2016

CHARACTERIZING HEALTH RISKS IN PRIVATELY-SUPPLIED DRINKING WATER DUE TO AGRICULTURAL PRACTICES IN RURAL WESTERN KENTUCKY

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ABSTRACT OF CAPSTONE

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The College of Public Health

University of Kentucky

2016

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ABSTRACT OF CAPSTONE

A Capstone project submitted in partial fulfillment of the requirements for the degree of Doctor of Public Health in the College of Public Health at the University of Kentucky

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ABSTRACT OF CAPSTONE

CHARACTERIZING HEALTH RISKS IN PRIVATELY-SUPPLIED DRINKING WATER DUE TO AGRICULTURAL PRACTICES IN RURAL WESTERN KENTUCKY

At least 400,000 people in Kentucky rely on private water wells or springs for drinking water. 551 households that rely on private water wells for drinking water were surveyed in 2009 about adverse health outcomes, including selected cancer incidence, adverse birth outcomes, and yearly incidence of diarrheal illness. Survey recipients were drawn from a population of well owners in the Jackson Purchase Region of Kentucky whose wells were tested for nitrate-nitrogen (NO3-N), triazine pesticides, and *E. coli* or total coliforms, by Kentucky Geologic Survey within the previous 15 years. 214 questionnaires were returned and matched to water quality data for analysis; the effective response rate was 39%. Of 211 wells in this study with NO3-N results available, 11 (5.91%) had NO3-N concentration above the MCL of 10 mg/L. Of 189 wells in this study with triazine pesticide results available, 1 (0.53%) had concentration above the MCL of 3 µg/L; 123 (65.08%) had undetectable concentrations of triazine pesticides. NO3-N and triazine levels were not independently distributed; shallower bored well construction was predictive of higher concentrations of both contaminants, consistent with other research. *E. coli* contamination was detected in 14.5% of wells tested in the study population, and total coliforms were present in 59.3%. Over one-fifth (21%) of wells in the study population were contaminated with all three, total coliforms, triazine pesticides and NO3-N, above background concentrations, indicating the wells' vulnerability to surface-level contamination that can result from well construction and agricultural land use practices. Survey respondents were asked about household incidence of non-Hodgkin's lymphoma and liver, stomach and breast cancers. SIRs were calculated to compare the study population with reference populations. Wilcoxon rank sum statistics comparing the distribution of nitrate in cancer-reporting household and non-cancer reporting households suggest an association between NO3-N exposure in drinking water and cancer incidence. Analysis of NO3-N concentration in the study population did not suggest an association between higher concentrations in drinking water and adverse birth outcomes including intrauterine death, miscarriage and premature birth; no statistically significant relationship was shown. Presence of *E.coli* or total coliform in the water from wells in the study population, whether modeled on a continuous or present/absent scale, was a poor predictor of yearly household incidence of gastrointestinal disease.

KEYWORDS: (Privately-supplied drinking water, water quality, rural, water wells, nitrate, nitrogen-N, triazines, herbicides, agriculture, *E. coli*, coliforms)

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ACKNOWLEDGEMENTS

The author wishes to thank the members of her committee, Drs. Glyn Caldwell, Steven Browning, and Gail Brion of the University of Kentucky, for their extensive guidance and patience. E. Glynn Beck, of the Kentucky Geologic Survey, served as a co-investigator and co-author on this project and provided much of the technical knowledge necessary for writing. This project was based on data collected over many years by the Kentucky Geologic Survey; the author thanks Drs. James Dinger and Stephen Fisher of the KGS for their assistance. Laboratory work associated with the project was performed at the Environmental Research Training Laboratories (E.R.T.L) at the University of Kentucky; the author thanks Trish and John for their guidance and help. The project was funded by a grant from the Southeast Center for Agricultural Health and Injury Prevention; thanks go to Dr. Robert McKnight and the Center for funding and material assistance. My thanks as well to Dylan Cummings, Florida Department of Health, who assisted with the map.

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CHAPTER 1

INTRODUCTION

Background

In 2016, public concerns over drinking water quality are high. Extended drought conditions in California have created contamination and shortages of public water supplies.[1,2] Hydraulic fracturing may contaminate some ground water used for privately-supplied drinking water with unknown chemicals and pollutants.[3,4] The discovery of dangerous amounts of lead in water supplied to homes in Flint, Michigan in 2014-2015 has brought attention to other places in the United States (U.S.) where aging infrastructure threatens drinking water quality.[5]

Clean water for drinking is essential to human life; the acquisition and provision of potable water is a policy matter in all communities, and it is a need for all families. Most people in the U.S. have drinking water available from a public water system.[6] Public water systems can rely on surface water or ground water as a source; there are more systems in the U.S. that use groundwater, but the ones using surface water serve more people.[7] All public water systems, including ground water wells, are regulated by the Safe Drinking Water Act (SDWA) of 1974 which sets health-based standards for naturally-occurring and man-made contaminants in drinking water.[8] Carrying out the SDWA is done at the state level.

In 2007, over 15 million households in the U.S. received drinking water from private wells.[9] Self-supplied water is water that is not provided by a public system; it's usually acquired by drilling a well and drawing up ground water. This water's quality is not regulated by the SDWA. Well owners are responsible for maintaining the water quality in their wells.[10] In Kentucky, newly constructed wells must be disinfected with chlorine and analyzed for fecal coliform bacteria, but there are no further requirements for water quality monitoring.[11]

The proportion of people in the U.S. who rely on a private water supply from a ground water well has decreased from 38% in 1950 to 14% in 2005; this change tracks increasing urbanization

in the U.S. in the same time period and a decrease in the number of U.S. workers living and working on farms.[12, 13]

Private water wells used by many farm families and rural populations are not regulated by public health or environmental agencies. Public water supplies are monitored for nitrate levels and bacterial indicator organisms, but private well users are responsible for testing their own drinking water.[10] At least 400,000 people in Kentucky rely on private water wells or springs for drinking water.[14, 15]

This study is focused on possible adverse health effects resulting from contaminated drinking water that are experienced by people living in a region that is mostly rural, heavily agricultural, and where many people draw drinking water from water wells.

Excess nitrate in drinking water is a contaminant that is known to cause methemoglobemia in infants and has been linked to some cancers in adults. Although nitrate is present in almost all drinking water sources, excess nitrate is considered the most widespread contaminant in groundwater. It is introduced into groundwater sources by the use of nitrogen fertilizer in agriculture and by leaking or abandoned septic tanks. (Other common sources in rural areas are animal feedlots.) In 1990, the U.S. Environmental Protection Agency (EPA) estimated that 2.4% of rural private water wells had nitrate-nitrogen concentrations above the maximum contaminant limit (MCL) of 10 mg/L, and that 4.2% would have at least one pesticide detectable in the water.[16]

Triazines are herbicides used in agriculture; atrazine is the most commonly used triazine. Triazines can leak into water wells from the top when spilled or improperly disposed of near a wellhead. In animal trials, triazines have been shown to be endocrine disruptors that may interfere with reproduction.[17]

Total coliforms (TC) are bacteria found in fecal matter and in other places in soil and on plants. They do not cause disease in humans, but when high numbers of them are found in water, they indicate sewage contamination and the possible presence of bacteria that do cause disease. *Escherichia coli* is a coliform found in the feces of warm-blooded animals and humans that is usually harmless, but its presence in water indicates contamination from sewage. TC and *E. coli*

can enter water wells by seeping through shallow ground into inadequately sealed wells from leaking septic tanks or because of manure application to ground crops.[18, 19]

Purpose of the study

This study seeks to determine if the population of the Jackson Purchase Region (JPR) of Kentucky that depends on water wells for drinking water experience disproportionate rates of cancers, birth defects or gastrointestinal illnesses that are possibly related to drinking water contamination by nitrate, herbicide, or bacterial contamination common in agricultural areas.

Specifically, the following questions are considered: Does the population of the JPR experience a higher incidence of Non-Hodgkin's lymphoma (NHL), stomach cancer, liver cancer, or breast cancer than referent populations and is any higher incidence associated with nitrate contamination in privately-supplied drinking water? Are those associations between nitrate in drinking water and cancer incidence affected by triazine contamination in privately-supplied drinking water? Do families whose well water was found to be contaminated by TC or *E. coli* have greater incidence of diarrheal illness than families in the same region whose wells were not similarly contaminated?

Overview of project processes

Households in the JPR that rely on private water wells for drinking water and whose wells were tested for nitrate-nitrogen (NO3-N), triazine pesticides, and E. coli or total coliforms, by the Kentucky Geologic Survey (KGS) within the 15 years prior to 2009 were surveyed by mail in 2009 about adverse health outcomes, including selected cancer incidence, adverse birth outcomes, and yearly incidence of diarrheal illness. Rates were calculated for the study population and were compared to standard rates for Kentucky and the U.S.

Scope and importance of the study

This survey project attempted to collect self-reported health information for an estimated 1,322 persons living in an 8-county region of Kentucky, a region that is mostly rural, heavily agricultural, and where many people draw drinking water from wells. Although groundwater is cleaner than the surface water sources that many municipalities with public water supplies use, public water supplies are cleaned and monitored for water contaminants on a regular basis. Private water supplies are not required to be monitored for water quality on a regular basis. Agricultural practices commonly introduce water contaminants into private water wells near or on the surface of the land. The households surveyed had had their water quality tested by the KGS between 1994 and 2009, making it possible to evaluate an association between disease rates and water quality. If it is found that water contaminants such as nitrate, triazine herbicides, or *E. coli* are associated with disproportionate amounts of disease in this region, further studies can be undertaken with the assistance of the KGS to understand what barriers exist that prevent households with private water wells from testing their water quality regularly.

CHAPTER 2

LITERATURE REVIEW

Purchase District demographics

The Purchase District of Kentucky is comprised of Ballard, Calloway, Carlisle, Fulton, Graves, Hickman, Marshall and McCracken counties. It is the portion of Kentucky farthest west; four of the counties border the Mississippi River. It contains 4.5% of Kentucky's population and 6% of the state's land area. It contains no metropolitan statistical area. 17.1% of the population in the Purchase District is 65 years old and older; Kentucky's percentage is 13.3%. It contains one urban cluster, at Paducah, the county seat of McCracken County. McCracken is the only county, among the eight in this district, that is not completely or majority-part rural.

The JPR is composed entirely of rural areas and micropolitan statistical areas (population 10,000-49,000). The JPR gained 1.5% in population (not quite 3,000 people) between 2000 and 2010.[20]

Local private sources of groundwater for drinking

At least 400,000 people in Kentucky rely on private water wells or springs for drinking water. (This is over 9% of the total population of Kentucky in 2010.)[14, 15]

The Purchase District generally lies over "semi-consolidated Cretaceous age and younger sand, silt gravel and clay deposits." The coarser sediments provide plenty of water for domestic water supply wells, but they are sensitive to contamination, especially at shallow depths. The relatively low-flow velocity in deeper saturation zones provides some protection from contamination. The sensitivity of groundwater in the region to contamination ranges from moderate to slight. In some areas where depth to water exceeds 30 m, groundwater sensitivity to contamination is the lowest in Kentucky.[21]

Sources of nitrate in well water

Nitrate (NO3-N) provides nitrogen to plants and animals. Nitrate is soluble and mobile; it is prone to leaching through soil with water.[22] Sources of nitrate in groundwater are plants and animal matter, human and animal fecal waste, household septic systems and fertilizers. Some

nitrate is present in almost all drinking water sources.[14] Relative background nitrate level is about 1 mg/L.[10, 22] Nitrate concentrations above 3 mg/L indicate human-made pollution.[10] Nitrate is considered the most widespread contaminant in groundwater. [22] In the National Water-Quality Assessment Program of the U.S. Geological Survey, a survey of 1,255 private water wells and 242 public water wells, nitrate was the chemical that was most frequently found in excess of its standard for drinking water, which is 10 mg/L.[23] Shallow groundwater generally has higher concentrations of nitrate than deeper groundwater, because nitrate sources are on or near the surface. Shallow ground water may be in contact with leaking septic systems, or it may be contaminated by frequent or heavy use of nitrogen-containing fertilizers.[14] Deeper groundwater is less likely to have excess nitrate because increased depth in aquifers is less favorable for nitrate accumulation and storage; deeper groundwater is older and may predate periods of increased fertilizer use (starting in 1950s); and with increasing depth, there is greater likelihood of less permeable layers that restrict downward movement of nitrate dissolved in water.[22, 24]

Levels of nitrate in well water are associated with source availability and regional environmental factors.[25, 26] In a 2005 study of factors that influence nitrate concentrations in water wells, the highest nitrate concentrations were predicted by high nitrogen fertilizer application, high water input, well-drained soils, fractured rocks or rocks with high effective porosity, and lack of attenuation processes. [22] In a 2005 study of groundwater chemistry on land previously used for agriculture, researchers found the highest concentrations of nitrate and atrazine in shallow wells at topographically low points. They speculated two reasons why this may be the case: runoff of applied fertilizers resulted in focused recharge of contaminants at topographic lows, and the water table may be more susceptible to contaminants applied at the surface in those locations because the water table is closer to the ground surface. Researchers also found that wells located outside cropped areas had lower concentrations of nitrate and atrazine.[27] In the early 1990s, researchers testing water wells in Iowa found 18.3% of private rural water wells had nitrate concentrations above the MCL, with much regional variation (The average nitrate concentration in the six regions ranged from 9.2% above 10 mg/L to 38.2% of wells above 10 mg/L).[10] In 1996, the Cooperative Private Well Testing Program sampled almost 35,000 wells in Ohio, Indiana, Illinois, West Virginia, and Kentucky; 3.4% of wells had nitrate-nitrogen concentration > 10 mg/L, and 23% had concentration > 1 mg/ L.[28]

