WSI-28	-	-	<10	<10	<0.05	7.31	<0.2	<0.01	188	<2	<2	40	5	<10	15.2
WSI-29	-	-	<10	<10	0.065	<0.1	<0.2	<0.01	296	<2	<2	57	15	<10	28.7
WSI-30	(+)	(+)	10	<10	<0.05	8.82	0.51	<0.01	302	<2	<2	39	17	<10	64.9
WSI-31	-	-	<10	<10	<0.05	7.25	<0.2	0.011	196	<2	<2	35	5	<10	23.4
WSI-32	(+)	-	<10	<10	<0.05	9.78	0.29	<0.01	230	<2	<2	28	12	26	38.7
WSI-33	-	-	<10	<10	<0.05	<0.1	<0.2	0.012	262	<2	<2	<2	103	<10	35.2
WSI-34	-	-	<10	<10	<0.05	6.41	0.28	<0.01	344	<2	<2	66	10	<10	46.4
WSI-35	-	-	<10	<10	<0.05	8.69	<0.2	<0.01	292	<2	<2	58	8	17	26.4

Table C-4. Background sampling results - April 2006.

Site ID#	Total coliform	<i>E. coli</i>	Fecal coliform	Fecal Strep.	Ammonia	Nitrate	TKN	Total Phosphorous	TDS	TOC	Arsenic	Calcium	Sodium	Zinc	Chloride
Laboratory Reporting Limit	n/a	n/a	10 cfu/100 mL		0.05 mg/L	0.1 mg/L	0.2 mg/L	0.01 mg/L	10 mg/L	2 mg/L	2 ug/L	2 mg/L	5 mg/L	10 ug/L	5 mg/L
WSI-04	-	-	<10	<10	0.489	<0.1	0.65	0.018	344	<2	<2	45	53	<10	39.9
WSI-19	-	-	<10	<10	<0.05	0.39	0.44	<0.01	484	<2	<2	107	16	<10	66.4
WSI-25	-	-	<10	<10	<0.05	7.07	<0.2	<0.01	250	<2	<2	57	10	<10	33.4
WSI-26	-	-	<10	<10	<0.05	5.75	0.31	<0.01	374	<2	<2	76	14	14	52.5
WSI-36	-	-	<10	<10	<0.05	3.09	<0.2	<0.01	334	<2	<2	74	8	<10	32.1

Table C-5. Sampling results for the Lauraland common collector outfall - April 2006.

Site ID#	Total coliform	<i>E. coli</i>	Fecal coliform	Fecal Strep.	Ammonia	Nitrate	TKN	Total Phosphorous	TDS	TOC	Arsenic	Calcium	Sodium	Zinc	Chloride
Laboratory Reporting Limit	n/a	n/a	10 cfu/100 mL		0.05 mg/L	0.1 mg/L	0.2 mg/L	0.01 mg/L	10 mg/L	2 mg/L	2 ug/L	2 mg/L	5 mg/L	10 ug/L	5 mg/L
WSI-37	(+)	(+)	270	1700	6.3	8.74	10	8.93	554	18	<2	49	97	27	106

Table C-6. QA/QC results - April 2006.

Site ID#	Total coliform	<i>E. coli</i>	Fecal coliform	Fecal Strep.	Ammonia	Nitrate	TKN	Total Phosphorous	TDS	TOC	Arsenic	Calcium	Sodium	Zinc	Chloride
Laboratory Reporting Limit	n/a	n/a	10 cfu/100 mL		0.05 mg/L	0.1 mg/L	0.2 mg/L	0.01 mg/L	10 mg/L	2 mg/L	2 ug/L	2 mg/L	5 mg/L	10 ug/L	5 mg/L
Blank	-	-	<10	<10	<0.05	<0.1	<0.2	<0.01	<10	<2	<2	<2	<5	<10	<5
WSI-09	-	-	<10	<10	<0.05	<0.1	0.27	0.016	306	<2	3.6	62	14	<10	48.2
WSI-20	(+)	-	<10	<10	<0.05	11.2	<0.2	0.01	182	<2	<2	27	<5	<10	13.7
WSI-22	(+)	-	<10	<10	<0.05	8.91	0.35	<0.01	288	<2	<2	38	19	40	63.8
WSI-29	-	-	<10	<10	0.065	<0.1	<0.2	<0.01	296	<2	<2	57	16	<10	28.6

Table C-7. Field parameters for the study area - April 2006.

Site ID#	Location	Colorimetric Strips		Temperature		Conductivity (umhos/cm)	pH	Oxidation Reduction Potential	TDS (mg/L)	Dissolved Oxygen (mg/L)
		Chlorine	Nitrate	Initial	Final					
WSI-01	Outside Tap	0	0	14.4	13	379	7.3	140	255	n/a
WSI-02	Outside Tap	0	3	n/a	11.3	314	6.6	244	146	n/a
WSI-03	Prior to Pressure Tank	0	4	11.2	11.8	408	6.8	n/a	n/a	9.7
WSI-05	Outside Tap	0	3	14.5	13.4	118	7	215	304	n/a
WSI-06	Outside Tap	0	0	14.4	12.7	430	7.4	1.95	291	n/a
WSI-07	Outside Tap	0	2	14.9	n/a	346	7.4	n/a	n/a	5.2
WSI-08	Outside Tap	0	5+	n/a	11.7	271	6.2	245	125	n/a
WSI-09	Outside Tap	0	0	12.7	11.9	546	7.8	n/a	n/a	4.6
WSI-10	Outside Tap	0	3	13.2	12.2	344	7.3	n/a	n/a	7.4
WSI-11	Outside Tap	0.5	2	12.5	12.1	558	7	n/a	n/a	n/a
WSI-12	Outside Tap	0	2.5	14.4	12.7	372	6.5	206	251	n/a
WSI-13	Outside Tap	0	0	14.1	n/a	487	7.3	n/a	n/a	2
WSI-14	Outside Tap	0	1.5	15.2	11.8	577	7	n/a	n/a	6.97
WSI-15	Outside Tap	0	3	11.9	11.7	392	6.9	n/a	n/a	5.81
WSI-16	Outside Tap	0	1	n/a	11.7	305	7.1	138	142	n/a
WSI-17	Outside rear	0	0	12.2	11.8	504	7.5	1	343	n/a
WSI-18	Prior to Pressure Tank	0	5	11.8	12	318	6.7	n/a	n/a	7.82
WSI-20	Pressure Tank	0	10	n/a	11.8	366	6.4	n/a	n/a	9.32
WSI-21	Outside Tap	0	10	13.1	12.5	470	7.2	n/a	n/a	n/a
WSI-22	Outside tap	0	2.5	15.5	13.8	447	6.7	224	302	n/a
WSI-23	Outside Tap	0	2	12.7	11.5	576	7	n/a	n/a	7.66
WSI-24	Outside Tap	0	4	12.5	n/a	428	7.7	n/a	n/a	9.3
WSI-27	Outside Tap	0	0	12.4	12	494	7.4	70	336	n/a
WSI-28	Outside Tap	0		15.1	11.5	336	6.9	200	156	n/a
WSI-29	Outside Tap	0	0	14.6	n/a	507	7.5	n/a	n/a	5
WSI-30	Outside Tap	0	0	13	12.5	346	8	n/a	n/a	3.91
WSI-31	Outside Tap	0	1	12.6	12	325	6.9	111	151	n/a
WSI-32	Pressure Tank	0	5	12.2	12.1	358	6.5	n/a	n/a	n/a
WSI-33	Outside Tap	0	0	14.2	12.4	446	7.9	n/a	n/a	3.9
WSI-34	Outside Tap	0	4+	11.7	11.5	542	7	n/a	n/a	8.97
WSI-35	Outside Tap	0	2	11.9	11.9	504	6.9	n/a	n/a	6.69

