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ENVIRONMENTAL EXPOSURE TO ATRAZINE AND BIRTH DEFECTS: AN ECOLOGICAL STUDY IN KENTUCKY, 2005-2014

Maria Dimitrios Politis
University of Kentucky, maria.politis@uky.edu

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Maria Dimitrios Politis, Student

Dr. Steven R. Browning, Committee Chair

Dr. Erin Abner, Director of Graduate Studies

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Maria Dimitrios Politis

College of Public Health

University of Kentucky

2018

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ABSTRACT OF CAPSTONE

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

By:
Maria Dimitrios Politis

Lexington, Kentucky

Director: Dr. Steven Browning
Lexington, Kentucky

ABSTRACT OF CAPSTONE

ENVIRONMENTAL EXPOSURE TO ATRAZINE AND BIRTH DEFECTS: AN ECOLOGICAL STUDY IN KENTUCKY, 2005-2014

Atrazine is one of the most widely used pesticides in the United States. Studies have shown that pesticides, in particular herbicides such as atrazine, may be associated with birth defects. The purpose of this study is to evaluate the association between potential environmental exposures to atrazine in water systems and prevalence rates of birth defects for the state of Kentucky. An ecological study using the Kentucky Birth Defects Registry Surveillance and the Kentucky Geological Survey databases from 2005 to 2014 was conducted. Poisson regression was used to estimate crude and adjusted rate ratios of the association between agricultural exposure metrics and birth defects. Overall, the results of this study support the majority of previous research reporting some or mixed association between atrazine and birth defects. Counties with high mean atrazine exposure had higher rates of all birth defects and genital birth defects than counties with low mean atrazine exposure. This study examining the association of atrazine and birth defects reported mostly statistically insignificant results. There was no evidence of increasing strength of association when the atrazine exposure was categorized into increasing exposure levels for mean concentration level, samples above the maximum containment level, and acres of corn planted. This research provides important information on how atrazine herbicide concentration in water systems affects birth defects prevalence. These results contribute to the existing literature and expand the understanding of endocrine disruptors in agrichemical exposures and the role they have on birth defects. Based on the findings from this study, future, more in-depth studies can be designed to examine individual measures of risk and exposures for birth defects.

KEYWORDS: Birth defects, atrazine, genital, musculoskeletal, central nervous system, mean concentration level, maximum contaminant level, limit of detection, acres of corn

Student's Signature: Maria Dimitrios Politis

Date: April 20, 2018

ENVIRONMENTAL EXPOSURE TO ATRAZINE AND BIRTH DEFECTS: AN ECOLOGICAL STUDY IN KENTUCKY, 2005-2014

By

Maria Dimitrios Politis

2018

Signature of Capstone Director: Dr. Steve Browning

Date: April 20, 2018

Signature of Director of Doctoral Studies: Dr. Erin Abner

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DEDICATION

I can do all things through Christ who strengthens me. – Philippians 4:13

And whatever you do, do it heartily, as to the Lord and not to men. – Colossians 3:21

This dissertation is dedicated to my parents, Dimitrios and Andromahe Politis. Thank you for teaching me the value of education and always supporting me in everything I do, especially my studies. Mom, you are not only my mother but my best friend. You have always been there for me, from the little achievements to the big ones, from my saddest moments to my happiest ones. Dad, I can always count on you to put a smile on my face. Your support has never once faltered and your encouragement has helped me through some difficult times. I cannot imagine my life without you both or even achieving my doctoral degree without you both by my side. Thank you both for your never-ending love. I have been truly blessed to have you both as my parents. I love you both so much.