Influence of well depth and construction on contamination

A water well is designed to collect or pull groundwater from below the water table using water-permeable materials where inflow is desired. Impermeable casings are parts of wells that prevent inflow in shallow depths, where water quality is worse, or at the surface, where accidents could introduce chemicals into the top of the well or contaminated surface water might collect or flow.[19, 29] Well casings that are ineffectively sealed and are permeable to shallow groundwater along their length can allow contaminated shallow ground water to travel downward to the well's intake area.[14]

Drilled wells, are much deeper than large-diameter wells, up to 762 meters in this study, are 4-6 inches in diameter, and are cased with plastic or steel, with the casing extending 12 inches or more above the ground. They are less likely to be contaminated, presumably because they draw from deep groundwater sources, but also because they are better designed to prevent surface and shallow contamination from entering the well.[29]

Other large-diameter wells (augered, bored or hand-dug), 24-34 inches in diameter in this study, are designed to store a lot of water in the well, because they are drawing from low volume water supplies. They are not generally as deep as drilled wells. The top portions (3-4.5 m) of casings are designed to allow water to seep in, so they can be contaminated with total and fecal coliforms and nitrate. A buried-slab well has water-tight (steel or plastic) casing along the first 10 feet underground, and the casing extends a minimum of 1 ft. above ground. Concrete tile casing allows water to seep into the well below the slab, which must be buried above the water table. This design in a bored well minimizes bacterial and chemical contamination from surface sources. [29]

Well depth influences the likelihood of water contamination with excess nitrate; the shallower the well, the more likely the water is to contain high concentrations of nitrate. [14, 22, 27] A water-well survey in Iowa conducted from 1993-1995 found well depth had less influence on contamination rates for measured contaminants than did well type, however; buried-slab wells were less likely to be contaminated with fecal coliform bacteria, nitrate or atrazine than other large-diameter wells, and there was no significant difference in incidence of those contaminants between the buried slab wells and drilled wells.[29]

Health effects from nitrate in drinking water

Oral absorption of nitrate is nearly 100%.[23] Most is absorbed through the small intestine, and it is distributed throughout the body, without targeting a specific organ. Nitrate is converted to nitrite in the body by bacteria in saliva, stomach and small intestine. The metabolism of nitrate and nitrite varies in human beings by age, health, and physiological factors. Neither nitrate nor nitrite has been shown to be directly carcinogenic. Amines and amides can interact in the body (in the stomach) with nitrite to form N-nitroso compounds (A substance that can interact with nitrite to form N-nitroso compounds is called nitrosatable.). Nitrosatable amines and amides are available in drugs, foods, agricultural chemicals, and tobacco; some are produced in the body.[30]

Ingesting sufficient quantities of nitrate can cause methemogloinemia (also known as blue-baby syndrome), excessive production of abnormal hemoglobin that reduces the blood's ability to carry oxygen. This primarily occurs in infants. The MCL for nitrate in drinking water was set by the U.S. EPA at 10 mg/L to protect against methemoglobemia in infants.[14, 22] Some animal and toxicological studies have raised the idea that maternal exposure to nitrate can be associated with miscarriage, intrauterine growth restriction or other birth defects, but the studies in humans are not conclusive.[31]

N-nitroso compounds (and nitrosamines) have been shown to cause cancer in animals (Nitrosamines are a subset of N-nitroso compounds.). Increased intake of nitrates can lead to corresponding increased production of N-nitroso compounds in the body. Nitrosamines must be metabolized in order to be carcinogenic; other N-nitroso compounds are directly carcinogenic. Most of the 300 known N-nitroso compounds have been shown to be carcinogenic in animals. They range in potency as carcinogens. Some antioxidants available in fruits and vegetables, such as ascorbic acid and α-tocopherol, inhibit nitrosation.[30]

Nitrate in drinking water has been associated in studies with several types of cancers, with concentrations as low as 2.5 mg/L.[22] Despite the associations seen in some ecologic studies, epidemiological studies of the relationship between nitrate in drinking water and cancers do not show consistent positive associations.[32]

Nitrate and Non-Hodgkin's lymphoma: NHL is a cancer that begins in the lymphocytes, which are white blood cells. Multiple types of lymphomas fall within this general description.

A population-based case-control study in Nebraska, with 156 cases and 527 controls, and using 32 years of nitrate concentration in community drinking water data, found elevated risk (OR=2.0; 95% CI 1.1-3.6) of NHL in the highest quartile for nitrate (≥4 mg/L). This study also measured nitrate levels in private wells for 51 cases and 150 controls at the time of interview; no association was found between nitrate level and risk of NHL after pesticide use on the property was taken into account.[33] A population-based ecological study in Yorkshire, England using 9 years of NHL incidence data and 6 years of nitrate concentration data in publically-supplied water, did not find evidence to support the hypothesis that nitrate in drinking water was associated with NHL incidence.[34] A population-based case-control study of 73 NHL cases and 143 controls in Minnesota, in which researchers estimated the average long-term exposure to nitrate in public drinking water supplies for a 28-year period, found no association between nitrate in drinking water supply and NHL within the range of 0.1-7.2 mg/L.[35] A population-based cohort study, part of the Iowa Women's Health Study, examined a cohort of 21,977 Iowa women who had been using the same water supply for 10 or more years and found no association between nitrate levels in drinking water and incidence of NHL. No data from private wells were available in this study, and women who drank from private wells comprised about 25% of the cohort; municipal water exposure data was used to characterize their intake. The nitrate exposure from water was divided into quartiles with cut points of 0.36, 1.01, and 2.46 mg/L of nitrate.[36] An ecologic study from an agricultural district in Slovakia, using 20 years of nitrate data and 9 years of cancer incidence data, found the data supported a possible positive association between incidence of NHL and increasing nitrate concentration in drinking water. Nitrate exposure was classed as low (0-10 mg/L), medium (10.1-20 mg/L) and high (20.1 mg-50 mg/L); p for positive trend in NHL incidence =0.021.[37] However, An ecological study in Sardinia, Italy, using 23 years of nitrate concentration data and NHL incidence for 19 years, did not show evidence of a positive association between increasing nitrate concentration and increasing NHL incidence; the average nitrate concentration was 4.57 mg/L, and 8 strata were used.[38] A case-control study in Iowa, with 181 cases and 142 controls, using 40 years of nitrate data, found no association between nitrate levels below 3 mg/L and risk of NHL. This study also used nitrate sampling of private well water at time of interview for 54 cases and 41 controls; no association was seen for this group either.[39] A case-control study from Taiwan, used 1,716 NHL cases and the same number of controls. One year of nitrate level for the municipal water system districts, ranging

from 0.0 to 2.86 mg/L, was used to characterize exposure. This study found no association between nitrate level in water and risk of death from NHL.[40] A case-control study in Nebraska with 140 NHL cases and 192 controls, measured nitrate in the public groundwater supply and translated it into a dichotomous variable of less than or equal to 2 mg/L, and more than 2 mg/L. The results showed no association between nitrate exposure and NHL (OR=0.6 and 95% CI 0.3-1.1) although the same study found an association between risk for NHL and exposure to a combination of nitrate and a dichotomous ever/never exposure to atrazine (OR=2.5 and 95% CI 1.0-6.2).[41] Appendix 2 summarizes the studies in table form.

Three of these studies were ecological; two of the three showed no association, and all used public water supply data to characterize nitrate exposure. The one that did show a positive association, grouped all nitrate concentrations from 0 to 10 mg/L into the lowest, referent category; the exposure categories are too high to be compared to this study.[38] Five studies were case-control, in which the exposure did precede the diagnosis of NHL. Four showed no association between NHL risk and nitrate in drinking water. All were studies of nitrate in publicly-supplied water, though two also analyzed well water separately.[33, 39] One of these did show an association, but only for publicly-supplied water; the association did not exist among study subjects receiving well water.[33] The other showed no association of risk for NHL to nitrate in water, either public or private.[38] Most of these studies use public water nitrate data to characterize the exposure for any subjects with well water supplies. They have the advantage, however, of using exposure categories that are low and representative of this study. A population cohort study, the Iowa Women's Health Study, also found no association between NHL risk and nitrate exposure in drinking water and diet, despite exposure categories that were high.[36] The literature does not provide evidence that low levels of nitrate in drinking water is associated with a greater risk for NHL.

Nitrate and stomach cancer: A case-control study in Wisconsin found no association between death from gastric cancer and well water as drinking water source compared to public water, where mean nitrate in well water was 2.41 mg/L compared with 0.95 mg/L. Address at death was used to classify water well users, and nitrate was measured in the well water after the subjects' deaths.[42] An ecological study in Valencia, Spain to find an association between nitrate in public drinking water and mortality from various cancers found increased risk of death from stomach

cancer in men and women aged 55-75 years (RRs of 1.91 and 1.81, respectively; $p < 0.05$) at nitrate exposure > 50 mg/L.[43] A prospective case-cohort study in the Netherlands examined 282 incident cases of stomach cancer and 3,500 non-cases. Nitrate from public drinking water was collected one year before follow-up started, and measured in quintiles that ranged from a mean of 0.02 mg/L to a mean of 16.5 mg/L. No association was found between risk for stomach cancer and higher exposure levels of nitrate.[44]

A case-control study in Taiwan found no association between death from stomach cancer and nitrate concentration in the water provided by the case or control subject's municipality; the OR for death from gastric cancer was 0.95 (95% CI 0.87-1.03) for subjects with nitrate levels between 0.23 and 0.44 mg/L, and 1.02 (95% CI 0.93-1.11) for subjects with nitrate levels ≥ 0.45 mg/L.[45] An ecological study in Yorkshire, England to examine the association between nitrate in drinking water and incidence of stomach, esophageal, or brain cancers found no association between stomach cancer incidence and nitrate concentration in water supply; the nitrate measures were contemporaneous with or later than the diagnoses.[46] An ecological study in Ontario, Canada that counted incident stomach cancer cases in agricultural districts in a five-year span found a negative association between stomach cancer and nitrate in multivariate models.[47] An ecological study in Hungary of the association between nitrate in drinking water and death from stomach cancer found significant elevated standard mortality ratios for subjects in nitrate exposure categories > 88 mg/L, and a significant positive trend of mortality with increasing log-transformed nitrate concentration. The nitrate exposure was based on the average nitrate concentration in water provided in a settlement, and the measurements were contemporaneous with the outcomes (mortality).[48] An ecological study in Slovakia examining associations between nitrate in drinking water and stomach cancer found increasing standardized mortality ratios with increasing nitrate exposure category (0–10, 10.1–20 and 20.1–50 mg/L) of 0.94 and 1.24. There was a significantly positive trend only in women. Nitrate measurements were averaged across public water-supply districts from 11 years before and through the outcome period.[37] A population-based case-control study of stomach cancer in Nebraska found no association between stomach cancer and nitrate concentration as measured in the public water supplies (or the private water wells for those cases and controls using wells); The odds ratio (OR) for stomach cancer was 1.2 with a 95% CI of 0.5-2.7. [49] Appendix 2 summarizes the studies in table form.

Of five ecological studies reviewed, three found no association between stomach cancer and nitrate in drinking water, and two found positive associations that were inconsistent or only seen in very high exposure categories that are not relevant to this study. Three case-control studies did not find an association between stomach cancer and nitrate in drinking water, one that made better-than-usual attempts to characterize intake correctly and included well water in a separate analysis.[49] A prospective study also did not show any association. The literature does not support any association between stomach cancer and nitrate in drinking water.

Nitrate and breast cancer: A population-based cohort study followed a cohort of 21,977 Iowa women who had been using the same water supply for 10 or more years and found no association between nitrate levels in drinking water and incidence of breast cancer. Municipal water exposure data was used to characterize the nitrate exposure for the women using well water. The nitrate exposure from water was divided into quartiles with cut points of 0.36, 1.01, and 2.46 mg/L of nitrate.[36] A case-control study in Massachusetts examining the relationship between breast cancer incidence and nitrate concentration in municipal ground water supplies found no association between breast cancer incidence and average nitrate exposure, summed nitrate exposure over years of exposure, or number of years exposed to nitrate in water >1 mg/L. Private well users were excluded from the study. Nitrate measurements were taken from 16 years before the outcome period to the end of the outcome period.[50] Appendix 2 summarizes the studies in table form. The literature does not support an association between nitrate in drinking water and breast cancer.

Nitrate and other cancer sites: A 2012 meta-analysis examining exposure to nitrate in drinking water and bladder cancer, including two cohort studies, two case-control studies and one ecological study, found no sufficient evidence for an association between the two.[51] The Iowa Women's Health Study, a population-based cohort study of 21,977 women in Iowa, found increased risk of ovarian cancer with increased nitrate in their drinking water, OR=2.34, 95% C.I. (1.42, 3.84) for the highest exposure quartile, 2.98-25.34 mg/L, compared to the lowest quartile, 0.01-0.47 mg/L. Municipal water exposure data was used to characterize the nitrate exposure for the women using well water.[52] The same cohort study also found an increased risk of thyroid cancer, RR=2.6, 95% C.I (1.1-6.2) for five or more years of exposure to >5 mg/L of nitrate compared to baseline.[53] A case-control study of 475 colorectal cancer cases and 1447

population controls in Wisconsin found an increased risk only for proximal colon cancer in an exposure category of >= 10 mg/L; there was no increased risk association with nitrate for other types of colorectal cancers in the study.[54] These studies are briefly described here to show that some epidemiological studies have found associations between nitrate in drinking water and other types of cancers, but the results overall are not consistent.