Table C-8. Field parameters for background samples - April 2006.

Site ID#	Location	Colorometric Strips		Temperature		Conductivity (umhos/cm)	pH	Oxidation Reduction Potential	TDS (mg/L)	Dissolved Oxygen (mg/L)
		Chlorine	Nitrate	Initial	Final					
WSI-04	Outside Tap	0	0	13.5	12.9	634	7.3	n/a	n/a	n/a
WSI-19	Outside Tap	0.5	0	12.7	12.8	817	7.1	n/a	n/a	n/a
WSI-25	Outside Tap	0	5+	n/a	12	490	7.1	216	230	n/a
WSI-26	Tap at Wellhead	0	2	11.7	11.6	617	7.1	n/a	n/a	n/a
WSI-36	Kitchen faucet	0	2	16	13.1	302	7.1	n/a	n/a	n/a

Appendix D

Phase 2 Investigation Summary and Results June 2006

Phase 2 Sampling

Phase 2 sampling involved one team from USGS and DDAGW collecting all samples following USGS and DDAGW procedures. USGS monitored field parameters; temperature, pH, conductivity and dissolved oxygen. At sites where a wastewater compound sample was collected it was the first to be collected. Afterward any other filtered samples – bromide (USGS), chloride and oxygen/nitrogen isotopes (DDAGW) – were collected. One unfiltered sample was collected in a one quart cubitainer preserved with sulfuric acid. Prior to collecting microbiology samples the sampling tap was decontaminated; microbiological samples were then collected – a 100 mL was collected at all locations and a 3 liter sample was collected for DNA marker analysis where indicated. Household sewage treatment system effluent samples were collected and five locations using a peristaltic pump. One 60 mL sample was collected at each location, composited in a cubitainer and decanted into one 60 mL for oxygen/nitrogen isotope analysis. USGS prepared a 3 liter composite sample for DNA marker analysis. Optical brightener sampling was conducted by placing a plastic mesh cage holding an untreated cotton pad in a toilet reservoir tank at six of the homes. The pads were collected by the Wayne County Health Department after at least one week. DDAGW did not collect a field blank sample but did collect a duplicate of all sample types except optical brighteners and oxygen/nitrogen isotopes at one location. All samples were analyzed using standard US EPA and USGS methods. Optical brightener samples were screened using a long wave (365 nanometer) 4-watt ultraviolet light.

Summary of Results

The following sections summarize some of the results of Phase 2 sampling and additional information not discussed in the Unsafe Water Supply Investigation Report. Complete field and analytical data can be found in Tables D-1 through D-10.

Optical Brighteners

Sampling during Phase 2 was designed to increase the volume of water the sampling pads were exposed to through long term placement in toilet tanks. The results for this round were inconclusive - they did not fluoresce. The concentration of optical brighteners in ground water may be too low to detect with the limited exposure time used in this study and the limited sensitivity of the method.

Table D-1. Field parameters and inorganic sampling results - June 2006.

Site ID	Collection Date	Temperature (°C)	pH	Conductivity (umhos/cm)	Dissolved Oxygen		Ammonia (mg/L)	Chloride (mg/L)	Nitrate (mg/L)	Bromide (mg/L)	Wastewater Compounds
					mg/L	Percent Saturation					
WSI-02	06/21/06	11.34	6.49	298	6.77	61.8	< 0.05	13	10.9	0.09	n/a
WSI-03	06/19/06	12.91	6.84	422	4.95	42.9	< 0.05	44.5	5.08	0.05	n/a
WSI-05	06/19/06	13.87	6.45	464	6.51	63.3	< 0.05	61.4	9.24 ¹	0.18	Metolachlor ² Caffeine ²
WSI-06	06/20/06	13.46	6.98	466	2.25	21.6	< 0.05	37.1	2.42	0.06	n/a
WSI-07	06/21/06	12.19	6.9	430	1.26	11.8	< 0.05	39.7	2.34	0.07	n/a
WSI-12	06/20/06	12.59	6.51	355	4.04	38.3	< 0.05	46.8	5.88	0.08	Metolachlor ²
WSI-20	06/20/06	13.05	6.25	263	7.65	72.7	< 0.05	12.6	11.3	0.16	Metolachlor ²
WSI-21	06/21/06	13.04	7.03	485	9.95	89.9	< 0.05	43.6	11.9	0.19	n/a
WSI-22	06/20/06	13.21	6.46	361	6.73	64.1	< 0.05	37.2	8.66	0.12	Metolachlor ²
WSI-23	06/19/06	12.84	6.98	554	4.96	46.9	< 0.05	28	6.5	0.05	n/a
WSI-26	06/19/06	12.67	6.99	616	7.15	67.5	< 0.05	53.2	6.38	0.04	n/a
WSI-30	06/19/06	13.1	6.26	417	8.56	81.6	< 0.05	59.2	9.87	0.14	Metolachlor ²

1-Nitrate value estimated - matrix interference

2-Present below reporting limit

Table D-2. Field parameters and inorganic sampling results for QA/QC samples - June 2006.