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CHAPTER 1

INTRODUCTION

Background

Atrazine (6-chloro-N-ethyl-N'-isopropyl-1,3,5-triazinediyl-2,4-diamine) is one of the most widely used pesticides in the United States. Pesticides include insecticides, herbicides, and fungicides, all chemical substances that are used to control various types of pests. Atrazine was first registered in 1958 as a selective herbicide and was originally developed to control broadleaf and grassy weeds.[1] The Environmental Protection Agency (EPA) has estimated that 76.4 million pounds of atrazine are applied annually.[2] Application on corn accounts for 86% of total United States usage, followed by sorghum at 10%, and sugarcane at 3%. In Kentucky, 1,333,000 pounds of atrazine were applied on corn in 2014.[3]

Atrazine is most commonly used as an agricultural herbicide in the United States, however, it has also been used on residential lawns from persons who are licensed as certified herbicide users who are permitted to purchase or use atrazine.[1,2] It is typically applied in spring and early summer, and can be sprayed prior to the crops starting to grow, during the growth process, or after they have emerged from the soil.[2] Atrazine has a half-life of approximately 60 days in warm, moist soils. It also has the ability to migrate from upper soil surfaces to deeper soil layers and enter the groundwater.[2] Atrazine has the ability to be broken down in the soil or taken up by plants, however, any amount of rain after application can cause atrazine to run off into waterways such as streams, lakes, and rivers.[2]

When detected in groundwater and waterways, atrazine is more persistent than compared to atrazine remaining in soil.[2] The half-life of atrazine identified in surface waters can be greater than 200 days.[4] Because of its ability to persist in soils and move through water, atrazine is the most commonly detected pesticide in surface waters. Seasonal usage patterns of atrazine can also cause variations in the concentrations in surface soils. These patterns as well as mobility of atrazine can also cause peak concentrations in surface and ground waters immediately before and after the planting season (April-July). Rural populations are at an increased risk of exposure to atrazine and triazine herbicides via drinking water.[5] This is extremely important in Kentucky because the U.S. Department of Agriculture reports that approximately 44% of the state's population lives in rural areas.[6] Atrazine is most applied in heavy corn-producing locations, which are primarily in western regions of Kentucky.[7] In 2007, atrazine was applied to 70% of the corn planted in Kentucky.[1]

The EPA, under the Safe Drinking Water Act, assessed atrazine drinking water monitoring data and set a maximum contaminant level (MCL) for atrazine at 0.003 μg/mL.[8] The MCL is the highest level of a contaminant that is permitted in drinking water.[9] It is an enforceable standard that is applied to public water systems. For atrazine, the MCL was set to prevent potential health effects from long-term exposure including cardiovascular system or reproductive health problems, specifically due to runoff from herbicide used in row crops.[8] The Kentucky Groundwater Data Repository, maintained by the Kentucky Geological Survey, analyzed groundwater data for atrazine in Kentucky regions collected from 1990 to 2005. Atrazine was detected at 91 sites, including 75 springs and 15 wells.[10] Twenty-seven atrazine measurements at 10 sites were greater than the MCL of 0.003 μg/mL. Several monitoring projects in Kentucky from the United States Geological Survey identified atrazine in water samples over 2-year periods.[7] Western Kentucky counties of Meade and Breckenridge from 2004 to 2005 had simazine and atrazine identified most frequently in spring and surface water. Of the samples that were collected in April, May, and June, 24% exceeded the MCL and the overall median concentration increased from 2004 to 2005. Fifteen out of the 24 pesticides that were detected in the Little River Basin in Western Kentucky were herbicides, and atrazine exceeded the MCL in 17% of the samples.[7]

Epidemiologic and environmental research suggest that agrichemical exposures may contribute to the occurrence of birth defects and congenital abnormalities. In ecologic and cross-sectional studies, congenital abnormalities, including neurologic, circulatory, genital, musculoskeletal, and respiratory defects, have been linked with amount of pesticide usage or levels of pesticides in local drinking water sources.[8-13] However, these studies collectively have not provided adequate evidence to support the conclusion that birth defects are associated with atrazine exposure.[17] Studies have examined the association of pesticide exposures in general and birth defects. Few studies have specifically explored the association between atrazine exposures and birth defects and the findings of these studies have been mixed. Few epidemiologic studies examining atrazine alone have been conducted. Results among the studies that examined atrazine exposure and birth defects have also been inconsistent, possibly due to small sample sizes, rare birth defect outcomes, the absence of exposure history, and weak methods for assessing exposure.[17]