Nitrate and adverse reproductive outcomes: An ecological study in the U.S. relating incidence of all birth defects in live births in 1996-2002 found statistically significant increased odds of any kind of birth defect for conception dates in April-July, when agricultural chemicals are being applied to row crops. While the authors demonstrated that the mean nitrate in those months is higher, atrazine and other pesticides were also higher. Nitrate and triazine contamination in wells is often coincident, and this study could not differentiate between the contaminants.[55] A cohort study in France of 11,446 births found a positive association between nitrate in drinking water exposure at or above 15 mg/L during the second trimester and small-for-gestational-age (SGA, also known as intrauterine growth retardation), in the absence of atrazine exposure. The nitrate measurements were specific to individuals and were taken through pregnancy.[56] Upon examination, however, this association was specific to higher socioeconomic geographical areas.[57] These two studies are presented in table form in Appendix 3. With the two studies to go by, the evidence can be considered suggestive of an association between nitrate in drinking water and adverse birth outcomes, but not supportive.

Sources of triazines in well water

Triazines are herbicides used in agriculture to control broad-leafed weeds and grasses from row crops. Atrazine is the most commonly used; others are simazine (used in agriculture) and propazine (not used in agriculture).

Triazines and other pesticides can enter wells if there is a spill or improper disposal near a well, via runoff into the top of an improperly constructed well, and by downward movement through the soil after application.[58] Atrazine is moderately soluble, has high to medium mobility through soil, and does not rapidly degrade.[17, 59] The probability of detecting atrazine in well water is not related to well depth.[16] In 1996, the Cooperative Private Well Testing Program sampled almost 35,000 wells in Ohio, Indiana, Illinois, West Virginia, and Kentucky; 4.9% of wells had atrazine detected in the water, and 0.1% had concentration > 0.3 mcg/L.[60] Samples taken from 171 rural

domestic wells in eastern North Carolina in 1995 found atrazine in 8.2% of the wells. No correlation was found with distance to the nearest application site or distance to the nearest pesticide handling and storage area. For half the wells in which atrazine (or alachlor, another pesticide with similar properties) was detected, it had not been used on the farm associated with the well in 3 or more years. This indicates that atrazine can be transported through groundwater or that it persists for a long time in the environment.[61] Atrazine and nitrate frequently occur together in rural well water.[16, 27]

Health effects from triazines in drinking water

When ingested, triazines are readily absorbed from the gastrointestinal tract; 80% or more is absorbed into the body. Triazines are endocrine disruptors in animals and can have reproductive effects. In rats, triazines have been found to suppress the surge of luteinizing hormone during ovulation, delay onset of puberty, alter the estrus cycle and affect maintenance of pregnancy. Triazines are not estrogenic; they disrupt hypothalamic-pituitary-gonadal function. Atrazine exposure in humans may disrupt the feedback loop of the gonads, hypothalamus and pituitary gland in a similar way. The hormone imbalance may be related to reproductive outcomes such as pre-term delivery and low birth weight infants.[17] The effect of nitrate on the reproductive toxicity of triazines is uncertain. The MCL for Atrazine is 0.3 µg/L and the MCL for simazine is 0.4 µg/L. In 2003, The Agency for Toxic Substances and Disease Registry derived an intermediate oral MRL of 0.3 µg/kg/day based on reproductive effects (delayed onset of estrus) in pigs.[23] The drinking water standard for atrazine, the most commonly used triazine, is 3 ppb, or 3 mcg/L.

The U.S. EPA has determined that atrazine is a possible human carcinogen, based on limited evidence from animal studies.[59] Triazines are not considered to be directly carcinogenic.[62] Triazines can react with nitrite (a nitrate metabolite) in the environment and the body to form N-nitroso compounds. Structure and activity of these compounds suggest they may be carcinogenic, but research has not been conducted that answers the question.[23]

Triazines and adverse reproductive outcomes: An ecological study in Iowa compared rates of premature birth (birth before 37 weeks), low birth weight (<2,500 g) and intrauterine growth retardation (IUGR, birth weight below the 10th percentile for gestational age) in an area with known elevated levels of triazine herbicides in the water system to other communities without elevated levels. Low birth weight was slightly more frequent in the affected areas, premature

birth was slightly less frequent, neither were statistically significant. The risk for IUGR in the affected areas was 1.8 times the risk in the unaffected areas, 95% C.I. (1.2-2.6).[63] A retrospective cohort study in an agricultural area of France examined 3,510 births in an area with strong seasonal variation in the levels of atrazine detected in the public water supply. Exposure was characterized by area of residence compared to water system measurements. First, second and third trimester high exposures (during May through September) were compared to the same trimesters occurring during low exposure time periods (October through April). No association was seen between seasonal third-trimester exposure for prematurity or low birth weight, but a positive association was seen for seasonal exposure to elevated atrazine in water during the third trimester and SGA (which is identical to IUGR), OR 1.37 (95% C.I. 1.04-1.81).[64] A retrospective cohort study in Indiana examined 24,514 births. Exposure to atrazine was characterized by area of residence compared to water system measurements. Third trimester exposure to atrazine above 0.103 mcg/L or entire pregnancy exposure to atrazine above 0.644 mcg/L was positively associated with IUGR or pre-term birth at statistically significant levels; prevalence ratios were 1.14 for the entire pregnancy exposure and 1.19 for the third trimester exposures.[65] A case-cohort study of 579 births in France, with 180 affected by fetal growth retardation (FGR, below 5% of the expected weight for gestational age), found increased odds (or=1.5; 95% C.I. 1.0-2.7) of atrazine or its metabolites detected in the urine of a mother of a child affected by FGR compared with the mothers of children unaffected. No association was seen between atrazine in urine and risk for congenital defects.[66] A county-level ecological study of premature birth prevalence and atrazine in public water supplies by Kentucky found increased risk (OR=1.22, 95% C.I. 1.16-1.29) for premature birth in counties with high (>=0.081 mcg/L) levels of atrazine in the water.[67] These studies are presented in table form in Appendix 4.

Despite the association of triazine herbicide level in drinking water and pre-term birth seen in the ecological study in Kentucky, stronger study designs found no association for this outcome alone, or an association of either pre-term birth or IUGR.[65, 67] One ecological study and three retrospective cohort studies found a consistent positive association between triazines in drinking water and IUGR, with odds ratios between 1.17 and 1.5.[63, 64, 65, 66] Although two of these studies characterize exposure by area of residence from public water supply measures, they have the advantage of getting a measurement at a time that is relevant to the outcome. One study was able to use individual measurements of atrazine or its metabolites in urine, which is the best

characterization of exposure available in the studies examined. None focused on well water, but all exposure levels of triazine, mostly below the MCL of 0.1 mcg/L, were meaningful for this study. The literature supports some increased risk of IUGR with elevated levels of triazine herbicide in drinking water during pregnancy, especially during the third trimester, but not for pre-term birth or any other adverse pregnancy-related outcome.

Triazines and cancer: Two reviews, published in 2011 and 2013, of the carcinogenicity of atrazine came to the conclusion that the epidemiological evidence does not support any causal relationship between atrazine and any type of cancer.[68, 69] This was echoed by a review in 2011 that focused on breast cancer, but came to the same conclusion that atrazine is "not likely to be carcinogenic."[70]

This author examined three studies, two ecological and one case-control, from Kentucky and Wisconsin.[71, 72, 73] The focus was breast or ovarian cancers, consistent with the evidence in animals that triazines affect hormone production. An ecological study in Kentucky that compared breast cancer incidence by county to atrazine exposure measures by county found a marginal increased risk for breast cancer with increased triazines in drinking water.[71] A second ecological study from Kentucky with exposure and incidence data at a district level (there are 15 in Kentucky) found no association between atrazine exposure and breast or ovarian cancer incidence.[72] A case-control study of breast cancer (3,275 cases) in Wisconsin found a way to estimate atrazine exposure by assigning subjects the atrazine measurement in the geographically nearest water well, using geocoding. This technique and design was a superior level of evidence, but this study found no increased risk of breast cancer associated with higher levels of atrazine in ground water.[73] The literature does not provide any support for the hypothesis that triazines in drinking water cause cancer.

Fecal contamination of well water

TC include both fecal and non-fecal coliform bacteria. (Coliform bacteria are rod-shaped Gram-negative bacteria that are normally found in the in the feces of warm-blooded animals including humans.) TC are found in soil, surface water, human and animal waste. TC do not cause disease, but their presence in well water is a sign that water has entered the well from the surface or along the top 3-4.5 m of the well below the surface. Fecal material can carry pathogens that cause human disease.[18, 74]

The presence of coliform bacteria in water indicates possible sewage pollution; it is a principal microbiological parameter used to determine water quality.[19] *E. coli* is an effective and accessible indicator organism for fecal contamination of water because it is found in sufficient quantity is all animal feces, but it doesn't multiply outside the intestine. It survives in water for 4-12 weeks.[75] Fecal coliform bacteria in water indicate that human or animal fecal material has gotten into the water.[18] Fecal bacteria applied to the ground, as in crop fertilization with liquid manure, move down below the crop roots in response to rainfall. The potential for fecal bacteria to contaminate groundwater depends on soil structure and water flow. *E. coli*, one type of coliform bacteria, move rapidly through well-structured soils with moderate to high input of water. Poorly-constructed water wells that are vulnerable to having inflow of water at the top or in shallow depths and are located near areas of manure application are a concern for human health.[76]

Septic systems may be a source of coliform bacteria and nitrate contamination of wells. In a study in Frederick, Maryland (not an agricultural area), incidence of coliforms found in well water was correlated with small lot size, meaning the distance between the septic system and the water well was at a minimum. Incidence of coliform bacteria contamination was highest when well casing length was shortest.[16, 18]

Serial testing of 78 wells in 1980 in Oregon found that coliform contamination was higher following rainfall, indicating that surface water was leaking into the wells, and the amount of coliforms in the water was higher in the summer months.[77]

In summer 2009, a sample of 180 private wells in northeastern Ohio was assessed for microbial water quality. 45% were positive for TC, and 9% were positive for *E. coli*. *E. coli* O157:H7, which is a human pathogen, was found in 4% of the specimens.[78] A 2011 sampling program of 538 private wells in Virginia found TC in 41% and *E. coli* in 10%. Unlike the Ohio sample in which contamination was not found to be related to well structure, geography or soil; well depth and location on a farm were predictive of TC contamination.[79]

Health effects of fecal contamination in well water

In 2007-2008, 13 disease outbreaks related to untreated groundwater were reported to the Centers for Disease Control and Prevention, which is over 60% of the total number of disease

outbreaks related to drinking and recreational water during that period.[78] Several enteric bacterial species can be ingested in water and can cause disease, including *E. coli*, *E coli* O157:H7 *Salmonella* spp., and *Shigella* spp., These organisms can cause infective diarrhea in humans, or acute gastrointestinal illness (AGI).[80]

A prospective cohort study conducted with 235 rural households in Ontario, in which incidence of AGI was measured by a questionnaire, and drinking water was sampled and tested concurrently, found no statistically significant relationship between TC or *E. coli* contamination of the water and incidence of AGI, although 20% of the households had a water sample that exceeded bacterial standards for safe drinking water.[81]

Summary

Many epidemiological studies have been conducted to test the hypotheses that nitrate or triazine pesticides contribute to the risk of various cancers. However, they have provided a lack of support overall. For each specific cancer that this study includes as a possible outcome, the literature provides no clear evidence of a link to either nitrate or triazine herbicides in drinking water. It is difficult to get sufficient power for a test with rare outcomes such as cancer, and misclassification is a burden, because of latency issues, because of differences in drinking habits or quantities at home that go unmeasured, and because most studies use an approximation method for the exposure.

It is likewise for adverse reproductive outcomes due to either nitrate or triazines (usually atrazine) or both. Some studies this author examined had good techniques for matching to time or exposure with the outcome, which is easier to do with pregnancy and birth than with cancers, but exposure measurements were still approximated across geographical areas, not made individually. The outcomes that this study measured are not linked to nitrate and triazine contamination in drinking water by epidemiological evidence so far.

The potential for gastrointestinal illness caused by fecal contamination of drinking water is a well-known one, and the success of *E. coli*, or total coliforms, as indicator organisms has been validated. Studies show that fecal contamination of private well water is common in the U.S.

CHAPTER 3

METHODOLOGY

Study design

This is an observational cross-sectional study in which measures of nitrate, triazines, and total coliform or *E. coli* are exposures and self-reported incidence of selected cancers, adverse reproductive outcomes, and gastrointestinal illness are outcomes.

Data sources/measurement

A survey was mailed to 551 unique households in the JPR that had their private well water tested by the KGS between January 1, 1994 and December 31, 2009. (The survey is attached in Appendix 1.) To maximize the response rate, a cover letter was included with each mailing, and a self-addressed stamped envelope for returning the questionnaire. One month after the initial mailing, a second identical mailing was sent to all households that had not responded. Both mailings occurred during the summer of 2009. Returning the survey constituted consent to participate in the study. Questionnaires that were returned were matched with data from the private wells from KGS based on name and address. Only wells that belonged to households that responded to the survey were included in this analysis. One member of a household was asked to complete a survey for all members about events that happened over a span of many years. This study had Institutional Review Board approval through the University of Kentucky. Questions included on the survey were developed by the researcher after a literature review designed to determine the adverse outcomes most commonly associated with the contaminants under study. The initial mailing served as a pilot test for the survey, but no changes were introduced to the survey as a result, before the second mailing.