Site ID	Collection Date	Temperature (°C)	pH	Conductivity (umhos/cm)	Dissolved Oxygen		Ammonia (mg/L)	Chloride (mg/L)	Nitrate (mg/L)	Bromide (mg/L)	Wastewater Compounds
					mg/L	Percent Saturation					
WSI-20	06/20/06	13.05	6.25	263	7.65	72.7	< 0.05	61.6	9.15	n/a	n/a
WSI-05	06/19/06	13.87	6.45	464	6.51	63.3	n/a	n/a	n/a	n/a	n/a

Table D-3. Microbiological indicator sampling results for study area samples - June 2006.

Site ID	Date	Total coliform	<i>E. coli</i>	<i>Enterococci</i>	Total coliform	<i>E. coli</i>	<i>Enterococci</i> esp marker	<i>Bacteroidales</i>		
								fecal	human	ruminant
					<i>cfm/100 mL</i>					
WSI-02	06/21/06	(+)	(+)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
WSI-03	06/19/06	(+)	-	< 1 g	4 Ek	< 1	n/a	-	n/a	n/a
WSI-05	06/19/06	(+)	(+)	2 E	32 E	1 Ek	-	(+)	-	-
WSI-06	06/20/06	(+)	-	< 1 g	2 Ek	< 1	n/a	n/a	n/a	n/a
WSI-07	06/21/06	(+)	-	< 1 g	14 Ek	< 1	n/a	-	n/a	n/a
WSI-12	06/20/06	(+)	(+)	2 Ek	16 Ek	< 1	-	(+)	-	-
WSI-20	06/20/06	(+)	(+)	2 Ek	28 Ek	2 Ek	-	-	n/a	n/a
WSI-21	06/21/06	(+)	(+)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
WSI-22	06/20/06	(+)	(+)	2 Ek	26 E	< 1	-	(+)	-	-
WSI-23	06/19/06	(+)	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a
WSI-26	06/19/06	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a
WSI-30	06/19/06	(+)	(+)	17 Ek	200 Ek	1 Ek	-	(+)	-	-

Table D-4. Microbiological indicator sampling results for background sample - June 2006.

Site ID	Date	Total coliform	<i>E. coli</i>	<i>Enterococci</i>	Total coliform	<i>E. coli</i>	<i>Enterococci</i> esp marker	<i>Bacteroidales</i>		
								fecal	human	ruminant
					<i>cfm/100 mL</i>					
Septic Tank	06/19/06 - 06/21/06	n/a	n/a	51,000 E	n/a	n/a	-	n/a	(+)	(+)

Table D-5. Microbiological indicator sampling results for QA/QC samples - June 2006.

Site ID	Date	Total coliform	<i>E. coli</i>	<i>Enterococci</i>	Total coliform	<i>E. coli</i>	<i>Enterococci</i> esp marker	<i>Bacteroidales</i>		
								fecal	human	ruminant
					<i>cfm/100 mL</i>					
WSI-05	06/19/06	(+)	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a
WSI-20	06/20/06	n/a	n/a	2 Ek	9 Ek	< 1	n/a	-	-	n/a

Counts in col/100mL; E-estimated, k - non ideal count range, g - present at concentrations less than 0.5

Table D-6. Stable isotope sampling results for study area samples - June 2006.

Site ID	d18O vs. VSMOW	d15N vs. At. Air
WSI-02	6.382	13.235
WSI-03	2.238	8.123
WSI-05	4.612	9.435
WSI-06	2.348	10.235
WSI-07	7.235	13.735
WSI-12	8.734	15.458
WSI-20	5.123	12.372
WSI-21	3.476	8.230
WSI-22	3.173	9.235
WSI-26	1.942	9.458
WSI-30	2.376	9.395

Table D-7. Stable isotope sampling results for the septic tank sample - June 2006.

Site ID	d18O vs. VSMOW	d15N vs. At. Air
WSI-99	12.235	21.124

Table D-8. Stable isotope sampling results for QA/QC samples - June 2006.

Site ID	d18O vs. VSMOW	d15N vs. At. Air
WSI-05	4.328	9.023

Table D-9. Stable nitrogen isotope standard results - June 2006.

Standard Data		
Sample ID	Client	d15N vs. At. Air
CLW	COIL standard	5.153
CLW	COIL standard	5.327
CLW	COIL standard	5.403
	Mean	5.294
	SD	0.128
$\delta^{15}\text{N-NO}_3$	COIL standard	4.624
$\delta^{15}\text{N-NO}_3$	COIL standard	4.535
$\delta^{15}\text{N-NO}_3$	COIL standard	4.865
	Mean	4.674
	SD	0.170

Table D-10. Stable oxygen isotope standard results - June 2006.

Quality Control Standards			
Sample ID	$\delta^{18}\text{O}$ vs. VSMOW		
$\delta^{18}\text{O-NO}_3$	6.854		
$\delta^{18}\text{O-NO}_3$	6.615		
$\delta^{18}\text{O-NO}_3$	6.592		
		Actual	Diff
Mean	6.687	6.7	-0.013
SD	0.145		
CLW	3.013		
CLW	2.923		
CLW	2.891		
		Actual	Diff
Mean	2.943	2.9	0.043
SD	0.063		

Appendix E

USGS Data Report:

**USGS Data Report: Use of DNA Markers for Investigating Sources of
Bacteria in Contaminated Ground Water: Wooster Township, Wayne
County, Ohio**

Denise H. Dumochelle, 2006



Prepared in cooperation with the Ohio Environmental Protection Agency

Use of DNA Markers for Investigating Sources of Bacteria in Contaminated Ground Water: Wooster Township, Wayne County, Ohio

By Denise H. Dumouchelle

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Conversion Factors

Multiply	By	To obtain
Length		
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
Volume		
gallon (gal)	3.785	liter (L)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
 $^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$

Small sample volumes are reported in milliliters (mL) and microliters (μL). Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L), micrograms per liter ($\mu\text{g/L}$), or nanograms per microliter (ng/ μL).

Bacteria concentrations are given in colony-forming units per 100 milliliters (CFU/100 mL).