Birth defects are structural or functional changes that can affect any part or parts of the body and can be present at birth or acquired after birth. They can vary from mild to severe. The well-being and lifespan of infants and children affected with birth defects depends on which organ or body part is involved and the severity of the defect. Birth defects can occur during any stage of pregnancy; however, most birth defects occur in the first three months of pregnancy—first trimester. This is the fetal stage when the organs of the fetus are forming and an important stage for development.

In the United States, birth defects affect one in every 33 babies born each year.[18] Birth defects are the leading cause of infant mortality in the United States, accounting for 20% of all infant deaths.[19] Worldwide, about 7% of all neonatal deaths were caused by birth defects.[20] In the Commonwealth of Kentucky, birth defects are more common than in the entire United States. Each year, about 4,900 infants are born in Kentucky with a major or minor birth defect.[21] This translates into about 1 in every 12 births.[21] Kentucky has the same infant mortality as the nation, about 20% of all infant deaths caused by birth defects, which in Kentucky is equivalent of about 81 cases per year.[22] Birth defects are also implicated in about 45 stillbirths per year in Kentucky (about 13% of all reported stillbirths).[22]

Birth defects disproportionately affect infants born to mothers who are residents of rural counties. Infant mortality due to birth defects generally increases with rurality, with 11.21 per 10,000 live births among residents of urban counties compared to 16.25 per 10,000 live births among residents of rural counties.[23] Birth defects, as well as infant mortality due to birth defects, may be due to differential exposures, as well as differential access to screening and risk-appropriate care across counties. Additionally, this may affect infants who are born into poverty or from families with low income. Socioeconomic status, including poverty and low income, increases the risk of infants being born with birth defects.[24-26] Maternal education, specifically lower educational attainment, is also a risk factor that can lead to increases in infants being born with birth defects, primarily central nervous system defects.[24,27,28] Lastly, agricultural compounds and chemicals in municipal and drinking water have been associated with birth defects.[29-32]

Statement of the Problem

Studies have shown that pesticides, in particular herbicides such as atrazine, may be associated with birth defects. Limited research has addressed this issue for the state of Kentucky, which is a relatively rural state with heavy use of agrichemicals, particularly in certain regions. Many previous studies have made numerous statistical comparisons exploring associations between several pesticides and herbicides and various birth defects, such that they have the potential for associations to have occurred by chance alone. Other studies have reported findings based on small sample sizes, were underpowered to detect small effects, used self-reported data, lacked detailed exposure information, and relied on proxies for exposure assessment. Additionally, few studies examined water data to assess atrazine exposure and its association with birth defects. Most studies only considered pesticide and herbicide exposure from agricultural data. Studies have not considered atrazine exposure from both water sources and agricultural data to allow for an additional index to assess exposures in statistical modeling.

Overview of the Study Process

An ecological study was conducted using county level data to evaluate the association between incidence rates of birth defects of infants in Kentucky and potential environmental exposures to atrazine herbicides. The study encompassed the years 2005-2014. Data used in this study were obtained from the Kentucky Birth Surveillance Registry (KBSR), the Kentucky Geological Survey (KGS), the Data Center at the University of Louisville, the Census of Agriculture, and the American Community Survey. First, a descriptive exposure analysis was conducted using the atrazine exposure database for Kentucky counties, which included the examination of measures of direct and indirect atrazine exposures. Second, an ecologic study was conducted to examine the association of birth defects and atrazine in Kentucky at the county level. Lastly, spatial analysis was conducted to determine if clusters of birth defects were present in areas of the state where high concentrations of atrazine were detected.