Setting

The JPR (Kentucky counties Ballard, Calloway, Carlisle, Fulton, Graves, Hickman, Marshall and McCracken.) was chosen because private well water quality data were available for many wells in the area, as part of the work of KGS for the Kentucky Groundwater Data Repository.

Participants

No survey recipient was contacted by the researcher outside of the mailed survey, although two recipients called the researcher, and one of those recipients completed a survey over the phone.

Variables

The following exposure variables were from the water well sampling done by KGS: Nitrate (mg/L), Triazine (mcg/L), Total coliform (cfu), and *E. coli* (cfu). Not all results are available for each address that was sent a survey. All water quality tests were carried out by the KGS. Water samples were tested for *E. coli* using EPA method 1603.[82] Water samples were tested for triazine concentration using EPA method 536.[83] Water samples were tested for NO3-N concentration using EPA method 1686.[84]

Table 1 shows the main variable collected by the survey. Not all participants answered every question of the survey. The complete survey is found in Appendix 1.

Table 1. Main variables collected by the survey with role in analysis

Variable Name	Planned Role in Analysis
Number of people in residence	Sample size
Water filter/conditioner (yes/no)	Effect modifier
Type of water filter	Additional information
Is well source of drinking water (yes/no)	Effect modifier
Does family drink bottled water at home(yes/no)	Additional information
Is bottled water primary source of drinking water (yes/no)	Effect modifier
Any cancer in household (yes/no)	Outcome
Any cancer in family (yes/no)	Outcome
Any Non-Hodgkin's lymphoma in household (yes/no)	Outcome
Any liver cancer in household (yes/no)	Outcome
Any stomach cancer in household (yes/no)	Outcome
Any breast cancer in household (yes/no)	Outcome
Any household pregnancy resulting in underweight (yes/no)	Outcome
Any household pregnancy resulting in neural tube defect (yes/no)	Outcome
Any household pregnancy resulting in congenital malformation (yes/no)	Outcome
Any household pregnancy resulting in intrauterine death (yes/no)	Outcome
Any household pregnancy resulting in miscarriage (yes/no)	Outcome
Did mother have multiple miscarriages? (yes/no)	Effect modifier
Any household pregnancy resulting in premature birth (yes/no)	Outcome
Number of people living at residence for prior 12 months	Sample size
Incidence of GI illness for each person in household in previous 12 months	Outcome
Number of people in household under 12 years old	Effect modifier
Diagnosed person's sex (male/female)	Effect modifier
Age at diagnosis	Effect modifier
Years at residence before diagnosis	Effect modifier
Does the diagnosed person smoke cigarettes (yes/no)	Effect modifier
Age of mother at time of birth	Effect modifier
Did the mother live at residence at time of birth (yes/no)	Effect modifier
Was the mother drinking from the well during pregnancy (yes/no)	Effect modifier
Was the mother smoking cigarettes during pregnancy (yes/no)	Effect modifier

Study size

A survey was mailed to 551 unique households in the JPR that had had their private well water tested by the KGS within 1994-2009. These were all available households with water well test results; this study used the entire population as a sample. Funding for this study was provided by the Southeast Center for Agricultural Health and Injury Prevention.

Quantitative variables – private well data

Nitrate measurements were used as a categorical variable; less than 1 mg/L, 1 to less than 10 mg/L, and greater than or equal to 10 mg/L; in the analysis of its association with triazine contamination. In that analysis, triazine measurements were used as a categorical variable; less than 0.1 mcg/L, and greater than or equal to 0.1 mcg/L. For the association of nitrate with cancer outcomes, nitrate measurements were used as a continuous variable.

Quantitative variables – survey

Cancer outcome was "yes" if the survey respondent reported any cancer in a member of the household while a resident or in someone who had moved away; it was no for the ones who answered both those questions in the negative. Adverse birth outcome was also a dichotomous "yes"/"no" variable in the association with nitrate analysis, where "yes" included any participant who reported an incident of premature birth, intrauterine death, or miscarriage.

TC and *E. coli* measurements were made into categorical variables; one or more cfu colony-forming units (cfu) was "detected," and zero cfu was "not detected." The correlation between TC or *E. coli* and incidence of gastrointestinal illness was calculated using TC and *E. coli* first as continuous variables and then as dichotomous variables.

Statistical methods

Each received survey was given an identification number recorded by the researcher on the survey document. The results from the survey were entered by the researcher into an Excel spreadsheet, which was converted to a SAS dataset for analysis. The data entry from the paper surveys into the Excel spreadsheet was repeated as a quality check. The surveys were matched to the well water testing data from KGS by address. Analysis was performed using SAS 9.2.

Not all wells were tested for all three contaminants; survey records were included in analyses only if the household matched a well that had been tested for the particular contaminant under study. For wells that were tested for bacterial contaminants on multiple occasions, the most recent value was used for analysis, because the relevant health outcome was the incidence of gastrointestinal illness in the previous 12 months. For wells that were tested for NO3-N and triazine concentration on multiple occasions, the values were averaged into a single value for analysis, because the relevant health outcomes may have taken place over decades.

Person-years at risk for standardized incidence ratio (SIR) calculation was not available from the sample. It was estimated at 20,560, which is 514 people total represented by the number of surveys in the analysis (214) multiplied by the average household size reported (2.4). Using U.S. census projected data for 2009, the median age for the Purchase district is 40 years old. The result, 20560 person years is (514 X 40). The exception is breast cancer; the person-years at risk was 11,130. Using U.S. census projected data for 2009, the median female age for the Purchase district is 42 years old, and females make up 51.5% of the population; these figures were used against the 524 estimated persons in the sample to calculate person-years at risk.

Fisher's exact test for association was performed for the association of nitrate contamination and triazine contamination in well water. ORs and 95% confidence intervals were calculated for the association of well type with TC, *E. coli*, and nitrate contamination. Wilcoxon rank sum statistics were calculated for the association of nitrate contamination with cancer and with adverse birth outcomes. Pearson correlation coefficients were calculated for the association of total coliform and *E. coli* contamination and incidence of AGI in the household.

CHAPTER 4

RESULTS

Participants

Five hundred fifty-one questionnaires were sent out. After two mailings, 220 were returned. This is a response rate of 39.9%. Of those, 218 were able to be matched with names and addresses in the available pool of well owner data. Three of the valid questionnaire responses indicated that the well owner was deceased or had stopped using the well; these records were not used in the analysis. One person responded that the household associated with the water well had no residents and provided no other questionnaire answers; this record was also not used for analysis. Two hundred fourteen survey responses were available for analysis (39.8%). No demographic information was collected from the participants. Aside from the number of persons in the household, and the number of those under 12 years old, no demographic information was collected about individuals. Figure 1 shows the number of participant households at each step before analysis. Figure 2 shows the geographical spread of the wells with contaminant data that were surveyed, and the households that responded.

Figure 1. Study Participants, Jackson Purchase Region, 2009

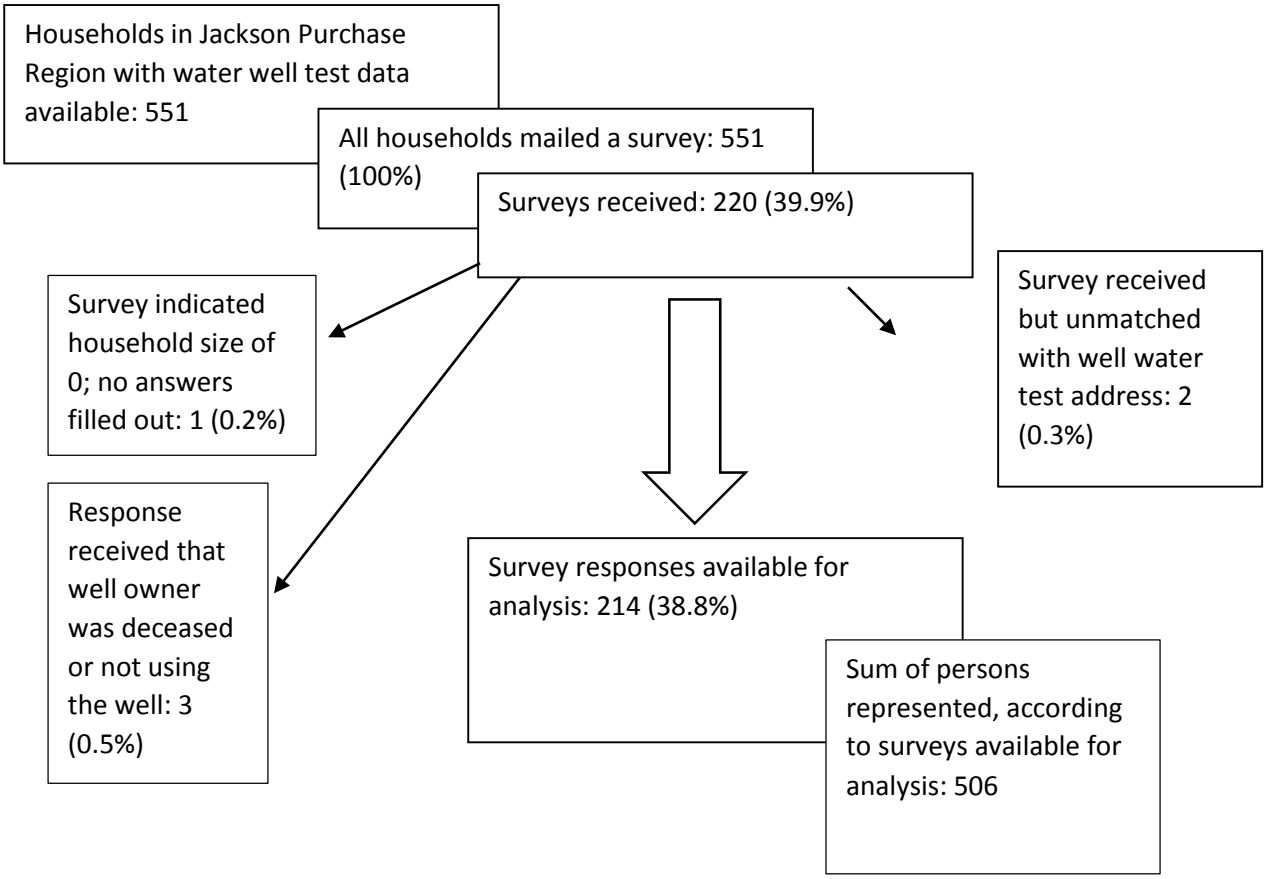
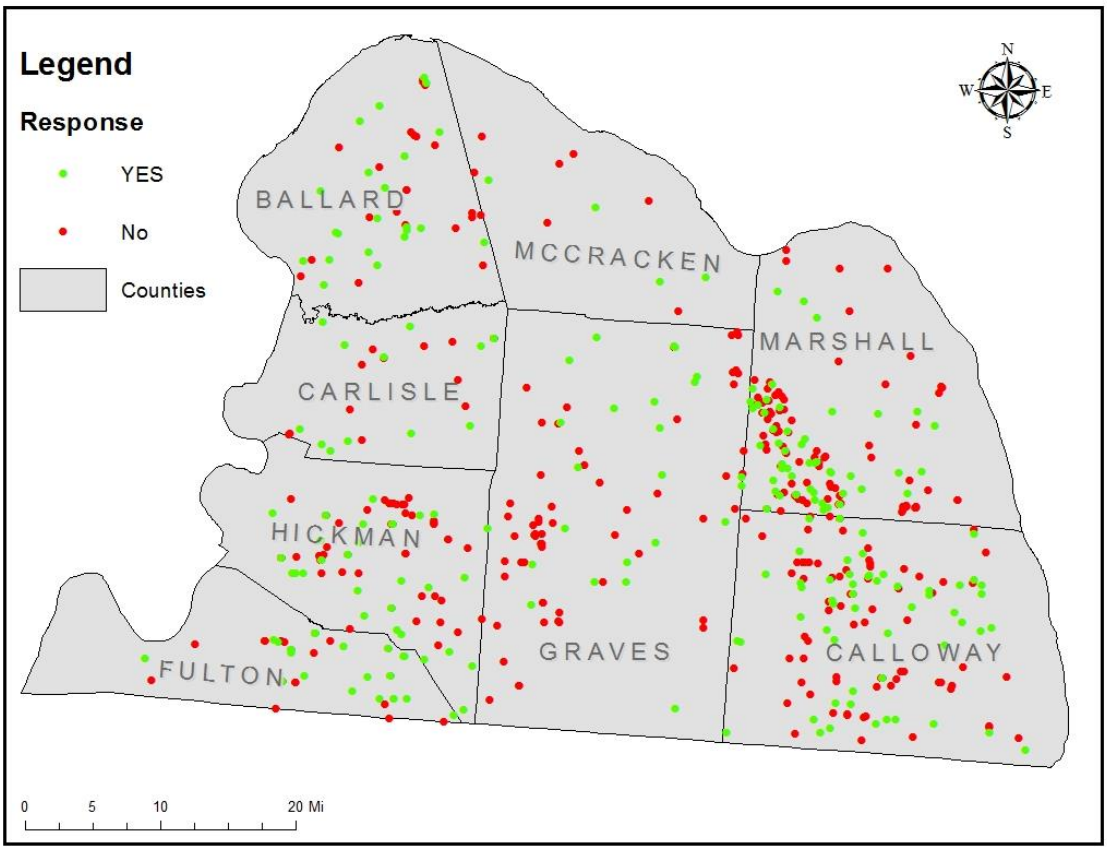


Figure 2. Water wells with available contaminant data and respondent households



Survey data

210 people answered the question "How many people reside in your household?" The mean was 2.4, the median was 2, and the range was 1 to 6.