Use of DNA Markers for Investigating Sources of Bacteria in Contaminated Ground Water: Wooster Township, Wayne County, Ohio

By Denise H. Dumouchelle

Abstract

In 2004, a public-health nuisance was declared by the Wayne County Board of Health in the Scenic Heights Drive-Batdorf Road area of Wooster Township, Wayne County, Ohio, because of concerns about the safety of water from local wells. Repeated sampling had detected the presence of fecal-indicator bacteria and elevated nitrate concentrations. In June 2006, the U.S. Geological Survey (USGS), in cooperation with the Ohio Environmental Protection Agency (Ohio EPA), collected and analyzed samples from some of the affected wells to help investigate the possibility of human-origin bacterial contamination. Water samples from 12 wells and 5 home sewage-treatment systems (HSTS) were collected. Bromide concentrations were determined in samples from the 12 wells. Samples from 5 of the 12 wells were analyzed for wastewater compounds. Total coliform, enterococci and *Escherichia coli* (*E. coli*) bacteria concentrations were determined for samples from 8 of the 12 wells. In addition, two microbial source-tracking tools that employ DNA markers were used on samples from several wells and a composite sample of water from five septic tanks. The DNA markers from the *Enterococcus faecium* species and the order *Bacteroidales* are associated with specific sources, either human or ruminant sources.

Bromide concentrations ranged from 0.04 to 0.18 milligrams per liter (mg/L). No wastewater compounds were detected at concentrations above the reporting limits. Samples from the 12 wells also were collected by Ohio EPA and analyzed for chloride and nitrate. Chloride concentrations ranged from 12.6 to 61.6 mg/L and nitrate concentrations ranged from 2.34 to 11.9 mg/L (as N).

Total coliforms and enterococci were detected in samples from 8 wells, at concentrations from 2 to 200 colony-forming units per 100 milliliters (CFU/100 mL) and 0.5 to 17 CFU/100 mL, respectively. *E. coli* were detected in samples from three of the eight wells, at concentrations of 1 or 2 CFU/100 mL. Tests for the human-specific marker of enterococci, the *esp* gene, were negative in the seven samples tested, including the composite sample of HSTS water. DNA with the general *Bacteroidales* marker was detected in samples from four wells, but the tests for both the human- and ruminant-associated markers were negative. The presence of the PCR (polymerase chain reaction) -detectable DNA for the general fecal *Bacteroidales* marker is indicative of fecal contamination and recently recharged water.

Introduction

In October 2004, the Wayne County Board of Health declared a public-health nuisance in the Scenic Heights Drive-Batdorf Road area of Wooster Township, Ohio (fig. 1) because of concerns about the safety of the local water supply. In January 2006, at the request of Wayne County officials, the Ohio Environmental Protection Agency (Ohio EPA) began an investigation. Repeated sampling of the private water wells in the area found elevated nitrate concentrations and total coliform and *Escherichia coli* (*E.*

coli) bacteria on multiple occasions (Ohio Environmental Protection Agency, 2006). One possible source of contamination is nearby home sewage-treatment systems (HSTS); other possible sources include domestic pets and wildlife (bacteria) and fertilizers (nitrate).

Several water-quality and microbial source tracking (MST) methods can be used to indicate sewage contamination. Unfortunately, other potential sources can complicate interpretation of the data from many of these methods. Therefore, studies have found that use of multiple methods, including chemical and microbiological indicators, is the best approach for evaluating potential sewage contamination (K. E. Hyer, U.S. Geological Survey, written commun., 2006; Dumouchelle, 2006; Noble and others, 2006). To help identify whether the observed bacterial contamination originated from a human source, the U.S. Geological Survey (USGS), in cooperation with the Ohio EPA, collected and analyzed samples from wells in the Scenic Heights Drive-Batdorf Road area. In particular, the USGS used two MST tools that employ human-associated DNA markers found in bacterial strains of the order *Bacteroidales* and the species *Enterococcus faecium* (Bernhard and Field, 2000; Dick and others, 2005; Scott and others, 2005). Additionally, the presence of the general *Bactroidales* marker was used as another indicator of fecal contamination. The USGS Ohio Water Science Center is testing MST methods for their utility in combination with more traditional tools for contaminant-source identification. The present study is one of several case studies in Ohio.

Purpose and Scope

The purpose of this report is to present data from ground-water samples collected from selected residential wells in the Scenic Heights Drive-Batdorf Road area and to describe the use of DNA markers for investigating the sources of bacteria in contaminated ground water. In June 2006, the USGS collected and analyzed water samples from 12 wells and 5 HSTS. Bromide concentrations were determined at all 12 wells. Wastewater compounds were analyzed in samples from 5 of the 12 wells. Total coliform, enterococci, and *E. coli* bacteria concentrations were determined for samples from 8 of the 12 wells. Analysis of DNA markers was done on water samples from seven wells. A composite sample of HSTS water from 5 of the 12 properties where wells were sampled was analyzed for DNA markers.

Ohio EPA concurrently collected water from the same 12 wells and analyzed the samples for chloride and nitrate concentrations. Data from samples collected and analyzed by the Ohio EPA also are presented.

Description of the Study Area

The study area is in Wooster Township, southwest of the city of Wooster in northeastern Ohio, near the intersection of Batdorf and Tolbert Roads (fig. 1). The land use is rural residential, with most homes on lots ranging from half an acre to more than an acre. The homes are served by residential water wells and leach-line HSTSs. The residential wells in the area are from 70 to 185 ft deep and are completed as open holes in fractured sandstone, siltstone, and shale. The Ohio Department of Health used a downhole camera in July 2003 to investigate a well in the vicinity of WN-36 (fig. 1), and numerous fractures and openings were observed both above and below the water level (Craig Smith, Ohio Environmental Protection Agency, written commun., 2006). In addition, during the sampling for the current study, cascading water was heard in one well (WN-40).