Of 213 responses to the question "Do you have a water filter?" Table 2 shows 177 households (83%) responded that they had no water filter and 34 households (16%) used one or more water treatment methods. (Table 2)

Table 2. Survey responses on home water filtration methods.

Responses to the question "Do you have a water filter?"		
	N	Percent
No home water filtration method	172	81%
One or more water filtration methods listed*	39	18%
"Don't know"	2	1%
Total	213	
Description of home water filtration system*		
	N	Percent
Active carbon filter	9	23%
Ion-exchange unit	1	3%
Reverse osmosis unit	3	8%
Water conditioner	7	18%
Distillation unit	2	5%
Counter-top unit or pour-through carafe/pitcher	4	10%
Faucet-mounted filter	8	21%
Whole-house sediment removal	19	49%
Other**	4	10%
*Total number of respondents to this question was 39, counting all respondents who described a water filtration system of any kind. Respondents could choose more than one method.		
** Other methods described were chlorine, strainer, filter and softener.		

213 answered the question, “Do members of your household use your well water as primary drinking water?” Of those, 179 (84.04%) answered “yes.” 213 households answered the question “Do members of your family purchase bottled water for drinking at home?” Of those, 87 (40.85%) answered “yes.” Responses to the question “Is bottled water your primary source of drinking water at home,” were mostly consistent with responses to the former question. (Table 3)

Table 3. Survey responses on home water filtration methods

Question	Yes
Do members of your household use your well water as primary drinking water?	179 (84.04%)
Do members of your family purchase bottled water for drinking at home?	87 (40.85%)

192 households provided an estimate above zero of how many years they had been drinking well water. The mean was 24 years; the median value was 20 years; the range was 0.5 to 100 years.

All of the information about length of time at the address, whether the family drank water from the well primarily, and what type of water filtration system they had was intended for use as effect modifying factors in multivariable models but the study power was insufficient to control for any of these.

Water contaminant data

211 households in the data set had nitrate levels available. Eleven (5.19%) had average values above the MCL of 10 mg/L. The values ranged from 0.002 mg/L to 25.04 mg/L. Quartile values were 0.678, 1.911 and 4.000 mg/L. 189 households in the data set had triazine levels available. 123 of those had levels below the detection limit of 0.06 µg/L (65.08%); 1 had a level above the MCL set by the EPA for atrazine of 3 mcg/L (0.53%). The range was 0.030 to 4.030 µg/L. 172 households in the data set had total coliform counts available. 70 of those (40.70%) were negative for total coliforms. Positive samples (59.30%) ranged from 1 cfu to 1011.2 cfu per 100 mL.

173 households in the data set had *E. coli* counts available. 149 of those (86.13%) had <1 cfu per 100 mL. Samples positive for *E. coli* contamination (13.87%) had cfu counts ranging from 1 cfu to 378 cfu per 100 mL. (Table 4)

Table 4. Water contaminants in the private water wells of study respondents in the Jackson Purchase Region, 1994-2009

Contaminant	Number wells sampled (N)	Mean (SE)	Median	Range	Number (%) of wells above MCL	Number (%) of wells less than background or below detection level
Nitrate	211	3.03 mg/L (0.24)	1.91 mg/L	0.00-25.04 mg/L	11	69 (32.7%) <1 mg/L
Triazines	189	0.17 mcg/L (0.03)	0.03	0.03 - 4.03 mcg/L	1	123 (65.1%) <0.06 mcg/L
Total coliform	172	104.34 cfu (22.50)	2.50	<1 - 2420 cfu	102 (59.3%)	71 (41.2%)
E. coli	173	8.87 cfu (3.45)	0.00	<1 - 378 cfu	25 (14.5%)	148 (85.5%)

Association between nitrate and triazines

Nitrate contamination in water wells among the survey respondents was not independently distributed from triazine contamination in those wells. Table 5 shows that triazine measurements above 0.1 mcg/L, which are 25% of the total, are more likely to occur in wells that have above-background (>1 mg/L) measurements of nitrate.

Table 5. Association between NO3-N and triazines.

	Triazine		
Nitrate	< 0.1 mcg/L	>= 0.1 mcg/L	Total
< 1 mg/L	63 (39.6%)	6 (11.5%)	69 (32.7%)
>= 1 and < 10 mg/L	89 (56.0%)	42 (80.8%)	131 (62.1%)
>= 10 mg/L	7 (4.4%)	4 (7.7%)	11 (5.2%)
Total	159	52	211

Fisher's Exact Test two-sided Pr <= P: <0.0001

Well depth and type and its association with contaminants

Among the water wells associated with survey respondents' households, there were 122 (57%) 4- or 6-inch diameter drilled wells, and 92 (43%) bored wells between 24 and 34 inches diameter.

Larger well diameter was associated with the likelihood of both *E. coli* and TC contamination in this sample, with greater likelihood of *E. coli* and TC being detected in the water from bored wells.

E. coli and TC data were grouped into zero ("not detected") and non-zero ("detected") categories. Table 6 shows that the larger-diameter bored wells were many times more likely to be contaminated with TC or *E. coli* than drilled wells.

Table 6. Odds of fecal contamination by well type

Well type	Coliform bacteria		*E. coli*	
	Odds Ratio	(95% Confidence Interval)	Odds Ratio	(95% Confidence Interval)
Drilled (4-6 in. diam.)	1.00	Referent	1.00	Referent
Bored (24-34 in. diam.)	8.14	(3.88-17.06)	8.63	(2.81-26.43)

Table 7 shows that the larger-diameter bored wells were more likely to have nitrate measurements above the MCL of 10 mg/L, but less likely to have nitrate in excess of background level of 1 mg/L than drilled wells. This is because most (62%) of the nitrate measurements were between 1 and 10 mg/L, those measurements were more evenly distributed in the bored wells. The odds of bored wells having nitrate above the MCL is not significant, however.

Table 7. Odds of nitrate contamination by well type

Well type	Nitrate above MCL		Nitrate above background	
	Odds Ratio	(95% Confidence Interval)	Odds Ratio	(95% Confidence Interval)
Drilled (4-6 in. diam.)	1.00	Referent	1.00	Referent
Bored (24-34 in. diam.)	3.68	(0.78-17.47)	0.44	(0.24-0.80)

The odds ratios for atrazine were not calculated because only 1 sample exceeded the MCL of 3 mcg/L.

Outcome data – Cancers

The survey asked if any member of the household had been diagnosed with cancer while living at the address, and then if any member had been diagnosed with a cancer after moving away. The survey specifically asked about incident cases of these types of cancers: NHL, liver, stomach, breast, bladder and ovarian. The answers for each of these questions included "yes," "no," and "don't know." There were zero reports of bladder or ovarian cancers. (Table 8)

Table 8. Reported cancers in participant households

Cancer outcome	Number (percentage of respondents)
Any cancer in household	54 (25.47%)
Any cancer in household member after moving	15 (12.40%)
Non-Hodgkin's lymphoma	7 (3.30%)
Liver	6 (2.83%)
Stomach	2 (0.94%)
Breast	16 (7.55%)

Reported incidences of NHL, liver cancer, stomach cancer and breast cancer among survey respondents were compared to historical rates of those cancers from the JPR, Kentucky, and the U.S. SIR and 95% confidence intervals are presented in Table 3. Person-years information is not available from the survey respondents; person-years at risk were estimated based on U.S. census projected population information for 2009 for the JPR. Comparisons were made to multiple reference populations in order to account for underlying incidence differences of these cancer outcomes between the local population and larger ones.

This survey did not collect the person-years at risk information required to calculate SIRs in a standard way. The researcher created person-years at risk for the study population: It was estimated at 20,560, which is 514 people total represented by the number of surveys in the analysis (214) multiplied by the average household size reported (2.4). Using U.S. census projected data for 2009, the median age for the Purchase district is 40 years old. The result, 20560 person years is (514 X 40). The exception is breast cancer; the person-years at risk was 11,130. Using U.S. census projected data for 2009, the median female age for the Purchase district is 42 years old, and females make up 51.5% of the population; these figures were used against the 514 estimated persons in the sample to calculate person-years at risk.

Table 9 shows that this study population experienced a higher than expected incidence of liver cancer, compared to the local, state and U.S. reference populations. No other reported type of cancer showed the same kind of excess incidence.

Table 9. Standardized Incidence Ratios for selected cancers in the study households.

		Purchase Region			
	Cases	Incidence*	Expected cases	SIR	95% C.I.
Non-Hodgkin's lymphoma	7	19.89	4	1.71	(0.69-3.52)
Liver cancer	6	2.79	1	**10.46**	**(3.84-22.77)**
Stomach cancer	2	4.33	1	2.25	(0.272-8.12)
Breast cancer	16	90.04	10	1.60	(0.91-2.59)
		Kentucky			
	Cases	Incidence*	Expected cases	SIR	95% C.I.
Non-Hodgkin's lymphoma	7	19.83	4	1.72	(0.69-3.54)
Liver cancer	6	4.33	1	**6.74**	**(2.47-14.67)**
Stomach cancer	2	5.83	1	1.67	(0.20-6.03)
Breast cancer	16	78.07	9	**1.84**	**(1.05-2.99)**
		U.S.			
	Cases	Incidence**	Expected cases	SIR	95% C.I.
Non-Hodgkin's lymphoma	7	19.6	4	1.74	(0.70-3.58)
Liver cancer	6	6.9	1	**4.23**	**(1.55-9.21)**
Stomach cancer	2	7.8	2	1.25	(0.15-4.51)
Breast cancer	16	122.9	14	1.17	(0.67-1.90)

*Age-adjusted incidence per year per 100,000 persons for 2003-2007 standardized to U.S. 2000 standard million from Kentucky Cancer Registry

**Age-adjusted incidence per year per 100,000 persons for 2003-2007 from U.S. Surveillance, Epidemiology, and End Results Program Registry

Bold type indicates a p-value ≤ 0.05.

Person-years at risk for SIR calculation was not available from the sample. It was estimated at 20560, which is 514 people total represented by the number of surveys in the analysis (214) multiplied by the average household size reported (2.4). Using U.S. census projected data for 2009, the median age for the Purchase district is 40 years old. The result, 20560 person years is (514 X 40). The exception is breast cancer; the person-years at risk was 11130. Using U.S. census projected data for 2009, the median female age for the Purchase district is 42 years old, and females make up 51.5% of the population; these figures were used against the 524 estimated persons in the sample to calculate person-years at risk.

Association of nitrate with cancer outcomes

It was intended to create logistic models for each of the four cancer outcomes in which any cases were reported as a function of nitrate contamination, but there were too few reported cases to perform this analysis. Because the nitrate measurements in this sample are not normally, distributed, a t-test comparison of mean nitrate values between cancer case households and non-cancer case households was not performed. The Wilcoxon Rank Sum test was performed to disprove the hypothesis that the distribution of nitrate measurements is identical between households with reported cancers and households without cancers. Table 10 shows the results of this test, where the participant households were classified based on their answers to two questions: 'Has any member of your household been diagnosed with cancer', or 'Has any member of your household been diagnosed with cancer after moving away'; affirmative answers to these two questions were merged into a 'Yes' category. NO3-N is a continuous variable.

Table 10. Association of nitrate with any reported cancer in a participant household.

				t approximation
	Class	N	Median NO3-N	Pr > |Z|
Response to questions: Any cancer in a member of your household?	No	151	1.68 mg/L	0.0241
	Yes	60	2.58 mg/L	

With incident cancers counted this way, the Wilcoxon two-sample test statistic is significant at 0.05; the distribution of nitrate is not identical between households with cancers and households without. The median nitrate measurement is higher for the households reporting a member with cancer.

If incident cancers are counted by summing the reported liver, stomach, breast cancers and NHL, the totals were 27 households reporting a cancer and 184 not; the same test generated a result that was not significant at 0.05.

Outcome data – Adverse birth outcomes

The survey asked if any member of the household had experienced one of the following adverse birth events: a low birth weight infant (<2500g), neural tube defect, congenital malformation, intrauterine death, miscarriage, or premature birth (birth before 37 weeks gestation). No neural tube defects were reported. One congenital malformation was reported. Neither of these adverse birth outcomes were included in the analysis. Reports of low birth weight and premature birth

were largely co-incident; it was concluded that the respondents were not differentiating between the two. Low birth weight was dropped from the analysis in favor of premature birth. The incidence of adverse birth outcomes in this population is shown in table 11. Information provided in the survey did not allow for calculation of standardized incidence ratios.

Table 11. Reported adverse birth outcomes in participant households

Adverse birth outcome	Number (percentage of respondents)
Intrauterine death	3 (1.40%)
Miscarriage	12 (5.61%)
Premature birth	4 (1.87%)

The survey asked if multiple instances of intrauterine death, miscarriage, or premature birth had occurred to the same woman or to other women in the same household. Positive responses were few, and these observations were not analyzed because they are possibly not independent events. Any "yes" answer for an instance of intrauterine death, miscarriage, or premature birth was combined into a yes/no answer for any adverse birth event. The Wilcoxon Rank Sum test was performed to disprove the hypothesis that the distribution of nitrate measurements is identical between households with reported adverse birth outcomes and households without. Table 12 shows the median nitrate measurement in households that reported an adverse birth outcome was higher, but the test statistic is not significant at 0.05, so it is not ruled out that the nitrate measurement distributions in the two groups are the same.

Table 12. Association of nitrate with any reported adverse birth outcome in a participant household.

				t approximation
	Class	N	Median NO3-N	Pr > |Z|
Response to questions: Any adverse birth outcome in a member of your household?	No	195	1.83 mg/L	0.4132
	Yes	16	3.40 mg/L	

Correlation between GI illness incidence and fecal contamination

Observed values for *E. coli* and TC, measured in colony-forming units (cfu) were transformed where necessary from "below detection limits" and "above detection limits" to numerical values. "Below detection limits" was observed as "<1"; to create a continuous numerical variable, these

were transformed to 0. “Above detection limits” was observed as either “>200.5” (31 instances for TC, 2 for *E. coli*) or “>2419.6” (2 instances for TC); to create a continuous numerical variable, these were transformed to 201 and 2420, respectively. (Two observed numerical values for *E. coli* were above 300; this is thought to be a difference in lab techniques used on these water samples.)

When a respondent gave a non-numerical answer for the incidence of AGI that could not easily be translated to an appropriate answer, that value was left missing. An example of an answer that was translatable is “6 or 7”; an answer like this would be recorded as 6.5. If a respondent did not indicate how many people were in the household, no household rate of AGI was calculated, and the record was not used for analysis of household rates. Despite whatever answer a respondent gave for how many persons lived in the house in the past 12 months, the household rate was calculated using the number of people for whom incidence of AGI was given.

Pearson correlation coefficients were generated for the relationship between both *E. coli* and TC, using the continuous scale, and household incidence of AGI, as a per-person rate. *E. coli* contamination approached, but did not quite reach, significance at alpha=0.05. TC contamination was not related to household incidence of AGI.

Correlation coefficients were generated for the relationship between both *E. coli* and TC, using the dichotomous variable (detected/not detected), and household incidence of AGI, as a per-person rate. TC contamination approached, but did not quite reach, significance at alpha=0.05. *E. coli* contamination was not related to household incidence of AGI.

Correlation coefficients were also generated for the relationship between both *E. coli* and TC, using the dichotomous variable (detected/not detected), and incidence of AGI in the previous 12 months reported by the survey respondent only. Neither *E. coli* nor TC contamination were related to incidence of AGI in the previous 12 months.

Table 13. Correlation coefficients for Acute Gastrointestinal Illness in household and fecal contaminants

E. coli and total coliform modeled as continuous variables		
	Pearson correlation coefficient Pr > |r|	
	E. coli	**Total coliform**
Household per person incidence of AGI in the past 12 months	0.16 0.06	-0.02 0.80
E. coli and total coliform modeled as dichotomous variables		
	E. coli	**Total coliform**
Household per person incidence of AGI in the past 12 months	0.08 0.36	0.06 0.06
Incidence of AGI in the past 12 months for primary survey respondent only	0.00 0.98	-0.02 0.83

CHAPTER 5

IMPLICATIONS FOR PUBLIC HEALTH

Key Results

Owners of domestic water wells in the JPR of Kentucky were surveyed about incidence of selected cancers, adverse birth outcomes and AGI in their households. The objective was to see if any disproportionate incidence of these diseases was correlated with nitrate, herbicide or bacterial contamination of well water which is common in agricultural areas.

This cross-sectional study showed a possible association between elevated levels of nitrate in privately supplied well water and cancer incidence in families living in the JPR of Kentucky, but due to many limitations of the study, the result should be viewed with skepticism. No association was seen between elevated nitrate in well water and adverse birth outcomes; including intrauterine death, premature birth, or miscarriage. No statistically significant association was seen between contamination with TC or *E. coli* and the incidence of gastrointestinal illness in this population.

The amount of *E. coli* found in well water had a small positive correlation with the incidence of AGI in the households that responded to this survey, but the correlation is not quite statistically significant at 0.05. The correlation could indicate a relationship that is plausible and expected.

Other results of interest

In this sample of 214 water wells, 5.19% had nitrate levels above the MCL of 10 mg/L. This is consistent with results from the 1996 Cooperative Private Well Testing Program study of wells in Ohio, Indiana, Illinois, West Virginia, and Kentucky (3.4%), and less than a sample of private water wells in Iowa (18.3%).

Only one household in this sample (0.53%) had well water with a triazine level above the MCL of 3 µg/L; it was detected in 66 (35%) of the samples. This is consistent with results from the 1996 Cooperative Private Well Testing Program study of wells in Ohio, Indiana, Illinois, West Virginia, and Kentucky (4.9% detection, 0.1% above the MCL), although the proportion of wells with detectable atrazine levels is higher in this sample.

59% of water wells in this sample had coliform bacteria detected in the water, and 14% had *E. coli* detected in the water. Both coliform bacteria and *E. coli* were several times more likely to be detected in the water from larger-diameter bored wells than the smaller-diameter drilled wells; the OR for detection of *E. coli* is 8.63, and for TC is 8.14.) This is consistent with other studies of the effect of poor well construction in the shallow depths on fecal contamination of water.

Nitrate and triazine contamination were positively associated in this sample of water wells, which is consistent with other studies and indicates the contaminants' common agricultural origin.

Most households surveyed reported drinking water from their wells. The median length of time the household had been drinking the well water was 20 years. This indicates long exposure times to well water

Limitations

The chief limitation of this study was its small size; it was predicted from the outset that with relatively rare outcomes such as cancers, the study would lack sufficient power to show meaningful results, which did occur. The small numbers of reported outcome measures also truncated the planned analysis. It was hoped that the incidence of cancer and adverse birth outcomes could be modeled as functions of both nitrate and triazine in the well water, using multivariate logistic regression, but there were too few reported outcomes or elevated triazine measurements to use adjusted models. Smoking status and age are important determinants of cancer incidence. These were collected by the survey, but they were not included in any analysis because small numbers of cancer outcomes prevented this. Similarly, respondents were asked if they relied primarily on their well for drinking water, how long they had been using that well, and whether they had any water treatment systems in place. Again, these potential strata of exposure could not be used in the analysis.

Next, the study design is cross-sectional, which can point toward possible associations between factors, but cannot demonstrate a causal relationship. Cross-sectional study assumes the putative exposures and outcomes measured at the same time, but another limitation of this study had to do with the long length of time over which the exposure measurements were taken. There was no effort made to ensure that the one measurement in time (or in some cases, a few) represented the

water quality during the entire time period that the survey asked about, or that it was specific to the time immediately before an adverse birth outcome. For each outcome in this study, cancers, adverse birth outcomes, and gastrointestinal illnesses, it's very possible the timing of a nitrate or coliform measurement did not match any purported period when that contaminant might have contributed to the outcome event. This may have led to misclassification, but most especially with bacterial contamination.

Well owners who were alerted their water had detectable coliforms or E. coli may have taken some action to clean their water, so the contamination might have no overlap in time with the "previous 12 months" asked about in the survey. Nitrate levels above the MCL that would negatively affect a pregnancy also need to exist at the same time as the pregnancy; no effort was made to match the date of nitrate test results with any adverse birth outcomes (Year of adverse birth outcome was collected. The small study size prevented this, also, from being used as planned in the analysis.) If it can be assumed that high nitrate level in well water would be consistent over many years because the source of nitrate (i.e. land use) would be unlikely to change and nitrate contamination would be difficult to fix, then the potential for misclassification of exposure would be least important for the relationship between nitrate in well water and the cancer outcomes, because cancers have long latency periods.

A prospective study of either adverse birth outcomes or incidence of AGI could reveal a real causal relationship between elevated nitrate (or nitrate and triazines together) in well water and adverse birth outcomes or between *E. coli* contamination and AGI incidence. A case control study would be more appropriate for the study of cancer outcomes because they are rare.

The lack of a control group in this study design also means that it is not capable of ruling out other factors as causes of the outcomes being measured, or to control for them. There are many suggested or unknown causes for cancers or adverse birth events. Although AGI outbreaks are often traced to drinking water sources, there are other explanations for AGI that were not taken into account by this study. For instance, one survey respondent called this researcher to explain that his frequent bouts of GI distress were caused by irritable bowel syndrome. Failure to collect alternative causes of AGI would mistakenly attribute all of any excess incidence to bacterial contamination of well water.

Selection bias was not an issue when the survey was sent out, because it was mailed to every household with well water testing results from the previous 15 years, an entire cohort not sampled on the basis of any health concern.

Non-response is a potential source of bias in survey studies such as this one; people that might be more likely to respond to the survey would include (1) people aware of contamination in their well water, (2) people whose wells were tested more recently, (3) people with greater numbers of adverse health outcomes among their family members, and (4) people whose adverse health outcomes are more recent. No effort was planned to assess or address this, aside from the second mailing of the survey to boost response. One member of a household was asked to complete a survey for all members about events that happened over a span of many years; this might have resulted in inaccurate recall that biased the study toward better information on more recent adverse health events.

Because cancers, birth outcomes and AGI are self-reported instead of observed, recall bias, or misclassification of outcome, is expected to be a limitation. If so, it would bias the results away from the null value of no relationship. Responses to questions about AGI in the previous twelve months were incomplete, and as expected, many were approximate estimates.

The information collected by the survey would have been more useful if it had included a demographic description of each member of the household, including age. With age, a true SIR could have been calculated for cancer incidence. But the survey was designed assuming this population would be uninclined to answer intrusive questions. 214 households, of 551 who were approached, responded to the survey and contributed records to the analysis. The effective response rate was 39%, which was higher than expected. One researcher associated with this project felt that people in the JPR would be less likely to want to participate in research associated with one of Kentucky's public universities, but they would also be a group of people motivated to cooperate with the KGS because they already had a relationship with that agency. (conversation, E. Glynn Beck) This issue with what to ask the potential participants, and the idea that they might be less willing to answer questions in the future, led to another weakness of the study design. The study should have focused on (1) a possible association between nitrate and triazine contamination and cancer incidence, or (2) the same set of exposures and adverse birth outcomes, or (3) bacterial contamination and AGI incidence.

Interpretation

The results seen in this study do not support any action focused on protecting persons relying on privately-supplied well water for drinking from common agricultural contaminants, including nitrate and triazine herbicides, or bacterial contamination from leaking septic tanks, manure applications, or feedlots; in the frequency and concentrations seen in this area of Kentucky. Because of study limitations that have to do with the study design, study size, non-control of potential confounders in the analysis, and some flaws in execution, any results that indicate a possible relationship between the contaminants measured and the outcomes examined should not be considered as strong evidence. The frequency of contamination of the wells in this sample with nitrate, triazine, TC, and *E. coli* are consistent with that of other agricultural areas in the U.S. For nitrate specifically, the results of this study are consistent with a 2006 statement that not enough evidence is available showing that levels of nitrate in water below the MCL have any association with long-term outcomes such as cancer.[35] This small study is consistent with the majority of studies this author examined that showed no relationship between nitrate in drinking water at low levels below the MCL and the incidence of various cancers or adverse reproductive outcomes. Triazine contamination in drinking water, although not linked in the literature to cancer incidence, or linked persuasively with adverse reproductive outcomes, was not analyzed in this study. This study, like the prospective cohort examined, did not show a statistically significant additional risk for AGI from water contamination with TC or *E. coli*, though that study was better designed because the testing was concurrent with the questionnaire.

Implications

Groundwater is a source less prone to contamination than surface water. In Kentucky, well owners are considered responsible for their own water quality, by law. Owners have access to water quality testing and to remediation if their water is contaminated. This study does not provide evidence to support intervening in this situation because it does not show any way in which the population suffers poor health outcomes because of contaminated well water.