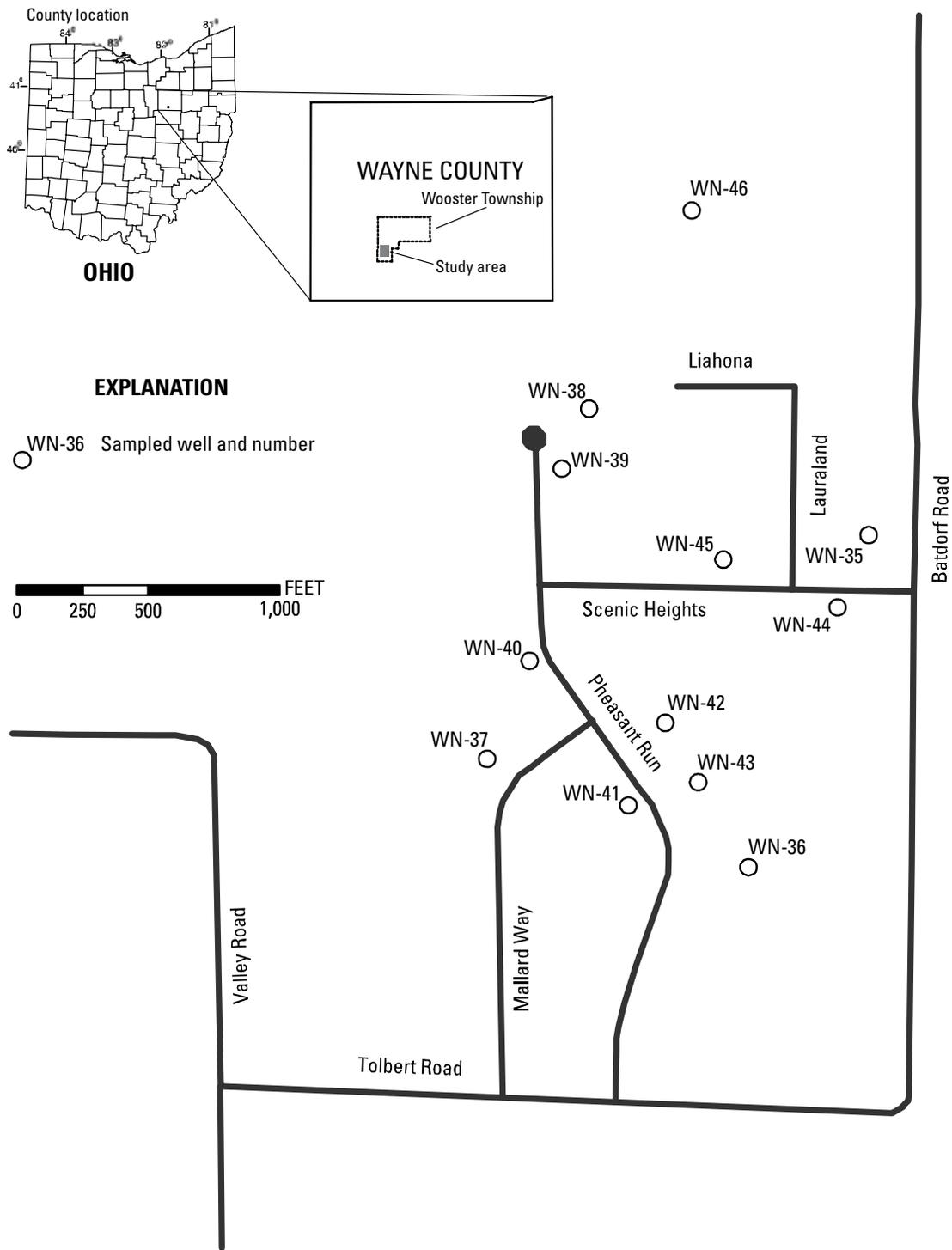


Figure 1. Location of study area and wells sampled in Scenic Heights Drive-Batford Road area, Wayne County, Ohio.

Sampling and Analytical Methods

Selection of wells was based on results of previous sampling by various agencies. Five wells with frequent detections of fecal-indicator bacteria were selected for analysis of wastewater compounds, bacteria, and bromide. Three additional wells were selected for analysis of bacteria and bromide. Another three wells were selected for the analysis of bromide only. A twelfth well, just north of study area, was sampled and analyzed for bromide to obtain background concentration data.

Homeowners of the selected wells gave permission for sampling and identified household spigots from which untreated water could be drawn. All samples were collected from these spigots as supplied by existing plumbing and well pumps. In most cases, an outside spigot was used as the sampling point. Sterile tubing and fittings were connected to the spigot for purging and sample collection. Although the sampled wells were in regular use, all wells were purged before sample collection. During purging, field measurements of pH, specific conductance, dissolved oxygen concentration, and temperature were made. Water samples were collected after the field measurements had stabilized.

The wastewater compounds and bromide analyses were done by the USGS National Water-Quality Laboratory in Denver, Colo. Bromide analyses were done by ion chromatography, with a reporting limit of 0.02 mg/L. The wastewater compounds and detection limits are given in table 1; analytical methods used are documented in Zaugg and others (2002). A field blank sample also was collected and analyzed for wastewater compounds to determine potential contamination from field and transport procedures.

Bacterial analyses were done at the USGS Ohio Water Microbiology Laboratory in Columbus, Ohio. Samples were analyzed within 26 hours of collection for total coliforms and *E. coli* by use of the MI membrane-filtration method (U.S. Environmental Protection Agency, 2002a) and for enterococci using the mEI membrane-filtration method (U.S. Environmental Protection Agency, 2002b). Because of the need to collect enterococci colonies for DNA analysis, up to 1 L of sample water was filtered, which is more than the standard 100 mL filtered for the method. The MST methods that can detect the presence of genetic markers within the order *Bacteroidales* and the species *Enterococcus faecium* were used to try to determine whether fecal contamination included a human source. Water samples were tested for the presence of a general fecal, human-associated, and ruminant-associated marker in the order *Bacteroidales*, as described in Dick and others (2005), according to the protocol originally described by Bernhard and Field (2000). Water samples were tested for the presence of *Enterococcus faecium* human-specific *esp* marker (enterococci marker) according to the protocol originally described by Scott and others (2005). About 500 mL of wastewater was collected from 5 septic tanks and combined into a single sample that also was analyzed for bacterial DNA markers.

Procedures for the microbiology quality-assurance/quality-control (QA/QC) laboratory practices are described in Francy and others (2005). In the laboratory, filter blanks were included for at least every third *E. coli* sample (and every sample for *Bacteroidales* or enterococci markers) to document that filtration equipment and buffered water were not contaminated.

Control DNA for human contamination sources (Delaware, Ohio, wastewater influent sample) and ruminant contaminant sources (Delaware, Ohio, cattle feces) were similarly processed for the presence of the *Bacteroidales* and enterococci markers. The presence of the general *Bacteroidales* marker (Bac32) was used as evidence that the polymerase chain reaction (PCR) was successful; in the absence of a positive response for Bac32, a matrix spike was included. For the enterococci marker, matrix spikes were included for each environmental sample, including the composite HSTS sample, to identify any possible matrix inhibition. PCR reagent blanks were included to test for contamination of PCR reagents with amplifiable target DNA.

Table 1. Wastewater-method compound names, USGS National Water-Quality Laboratory reporting limits, and possible compound uses (modified from Zaugg and others, 2002).

[Laboratory reporting limits are in micrograms per liter; F, fungicide; H, herbicide; I, insecticide; GUP, general-use pesticide; FR, flame retardant; WW, wastewater; Manuf, manufacturing; %, percent; >, greater than; CP, combustion product; UV, ultraviolet]

Compound name	Laboratory reporting limit	Possible compound uses or sources
1,4-Dichlorobenzene	0.5	Moth repellent, fumigant, deodorant
17beta-Estradiol	5	Estrogen replacement therapy, estrogen metabolite
1-Methylnaphthalene	0.5	2-5% of gasoline, diesel fuel, or crude oil
2,6-Dimethylnaphthalene	0.5	Present in diesel/kerosene (trace in gasoline)
2-Methylnaphthalene	0.5	2-5% of gasoline, diesel fuel, or crude oil
3beta-Coprostanol	2	Carnivore fecal indicator
3-Methyl-1H-indole (skatol)	1	Fragrance, stench in feces and coal tar
3-tert-Butyl-4-hydroxyanisole (BHA)	5	Antioxidant, general preservative
4-Cumylphenol	1	Nonionic detergent metabolite
4-n-Octylphenol	1	Nonionic detergent metabolite
4-tert-Octylphenol	1	Nonionic detergent metabolite
5-Methyl-1H-benzotriazole	2	Antioxidant in antifreeze and deicers
Acetophenone	0.5	Fragrance in detergent and tobacco, flavor in beverages
Acetyl-hexamethyl-tetrahydro-naphthalene (AHTN)	0.5	Musk fragrance (widespread usage) persistent in ground water
Anthracene	0.5	Wood preservative, component of tar, diesel, or crude oil, CP
Anthraquinone	0.5	Manuf dye/textiles, seed treatment, bird repellent
Benzo[a]pyrene	0.5	Regulated PAH, used in cancer research, CP
Benzophenone	0.5	Fixative for perfumes and soaps
beta-Sitosterol	2	Plant sterol
beta-Stigmastanol	2	Plant sterol
Bisphenol A	1	Manuf polycarbonate resins, antioxidant, FR
Bromacil	0.5	H (GUP), >80% noncrop usage on grass/brush
Bromoform	0.5	WW ozonation byproduct, military/explosives
Caffeine	0.5	Beverages, diuretic, very mobile/biodegradable
Camphor	0.5	Flavor, odorant, ointments
Carbaryl	1	I, crop and garden uses, low persistence
Carbazole	0.5	I, Manuf dyes, explosives, and lubricants
Chlorpyrifos	0.5	I, domestic pest and termite control (domestic use restricted as of 2001)
Cholesterol	2	Often a fecal indicator, also a plant sterol
Cotinine	1	Primary nicotine metabolite
Diazinon	0.5	I, > 40% nonagricultural usage, ants, flies
Dichlorvos	1	I, pet collars, flies, also a degradate of naled or trichlofon
d-Limonene	0.5	F, antimicrobial, antiviral, fragrance in aerosols
Equilenin	5	Hormone replacement therapy drug
Estrone	5	Biogenic hormone

Table 1. Wastewater method compound names, USGS National Water-Quality Laboratory reporting limits, and possible compound uses.— Continued

Compound name	Laboratory reporting limit	Possible compound uses or sources
Ethynyl estradiol	5	Oral contraceptive
Fluoranthene	0.5	Component of coal tar and asphalt (only traces in gasoline or diesel fuel), CP
Hexahydrohexamethyl-cyclopentabenzopyran (HHCB)	0.5	Musk fragrance (widespread usage) persistent in ground water
Indole	0.5	Pesticide inert ingredient, fragrance in coffee
Isoborneol	0.5	Fragrance in perfumery, in disinfectants
Isophorone	0.5	Solvent for lacquer, plastic, oil, silicon, resin
Isopropylbenzene (cumene)	0.5	Manuf phenol/acetone, fuels and paint thinner
Isoquinoline	0.5	Flavors and fragrances
Menthol	0.5	Cigarettes, cough drops, liniment, mouthwash
Metalaxyl	0.5	H, F (GUP), mildew, blight, pathogens, golf/turf
Methyl salicylate	0.5	Liniment, food, beverage, UV-absorbing lotion
Metolachlor	0.5	H (GUP), indicator of agricultural drainage
N,N-diethyl- <i>meta</i> -toluamide (Deet)	0.5	I, urban uses, mosquito repellent
Naphthalene	0.5	Fumigant, moth repellent, major component (about 10%) of gasoline
Nonylphenol, diethoxy- (total, NPEO2)	5	Nonionic detergent metabolite
Octylphenol, diethoxy- (OPEO2)	1	Nonionic detergent metabolite
Octylphenol, monoethoxy- (OPEO1)	1	Nonionic detergent metabolite
<i>para</i> -Cresol	1	Wood preservative
<i>para</i> -Nonylphenol (total)	5	Nonionic detergent metabolite
Pentachlorophenol	2	H, F, wood preservative, termite control
Phenanthrene	0.5	Manuf explosives, component of tar, diesel fuel, or crude oil, CP
Phenol	0.5	Disinfectant, manuf several products, leachate
Prometon	0.5	H (noncrop only), applied prior to blacktop
Pyrene	0.5	Component of coal tar and asphalt (only traces in gasoline or diesel fuel), CP
Tetrachloroethylene	0.5	Solvent, degreaser, veterinary anthelmintic
Tri(2-chloroethyl) phosphate	0.5	Plasticizer, flame retardant
Tri(dichloroisopropyl) phosphate	0.5	Flame retardant
Tributyl phosphate	0.5	Antifoaming agent, flame retardant
Triclosan	1	Disinfectant, antimicrobial (concern for acquired microbial resistance)
Triethyl citrate (ethyl citrate)	0.5	Cosmetics, pharmaceuticals
Triphenyl phosphate	0.5	Plasticizer, resin, wax, finish, roofing paper, FR
Tri(2-butoxyethyl) phosphate	0.5	Flame retardant

Ground-Water-Quality Data

Data on the wells and the onsite measurements (pH, specific conductance, temperature, and dissolved oxygen (DO)) are listed in table 2. The pH, specific conductance, and temperature values are typical for ground water from bedrock in northeastern Ohio (Eberts and others, 1990; Jagucki and Darner, 2001); however, the DO concentrations are unusually high for ground water. Although the use of the residential well pumps in the sample collection could introduce some oxygen into the water, the DO concentrations are still unusually high. In general, DO concentrations in ground water are expected to be less than 0.1 mg/L because buried organic matter and oxidizable minerals deplete available oxygen quickly; however, ground water in recharge areas can have relatively high DO concentrations, similar to concentrations in surface water (Hem, 1989; Freeze and Cherry, 1979). The DO concentrations measured in the wells in this study are consistent with recently recharged water.