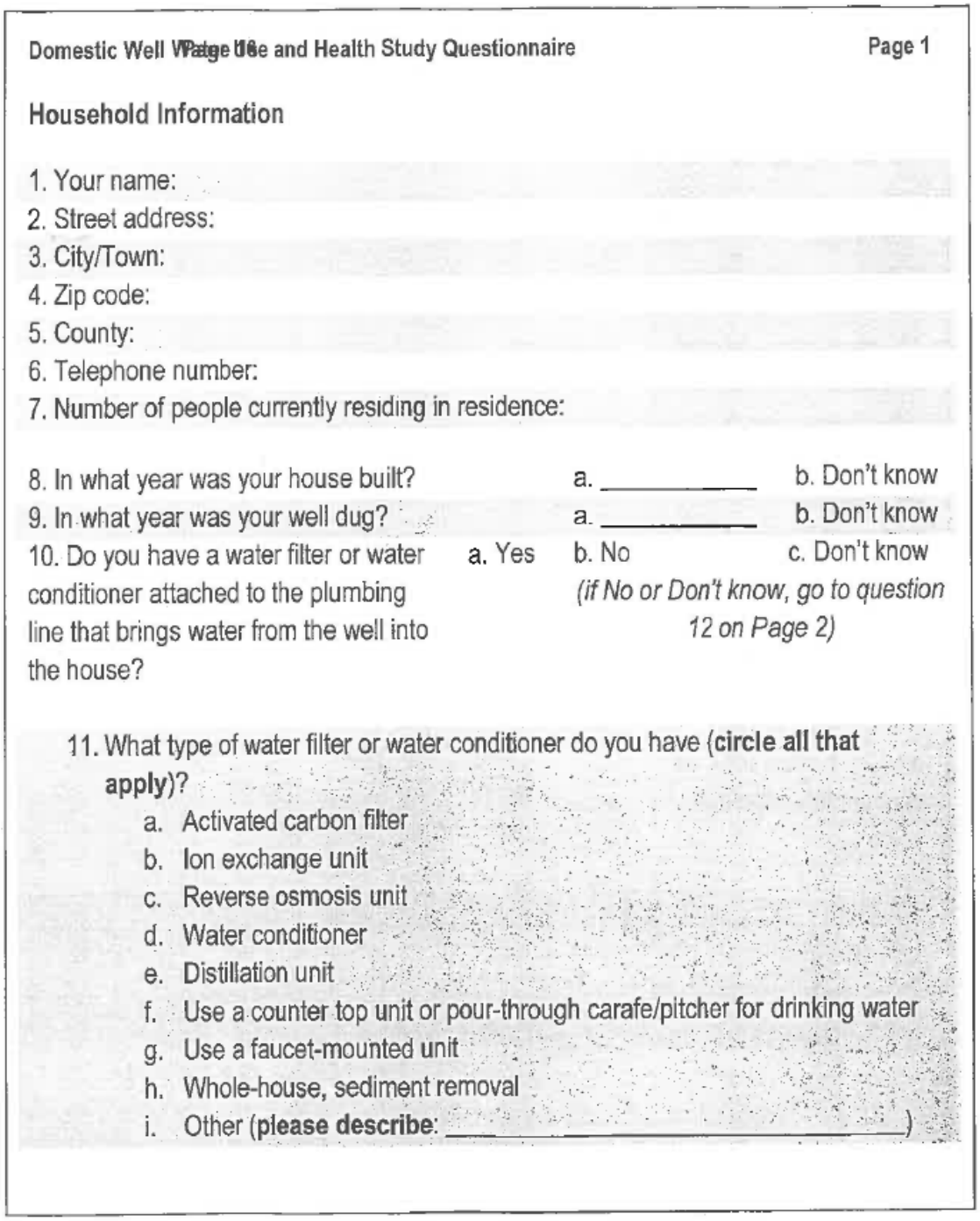
Urbanization is, over time, reducing the number of people who get their water from private wells, a trend that will continue as long as fewer people are required for agriculture. If well water could be considered a dangerous exposure, then the frequency is already being reduced and the scope of the problem is shrinking even now. Kentucky, however, is a more rural state than the

country as a whole, so this problem could be considered one over which our state has a unique kind of ownership. In Kentucky, 24.0% of persons live in rural areas; in the U.S., 6.3% of the population lives in rural areas.[23] Further research could be focused on evaluating whether Kentuckians who rely on private wells for drinking water are adequately informed about how to prevent contaminants from entering their wells, or how often water quality testing might benefit them. Then policy makers should ensure that these citizens have access to testing, and remediation if necessary.

The association between fecal contamination of drinking water and intestinal illness is well established, and this study revealed that a high percentage of well owners in this area had indication of fecal contamination of their drinking water at some time in the recent past. Policy makers should make sure that financial constraints do not prevent this population from testing or remediating their drinking water.

APPENDIX 1

The Survey Instrument



Domestic Well Water Use and Health Study Questionnaire Page 1

Household Information

1. Your name:
2. Street address:
3. City/Town:
4. Zip code:
5. County:
6. Telephone number:
7. Number of people currently residing in residence:

8. In what year was your house built? a. _____ b. Don't know
9. In what year was your well dug? a. _____ b. Don't know
10. Do you have a water filter or water conditioner attached to the plumbing line that brings water from the well into the house? a. Yes b. No c. Don't know *(if No or Don't know, go to question 12 on Page 2)*

11. What type of water filter or water conditioner do you have (**circle all that apply**)?
    a. Activated carbon filter
    b. Ion exchange unit
    c. Reverse osmosis unit
    d. Water conditioner
    e. Distillation unit
    f. Use a counter top unit or pour-through carafe/pitcher for drinking water
    g. Use a faucet-mounted unit
    h. Whole-house, sediment removal
    i. Other (**please describe**: _____)

12. Do members of your household use your well water as primary (50% or more of use) drinking water?	a. Yes	b. No	c. Don't know
13. Do members of your household purchase bottled water for drinking at home?	a. Yes	b. No *(if No, go to question 16)*	
14. Is bottled water your primary (50% or more of use) source of drinking water at home?	a. Yes	b. No *(if No, go to question 16)*	
15. If Yes, for how many years have you been drinking bottled water as your primary source at home?	a. _____ years	b. Don't know	
16. For how many years have you been drinking water from your household well?	a. _____ years	b. Don't know	

The following pages ask questions about the health status of your family.

Cancer Outcomes

17. Has any member of your household been diagnosed with **any type of cancer** while living at your present location?	a. Yes	b. No *(if no, go on to question 60 on Page 10)*	c. Don't know
18. Has any person who previously lived with your family at your current location been diagnosed with **any type of cancer** after he or she moved away?	a. Yes	b. No *(if no, go on to question 60 on Page 10)*	c. Don't know

Non-Hodgkin's Lymphoma: a cancer affecting white blood cells.

19. Has anyone in your family who lives, or lived, at your present location, been diagnosed with **Non-Hodgkin's lymphoma**?	a. Yes	b. No *(If No or Don't know, go on to question 26 on Page 5)*	c. Don't know
20. If yes, is the person diagnosed with lymphoma cancer male or female?	a. Male	b. Female	
21. What calendar year was this person born?	a. _____		b. Don't know
22. What year was this person diagnosed with **Non-Hodgkin's lymphoma**?	a. _____		b. Don't know
23. How long did this person live at your present location before receiving a diagnosis of **Non-Hodgkin's lymphoma**?	a. _____ years		b. Don't know
24. Does this person smoke cigarettes?	a. Yes	b. No	c. Has quit
25. Have any other members of your household been diagnosed with **Non-Hodgkin's lymphoma**?	a. Yes	b. No	c. Don't know

Bladder Cancer

26. Has anyone in your family who lives, or lived, at your present location, been diagnosed with **bladder cancer**?	a. Yes	b. No *(If No or Don't know, go on to question 33 on Page 6)*	c. Don't know
27. If yes, is the person diagnosed with **bladder cancer** male or female?	a. Male	b. Female	
28. What calendar year was this person born?	a. _____		b. Don't know
29. What year was this person diagnosed with **bladder cancer**?	a. _____		b. Don't know
30. How long did this person live at your present location before receiving a diagnosis of **bladder cancer**?	a. _____ years		b. Don't know
31. Does this person smoke cigarettes?	a. Yes	b. No	c. Has quit
32. Have any other members of your household been diagnosed with **bladder cancer**?	a. Yes	b. No	c. Don't know

Liver Cancer

33. Has anyone in your family who lives, or lived, at your present location, been diagnosed with **liver cancer**?	a. Yes	b. No *(If No or Don't know, go on to question 40 on Page 7)*	c. Don't know
34. If yes, is the person diagnosed with **liver cancer** male or female?	a. Male	b. Female	
35. What calendar year was this person born?	a. _____		b. Don't know
36. What year was this person diagnosed with **liver cancer**?	a. _____		b. Don't know
37. How long did this person live at your present location before receiving a diagnosis of **liver cancer**?	a. _____ years		b. Don't know
38. Does this person smoke cigarettes?	a. Yes	b. No	c. Has quit
39. Have any other members of your household been diagnosed with **liver cancer**?	a. Yes	b. No	c. Don't know

Stomach Cancer

40. Has anyone in your family who lives, or lived, at your present location, been diagnosed with **stomach cancer**?	a. Yes	b. No *(If No or Don't know, go on to question 47 on Page 8)*	c. Don't know
41. If yes, is the person diagnosed with **stomach cancer** male or female?	a. Male	b. Female	
42. What calendar year was this person born?	a. _____		b. Don't know
43. What year was this person diagnosed with **stomach cancer**?	a. _____		b. Don't know
44. How long did this person live at your present location before receiving a diagnosis of **stomach cancer**?	a. _____ years		b. Don't know
45. Does this person smoke cigarettes?	a. Yes	b. No	c. Has quit
46. Have any other members of your household been diagnosed with **stomach cancer**?	a. Yes	b. No	c. Don't know

Ovarian Cancer

47. Has anyone in your family who lives, or lived, at your present location, been diagnosed with **ovarian cancer**?	a. Yes	b. No *(If No or Don't know, go on to question 53 on Page 9)*	c. Don't know
48. What calendar year was this person born?	a. _____		b. Don't know
49. What year was this woman diagnosed with **ovarian cancer**?	a. _____		b. Don't know
50. How long did this woman live at your present location before receiving a diagnosis of **ovarian cancer**?	a. _____ years		b. Don't know
51. Does this woman smoke cigarettes?	a. Yes	b. No	c. Has quit
52. Have any other members of your household been diagnosed with **ovarian cancer**?	a. Yes	b. No	c. Don't know

Breast Cancer

53. Has anyone in your family who lives, or lived, at your present location, been diagnosed with **breast cancer**?	a. Yes	b. No *(If No or Don't know, go on to question 60 on Page 10)*	c. Don't know
54. If yes, is the person diagnosed with **breast cancer** male or female?	a. Male	b. Female	
55. What calendar year was this person born?	a. _____		b. Don't know
56. What year was this person diagnosed with **breast cancer**?	a. _____		b. Don't know
57. How long did this person live at your present location before receiving a diagnosis of **breast cancer**?	a. _____ years		b. Don't know
58. Does this person smoke cigarettes?	a. Yes	b. No	c. Has quit
59. Have any other members of your household been diagnosed with **breast cancer**?	a. Yes	b. No	c. Don't know

Birth outcomes: The following questions concern pregnancies and births in your household among any female members who lived at your present location while pregnant.

60. **Low birth weight** babies weigh 2500 grams or less when they are born; that is 5.5 pounds or less. Has any pregnancy in your household resulted in a baby born **underweight**?	a. Yes	b. No *(If No or Don't know, go on to question 68 on Page 11)*	c. Don't know
61. If yes, what year did the birth of the **low-birth-weight** infant occur?	a. _____		c. Don't know
62. Was the infant male or female?	a. Male		b. Female
63. What calendar year was the mother born, or how old was the mother at the time of the birth?	a. Mother's birth date: _____	b. Mother's age: _____	c. Don't know
64. Was the mother living at your present location during pregnancy?	a. Yes	b. No	c. Don't know
65. If the mother was living at your present location while pregnant, was she drinking water from the well?	a. Yes	b. No	c. Don't know
66. Did the mother smoke cigarettes during pregnancy?	a. Yes	b. No	c. Don't know
67. Have there been other **low-birth-weight** infants born to women living in your household?	a. Yes	b. No	c. Don't know

The following conditions are all kinds of **neural tube defects**. **Spina bifida** is a condition in which the spine is not completely formed. **Anacephaly** is a condition in which the skull is not completely formed. **Cranium bifidum** is a condition in which part of the brain is exposed through holes in the skull.

68. Has any pregnancy in your household resulted in a baby born with a **neural tube defect**?	a. Yes	b. No *(If No or Don't know, go on to question 75 on Page 12)*	c. Don't know
69. If yes, what year did the birth of the infant with **neural tube defect** occur?	a. _____		c. Don't know
70. Was the infant male or female?	a. Male		b. Female
71. What calendar year was the mother born, or how old was the mother at the time of the birth?	a. Mother's birth date: _____	b. Mother's age: _____	c. Don't know
72. Was the mother living at your present location during pregnancy?	a. Yes	b. No	c. Don't know
73. If the mother was living at your present location while pregnant, was she drinking water from the well?	a. Yes	b. No	c. Don't know
74. Have there been other infants born with **neural tube defect** to women living in your household?	a. Yes	b. No	c. Don't know

Congenital malformation

60. Has any pregnancy in your household resulted in a baby born with a **malformation** not mentioned in the previous questions?	a. Yes	b. No *(If No or Don't know, go on to question 69 on Page 13)*	c. Don't know
61. If yes, please describe briefly:	a. _____		
62. What year did the birth of the infant with **congenital malformation** occur?	a. _____		b. Don't know
63. Was the infant male or female?	a. Male		b. Female
64. What calendar year was the mother born, or how old was the mother at the time of the birth?	a. Mother's birth date: _____	b. Mother's age: _____	c. Don't know
65. Was the mother living at your present location during pregnancy?	a. Yes	b. No	c. Don't know
66. If the mother was living at your present location while pregnant, was she drinking water from the well?	a. Yes	b. No	c. Don't know
67. Did the mother smoke cigarettes during pregnancy?	a. Yes	b. No	c. Don't know
68. Have there been other infants born with **congenital malformation** to women living in your household?	a. Yes	b. No	c. Don't know