Bromide concentrations from 12 ground-water samples ranged from 0.04 to 0.18 mg/L (table 3). Samples were collected concurrently by Ohio EPA and analyzed by their laboratory for chloride and nitrate¹ concentrations (table 3). Chloride concentrations ranged from 12.6 to 61.6 mg/L. Nitrate concentrations ranged from 2.34 to 11.9 mg/L (as N). Water samples from three of the wells had nitrate concentrations that exceeded the Maximum Contaminant Level of 10 mg/L (U.S. Environmental Protection Agency, 2004). Samples from 10 of the wells had nitrate concentrations greater than 3.1 mg/L; in Ohio, this may indicate effects of human activity (Baker and others, 1989). High nitrate concentrations are not common in ground water because aquifers are normally low in oxygen, forcing soil bacteria to strip oxygen from nitrate. These nitrate concentrations are consistent with the high DO concentrations described earlier.

Samples from eight wells were collected and analyzed for bacteria concentrations (table 4). Enterococci bacteria were found in samples from every well, although the concentrations in samples from three wells were less than 0.5 CFU/100 mL (volumes filtered were greater than 100 mL). Total coliforms were found in samples from every well at concentrations from 2 to 200 CFU/100 mL. *E. coli* were found in samples from three wells at concentrations greater than 1 CFU/100 mL.

Concentrations of enterococci from wells WN-37, WN-39, WN-40, WN-42, and WN-43 did not allow for the collection of more than 100 colonies for the DNA-marker analysis. This is significant because Scott and others (2005) reported consistent detection of the *esp* gene in human-derived enterococci when at least 100 colonies were cultivated. Regardless, the colonies were tested for the presence of the *esp* gene. The presence of this gene indicates fecal contamination from a human source; the absence of the gene indicates either that fecal contamination is from nonhuman sources, or that the enterococci concentrations from human sources were below the detection limit, or that the marker was not present in the fecal source. The *esp* gene was not found in any of the samples, including the composite sample from five septic tanks (table 4). In another study, the *esp* gene was reported to be present in 8 of 10 septic-system samples that had enterococci concentrations of 58 ± 24 CFU/100 mL (Scott and others, 2005), so the failure to detect the marker in the composite sample with 51,000 CFU/100 mL is a strong indication that the gene would not have been detected in the ground-water samples even if the enterococci concentrations were greater. Therefore, the fecal contamination in the wells is likely from either human sources that did not have the marker or from nonhuman sources.

¹ Actual analysis was for nitrite plus nitrate; however, “nitrate” is used in this report for simplicity because the proportion of nitrite to nitrate is negligible in most ground water.

Table 2. Well data and water-quality field data for ground-water samples from wells in the Scenic Heights Drive-Batdorf Road area of Wayne County, Ohio, June 2006

[μ S/cm, microsiemens per centimeter at 25 degrees Celsius; deg. C, degrees Celsius; mg/L, milligrams per liter; U, unknown; <, less than; --, no data]

Site name	Date sampled	Approximate depth of well/length of casing (feet)	Depth to water, below ground surface (feet)	pH (standard units)	Specific conductance (μ S/cm)	Temperature, water (deg. C)	Dissolved oxygen (mg/L)
WN-35	6-21-2006	150*/U	102	7.0	485	13.0	9.45
WN-36	6-21-2006	118 / 70	59.7**	6.9	430	12.2	1.26
WN-37	6-19-2006	80*/U	29.4	6.3	417	13.1	8.56
WN-38	6-21-2006	140 / 116	94.8	6.5	298	11.3	6.77
WN-39	6-20-2006	140*/U	82.4	6.3	273	13.0	7.65
WN-40	6-19-2006	116 / 52	51.6	6.6	465	13.8	6.51
WN-41	6-20-2006	135 / 79	55.0	7.0	466	13.5	2.25
WN-42	6-20-2006	140*/U	65.8	6.5	361	13.2	6.73
WN-43	6-20-2006	100 / 79	63.0	6.5	355	12.6	4.04
WN-44	6-21-2006	140*/U	104.3	7.0	554	12.8	4.96
WN-45	6-19-2006	150 / 120	98.0	6.8	422	12.9	4.59
WN-46	6-19-2006	--	--	7.0	618	12.7	7.15

* Reported by the Ohio Environmental Protection Agency.

** Depth to water is below the top of casing.

Table 3. Results of bromide, chloride, and nitrate analyses for ground-water samples from wells in the Scenic Heights Drive-Batdorf Road area of Wayne County, Ohio, June 2006.

[Samples for chloride and nitrate analyses were collected and analyzed by the Ohio Environmental Protection Agency; mg/L, milligrams per liter]

Site name	Bromide, dissolved (mg/L)	Chloride, dissolved (mg/L)	Nitrite + nitrate, (mg/L as N)
WN-35	0.19	43.6	11.9
WN-36	.07	39.7	2.34
WN-37	.14	59.2	9.87
WN-38	.09	13.0	10.9
WN-39	.16	12.6	11.3
WN-40	.18	61.6	9.15
WN-41	.06	37.1	2.42
WN-42	.12	37.2	8.66
WN-43	.08	46.8	5.88
WN-44	.05	28.0	6.50
WN-45	.05	44.5	5.08
WN-46	.04	53.2	6.38

Table 4. Results of analyses of bacteria concentrations and bacterial DNA in ground-water samples from wells in the Scenic Heights Drive-Batdorf Road area of Wayne County, Ohio, June 2006.

[CFU/100 mL, colony-forming units per 100 milliliters; <, less than; E, results based on estimated colony count; k, results based on colony count outside of the ideal range, potentially reducing accuracy of estimates; g, present at concentrations less than 0.5 colonies per 100 milliliters; --, not determined; Pos., positive for detection of bacterial DNA marker; Neg., negative for detection of bacterial DNA marker]

Site name	Enterococci ^a (CFU/100mL)	Total Coliform ^a (CFU/100ml)	<i>E. coli</i> ^a (CFU/100mL)	<i>Enterococcus</i> <i>esp</i> marker ^b present	<i>Bacteroidales</i> ^c markers present		
					Fecal	Human	Ruminant
WN-36	g ^d	14 E, k	< 1	--	Neg.	--	--
WN-37	17 E, k	200 E, k	1 E, k	Neg.	Pos.	Neg.	Neg.
WN-39	2 E, k	28 E	2 E, k	Neg.	Neg.	--	--
WN-39 replicate	2 E, k	9 E, k	< 1	Neg.	Neg.	--	--
WN-40	2 E, k	32 E	1 E, k	Neg.	Pos.	Neg.	Neg.
WN-41	g ^d	2 E, k	< 1	--	--	--	--
WN-42	2 E, k	26 E	< 1	Neg.	Pos.	Neg.	Neg.
WN-43	2 E, k	16 E, k	< 1	Neg.	Pos.	Neg.	Neg.
WN-45	g ^d	4 E, k	< 1	--	Neg.	--	--
Septic composite	51,000 E	--	--	Neg.	--	Pos.	Neg.

^a Samples were processed within 26 hours of collection.

^b The *esp* marker is on a gene in the *Enterococcus faecium* DNA that is specific to bacteria from a human source. Presence of the marker indicates fecal contamination of human origin but does not indicate humans as a dominant source. Absence of the marker indicates either no human-origin fecal contamination or concentrations less than the minimum detection limit.

^c The presence of the general fecal *Bacteroidales* marker indicates fecal contamination. The presence of the human-associated or ruminant-associated marker indicates human or ruminant sources contributed to the fecal contamination. The absence of a marker indicates either no associated host-source for the fecal contamination or that the concentrations were less than the minimum detection limit.

^d Concentrations less than 1 CFU/100 mL were determined by filtering up to 1 liter of water.

Samples from seven wells were analyzed for the detection of the general fecal Bacteroidales marker; four were positive (table 4). DNA samples from these four wells were then tested for the host-specific, human- and ruminant-associated markers; all tests for host-specific markers were negative. The presence of the general fecal marker indicates fecal contamination. Although the presence of the human- or ruminant-associated marker indicates human or ruminant sources contributed to the contamination, the absence of the marker may indicate one or more of the following situations: (1) there was no fecal contamination from the host-associated sources, (2) the amount of contamination from the host-associated sources was below the detection limit of the method, or (3) the marker was not present in the fecal sources or was not present at expected concentrations.

Fecal bacteria of the order Bacteroidales are anaerobes and are expected to survive for only a relatively short time in oxygenated waters. A study by Kreader (1998) found that in river water at 4°C, PCR-detectable DNA from Bacteroidales was detectable for at least 2 weeks; at 14° C, the DNA was detectable for only 4 to 5 days. Given the temperatures in the sampled water in this study (11-13°C), the presence of detectable DNA from Bacteroidales in samples from four wells —WN37, WN-40, WN-42, and WN-43 — is indicative of recently recharged water.

Water from five wells — WN-37, WN-39, WN-40, WN-42 and WN-43 — was sampled and analyzed for wastewater compounds (table 1). Metolachlor, an herbicide and general-use pesticide and an indicator of agricultural drainage (table 1), was detected in all five samples but at concentrations below the laboratory reporting limit of 0.5 µg/L (data on file at the USGS Ohio Water Science Center). The estimated concentrations of metolachlor ranged from 0.0102 to 0.1160 µg/L. The only other compound detected was caffeine, in the sample from WN-40; the concentration was estimated at 0.120 µg/L, below the reporting limit of 0.5 µg/L.

Summary

In 2004, a public-health nuisance was declared by the Wayne County Board of Health in the Scenic Heights Drive-Batdorf Road area of Wooster Township, Wayne County, Ohio, because of concerns about the safety of water from local wells. Repeated sampling of the private water wells had found elevated nitrate concentrations and the fecal-indicators total coliform and *Escherichia coli* (*E. coli*). To help identify whether the bacterial contamination was from a human source, the U.S. Geological Survey (USGS), in cooperation with the Ohio Environmental Protection Agency (Ohio EPA), collected and analyzed samples from some of the affected residential water wells. The wells in the area are completed as open holes in fractured bedrock. In June 2006, the USGS collected water samples from 12 wells and 5 septic tanks. Bromide concentrations were analyzed in samples from all 12 wells. Samples from 5 of the 12 wells were analyzed for wastewater compounds. Total coliform, enterococci, and *E. coli* bacteria concentrations were determined in samples from 8 of the 12 wells. In addition, two microbial source tracking (MST) tools that employ DNA markers were tested on several samples from wells and a composite sample of water from 5 septic tanks. The DNA markers from the *Enterococcus faecium* species and the order Bacteroidales are associated with specific sources, either human or ruminant. The general Bacteroidales marker is also an indicator of fecal contamination from warmblooded animals.

Onsite measurements of pH, temperature, specific conductance, and dissolved oxygen were made during sampling. The unexpectedly high concentrations of dissolved oxygen, ranging from 1.26 to 9.45 mg/L, are indicative of recently recharged waters. Bromide concentrations in samples from 12 wells ranged from 0.04 to 0.18 mg/L. No wastewater compounds were detected at concentrations above the reporting limits in five wells. Metolachlor was detected in the five samples but at concentrations below the laboratory reporting limit of 0.5 µg/L. Caffeine was detected in one sample but also at a concentration below the reporting limit of 0.5 µg/L. Samples from the 12 wells were collected by Ohio EPA and analyzed for chloride and nitrate; chloride concentrations ranged from 12.6 to 61.6 mg/L and nitrate concentrations ranged from 2.34 to 11.9 mg/L.

Total coliforms were detected concentrations from 2 to 200 CFU/100 mL in eight wells. Similarly, enterococci bacteria were detected in all eight wells sampled, ranging in concentration from 0.5 to 17 CFU/100 mL. *E. coli* were detected in samples from only three of the eight wells, at concentrations of 1 or 2 CFU/100 mL. The test for the human-specific marker, the *esp* gene, of enterococci was negative in seven samples tested, including the composite sample of HSTS water. The general fecal Bacteroidales marker was detected in samples from four wells, but the tests for both the human- and ruminant-associated markers were negative. The presence of the PCR-detectable DNA for the general fecal Bacteroidales marker is indicative of fecal contamination and recently recharged water.

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