Intrauterine death

69. Has any pregnancy in your household ended when the baby **died while in the uterus**?	a. Yes	b. No *(If No or Don't know, go on to question 77 on Page 14)*	c. Don't know
70. What year did the death of the infant occur?	a. _____		b. Don't know
71. Was the infant male or female?	a. Male		b. Female
72. What calendar year was the mother born, or how old was the mother at the time of the infant's death?	a. Mother's birth date: _____	b. Mother's age: _____	c. Don't know
73. Was the mother living at your present location during pregnancy?	a. Yes	b. No	c. Don't know
74. If the mother was living at your present location while pregnant, was she drinking water from the well?	a. Yes	b. No	c. Don't know
75. Did the mother smoke cigarettes during pregnancy?	a. Yes	b. No	c. Don't know
76. Have there been other stillborn infants or infants who **died in the womb** in your household?	a. Yes	b. No	c. Don't know

Miscarriage

77. Has any pregnancy in your household ended in a **miscarriage**?	a. Yes	b. No *(If No or Don't know, go on to question 85 on Page 15)*	c. Don't know
78. If yes, what year did the **miscarriage** occur?	a. _____		b. Don't know
79. What calendar year was the mother born, or how old was she at the time of the **miscarriage**?	a. Mother's birth date: _____	b. Mother's age: _____	c. Don't know
80. Was the mother living at your present location during pregnancy?	a. Yes	b. No	c. Don't know
81. If the mother was living at your present location while pregnant, was she drinking water from the well?	a. Yes	b. No	c. Don't know
82. Did the mother smoke cigarettes during pregnancy?	a. Yes	b. No	c. Don't know
83. Has the same woman experienced multiple miscarriages?	a. Yes	b. No	c. Don't know
84. Have other women in your household experienced **miscarriage**?	a. Yes	b. No	c. Don't know

Premature birth

85. Has any pregnancy in your household ended in a **premature birth**?	a. Yes	b. No *(If No or Don't know, go on to question 93 on Page 16)*	c. Don't know
86. What year did the **premature birth** occur?	a. _____		b. Don't know
87. Was the infant male or female?	a. Male		b. Female
88. What calendar year was the mother born, or how old was the mother at the time of the infant's birth?	a. Mother's birth date: _____	b. Mother's age: _____	c. Don't know
89. Was the mother living at your present location during pregnancy?	a. Yes	b. No	c. Don't know
90. If the mother was living at your present location while pregnant, was she drinking water from the well?	a. Yes	b. No	c. Don't know
91. Did the mother smoke cigarettes during pregnancy?	a. Yes	b. No	c. Don't know
92. Have there been other **premature** infants born in your household?	a. Yes	b. No	c. Don't know

Stomach Ailments

93. How many people have been living in your household for the last 12 months? _____ people

94. Answering for each person you thought of, and including yourself, how frequently in the last 12 months does the household member experience **gastroenteritis**, which is **stomachache and diarrhea at the same time**?

a. Person 1 _____ times per year
b. Person 2 _____ times per year
c. Person 3 _____ times per year
d. Person 4 _____ times per year
e. Person 5 _____ times per year
f. Person 6 _____ times per year

95. How many of the people in your household are under 12 years old? _____ people

This is the end of the survey. Thank you for participating in this research on the health status of Western Kentuckians relying on private wells for drinking water.

Please return this survey to
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APPENDIX 2

Epidemiological studies reporting risk of selected cancers by nitrate in drinking water

Year, Author	Study Design	Cancer site	Number of cases	Study location	Concentrations	Exposure timing	Association
1996, Ward et al.	Population-based case control	NHL	156	Nebraska	>= 4 mg/L	1947-1975 until diagnosis	OR=2.0; 95% CI 1.1-3.6 (public supply; no association seen for private water supply)
1999, Law et al.	Ecological	NHL		Yorkshire, England	1.48 mg/L min., 11.86 mg/L mean	6 years	None
2000, Freedman et al.	Population-based case control	NHL	73	Minnesota	<=0.5 mg/L, 5.0 to <=1.5 mg/L, >1.5 mg/L	1947-1975 until diagnosis	None
2001, Weyer et al.	Population-based cohort	NHL	134 cases; cohort=21,977	Iowa	11.6-18.0 mg/L	10+ years before diagnosis	None; OR (95% CI)=0.88 (0.55-1.40)
2002, Gulis et al.	Ecological	NHL		Slovakia	0-10 mg/L, 10.1-20 mg/L, 20.1-50 mg/L	1975-1995	Positive trend in incidence by exposure category: P for trend of SIRs 0.13 for women and 0.017 for men
2003, Cocco et al.	Ecological	NHL		Sardinia, Italy	4.57 mg/L mean; range of <=2, 26.64 mg/L	23 years	None
2006, Ward et al	Population-based case control	NHL	181	Iowa	Quartiles, highest >2.9 mg/L	1960-2000 until diagnosis	None; OR=1.2, 95% CI 0.6-2.2

2010, Chih-Ching et al	Matched case-control	NHL	1,716	Taiwan	<=0.18 mg/L, 0.19-0.45 mg/L, 0.48-2.86 mg/L	1 year prior to diagnosis	None; ORs of 1.02 (95% CI 0.87-1.2) and 1.05 (95% CI)
2013, Rhoades et al	Population-based case-control (public groundwater source)	NHL	140	Nebraska	<=2 mg/L, >2 mg/L	1999-2002	None; *OR for risk for interaction of nitrate and atrazine 2.5 (95% CI 1.0-6.2)*
1992, Rademacher et al.	Matched case-control	Stomach	220	Wisconsin	Well water: yes/no; mean 2.41 mg/L	Nitrate measured after death	None; OR=1.09, 95% C.I (0.82, 1.47)
1995, Morales-Suárez-Varela et al	Ecological	Stomach		Valencia, Spain	>50 mg/L		RR 1.91 in men; 1.81 in women p<0.05
1996, Van Loon et al	Prospective case-cohort	Stomach	282 cases, 3500 in cohort	Netherlands	Quintiles from mean of 0.02 mg/L to mean of 16.5 mg/L	One year before study start	None; RR=0.88, 95% C.I. 0.59-1.32
1997, Yang et al	Population-based Case-control	Stomach	6,766	Taiwan	<=0.22 mg/L, 0.23-0.44 mg/L, >=0.45 mg/L	Deaths in 1987-1991; nitrate measure in 1990	None; OR 1.02, 95% C.I. 0.93-1.11 for highest tertile

1998, Barrett et al.	Ecological	Stomach	15,554	Yorkshire, England	Quartiles from 1.5 to 40.1 mg/L; median 8.1 mg/L	Cases in 1975-94; nitrate in 1990-95	None or negative; RR 0.91, 95% C.I. (0.87-0.95) in highest quartile
1999, Van Leeuwen et al.	Ecological	Stomach		Ontario, Canada	Range 0.05 to 7.79 mg/L, median 1.52 mg/L	Nitrate measured after case collection	Negative
2001, Sandor et al.	Ecological	Stomach	407	Hungary	10 deciles from 5.58 mg/L (referent) to 311 mg/L	Deaths in 1984-1993; nitrate in 1974-1993	Inconsistently positive; RR 1.79 95% C.I. 1.26-2.55 in 7th decile
2002, Gulis et al.	Ecological	Stomach		Slovakia	0-10 mg/L, 10.1-20 mg/L, 20.1-50 mg/L	1975-1995	Positive trend in incidence by exposure category for women only: P for trend of SIRs 0.10
2008, Ward et al.	Population-based case control	Stomach	170	Nebraska	Duration of use at >= 10 mg/L; Quartiles, range 0.5 to 12 mg/L	Cases 1988-1993; nitrate 1965-1985	None; OR=1.2, 95% C.I. 0.5-2.7 in highest quartile
2001, Weyer et al.	Population-based cohort	Breast	253 cases; cohort=21,977	Iowa	11.6-18.0 mg/L	10+ years before diagnosis	None; OR=0.99, 95% C.I. (0.83-1.19) for highest tertile
2006, Brody et al.	Population-based cohort	Breast	824	Massachusetts	<0.3, 0.3-0.6, 0.6-0.9, 0.9-1.2, >=1.2 mg/L	Cases 1988-95; nitrate from 1972 to diagnosis	None; OR=1.0, 95% C.I. (0.5-1.9) for >=1.2 mg/L compared to <0.3

APPENDIX 3

Epidemiological studies reporting risk of adverse reproductive outcomes by nitrate in drinking water

Year, Author	Study Design	Outcome	Number of cases	Study location	Concentrations	Exposure timing	Association
2009, Winchester et al.	Ecological	All birth defects		United States	Means of two categories: 1.94, 1.65 mg/L	Conception in April-July, compared to other months	Increased odds of defect associated with higher mean nitrate in water, $p<0.05$
2013, Migeot et al.	Cohort	Small for gestational age	985 cases; 11, 446 births	France	Terciles; 0-14, 15-26, 27+ mg/L, roughly	During pregnancy, all trimesters	Significant increased risk with above-baseline exposure during 2nd trimester

APPENDIX 4

Epidemiological studies reporting risk of adverse reproductive outcomes by triazines in drinking water

Year, Author	Study Design	Outcome	Number of cases	Study location	Concentrations	Exposure timing	Association
1994, Munger et al.	Ecological	IUGR/SGA		Iowa	Mean of 2.2 mcg/L compared to mean of 0.6 mcg/L	Births in 1984-90; triazines in 1986-87	PR 1.8, 95% C.I (1.2, 2.6) of IUGR for affected water source areas
2005, Villaneuva et al.	Retrospective cohort	IUGR/SGA	3510 births	France	Mean of 0.1 mcg/L compared to mean <0.04 mcg/L	Third trimester in May-Sept compared to Oct-Apr	OR=1.37, 95% C.I. (1.04-1.81) for 3rd trimester in May-Sept compared to Oct-Apr
2009, Ochoa-Acuna et al.	Retrospective cohort	IUGR/SGA and pre-term delivery	24,154 births	Indiana	>=0.103, >0.835 mcg/L and >=0.179, >0.644 mcg/L	Third trimester/entire pregnancy	OR 1.17 (1.03-1.34) for 3rd trimester; OR 1.14 (1.03-1.24) for entire pregnancy in highest tercile
2011, Chevrier et al.	Case-cohort	IUGR/SGA	579 births; 180 cases	France	Presence/absence of atrazine metabolite in mother's urine	Atrazine measure after birth	OR=1.5, 95% C.I. 1.0-2.7
2012, Rinsky et al.	Ecological	Pre-term birth		Kentucky	0 mcg/L, 0 to <0.0810, and >=0.0810 mcg/L	Births 2004-06; atrazine 2000-08	OR 1.22 (1.16-1.29)

IUGR/SGA is intrauterine growth retardation or small for gestational age; both are defined as below the 10th percentile of weight for gestational age.

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EDUCATION

2003 Rollins School of Public Health, Emory University

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EXPERIENCE

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Title: Reporting Unit Manager, Epidemiologist

I returned to my previous position within the Department, as a scientist and unit manager, supervising one epidemiologist and one data analyst.

February 2016 – September 2016 Florida Department of Health, Division of Community Health

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July 2014 – February 2016 Florida Department of Health, Bureau of Emergency Medical Oversight, Health Information and Policy Analysis Section

Title: Reporting Unit Manager, Epidemiologist

I supervised four epidemiologists and health data analysts. The unit is responsible for the analysis and reporting needs for all sections in the bureau. I develop and perform complex data analyses and write reports in the areas of emergency medical services, trauma care, and injury prevention.

July 2013 – July 2014 Florida Department of Health, Bureau of Communicable Disease, Tuberculosis Control

Title: Epidemiologist

I was responsible for surveillance and data quality activities in the TB Control Program, including reporting from the Tuberculosis case registry.

February 2011 – July 2013 Florida Department of Health, Bureau of Emergency Medical Services

Title: Epidemiologist

I worked with data and performed analysis with the Health Information and Policy Analysis Program in the Bureau of Emergency Medical Oversight. I was the primary epidemiologist for the Injury Prevention and Trauma Registry programs.

September 2009 – February 2011 Lincoln Trail District Health Department

Title: Regional Epidemiologist

As one of eighteen regionally-placed epidemiologists in the state of Kentucky, I focused on disease surveillance activities and disaster preparedness in an eight-county district. My job duties included leading field outbreak investigations, investigating cases of reportable disease, and supervising surveillance of reportable diseases for district.

August 2007 – May 2009 University of Kentucky College of Nursing

Title: Research Assistant

I assisted with research activities on a grant-funded research project funded by the National Institutes of Health. I collected data, conducted phone interviews and assisted in manuscript preparation. This was a half-time position while I attended graduate school.

June 2005 – August 2007 University of Kentucky Clinical Research Development and Operations Center

Title: Clinical Research Associate I

I coordinated clinical research studies. I scheduled patients and carried out research activities and data collection. I worked with patients, study sponsors and investigators. I prepared regulatory paperwork. I prepared estimates of time required on study tasks for creating budgets. I was involved in study projects from beginning to end.

GRANTS

- 2008-2009 Characterizing Health Risks in Privately-Supplied Drinking Water Due to Agricultural Practices in Rural Western Kentucky, Leading to an Intervention Study ***Southeast Center for Agricultural Health and Injury Prevention*** ($36,502).

PRESENTATIONS

- Card, Karen. 2012. Florida EMSTARS: *Success in bringing together information on traffic-related injury, pre-hospital patient care, and hospital patient care.* 38th International Forum on Traffic Records and Highway Information Systems, Biloxi, MS.
- Arrowood, K. and Beck, EG. 2009. *Characterizing Health Risks in Privately-Supplied Drinking Water Due to Agricultural Practices in Rural Western Kentucky, Leading to an Intervention Study*. USDA-CSREES National Water Conference, St. Louis, MO.