Scope and Importance of Study

This project provides important information on atrazine herbicide concentrations in water systems that affect birth defects prevalence. This study offers several novel approaches to examining the association between atrazine herbicides and birth defects. This is the first study to examine this association in the state of Kentucky for this time period using the Kentucky birth defects registry. Previous studies were conducted examining pesticide use and birth defects in other states; however, this would be the first in Kentucky for this specific time period.

The use of multiple exposure metrics is a novel approach in this study. Data from the Kentucky Geological Survey and the Census of Agriculture were used to estimate the atrazine herbicide concentration at the county level. The data obtained from the Census of Agriculture provided a novel way to measure atrazine exposure in the form of the number of acres of corn planted and the bushels of corn produced.

Another novel approach of this study was the selection of birth defects. Primarily, all analyses were conducted to examine all of the birth defects identified during the time period of 2005-2014. But the study also examined the birth defects that are more likely caused by endocrine disrupting chemicals, such as atrazine. The birth defects that were examined in this included central nervous system defects, musculoskeletal birth defects, and genital defects.

Finally, this study conducted spatial analyses to determine if clusters of birth defects were present in areas of the state where high concentrations of atrazine were detected. Spatial analysis examining the association between potential environmental exposures to atrazine herbicides in water systems and prevalence rates of birth defects for the state of Kentucky has not been done. Additionally, no spatial analysis has been conducted in general examining the association between atrazine exposure and birth defects at a county level.

This study contributes to the existing literature and expands the understanding of endocrine disruptors in agrichemical exposures and the role they have on birth defects. Based on the findings from this study, more in-depth studies can be designed to examine individual measures of risk and exposures for birth defects.

Purpose and Hypothesis of Study

The purpose of this study is to evaluate the association between potential environmental exposures to atrazine herbicides in water systems and prevalence rates of birth defects for the state of Kentucky.

The research hypothesis of this proposal is that the use of agrichemicals—particularly atrazine herbicides—and their presence in water systems by counties are associated with regional variation in prevalent birth defects.

Specific Aims of Study

The specific aims of this study are to:

1. Evaluate the correlations between agricultural activities—such as acres of corn planted and bushels of crops produced—and concentrations of atrazine herbicides in surface and ground water systems using counties as analytical units.
2. Create a variety of metrics to estimate the environmental atrazine herbicide exposure.
3. Evaluate the association between atrazine herbicide exposure and birth defect prevalence rates, accounting for covariates at the county level (mother's age, mother's education, mother's race, poverty level, medium household income, and public water usage) using an ecological study design.
4. Conduct spatial analysis analyzing if clusters of birth defects are present in areas of the state where high concentrations of atrazine are detected.

CHAPTER 2

LITERATURE REVIEW

Biological Plausibility

Studies have shown that pesticides (and their metabolites) can be considered as endocrine-disrupting chemicals because of their capability to interact with hormone receptors. One potential biological mechanism by which atrazine may influence fetal development in utero is by acting as an antagonist and interfering with hormone receptors.[33] One study examined how pesticides were capable of binding to estrogen and androgen receptors as xenoestrogens and antiandrogens, respectively, preventing estrogen and androgens, like testosterone, from mediating their biological effects in the body.[34] This mechanism is further supported by research demonstrating that 2-chloro-s-triazine herbicides (which includes atrazine) induce aromatase activity and act as a stimulant of the protein kinase-A pathway that mediates the induction of aromatase in cells.[35] The mechanism of the induction of aromatase, which is the rate-limiting enzyme in the conversion of androgens to estrogens, may be the underlying explanation for reported hormonal disruption due to atrazine. A recent study found that the G protein estrogen receptor, a seven-transmembrane receptor, is involved in particular biological responses such as gene expression changes and growth effects that are induced by atrazine.[36] Another potential mechanism by which atrazine may influence fetal development is by inhibiting cyclic 3', 5'-adenosine monophosphate (cAMP) specific phosphodiesterases, which leads to an increase in intracellular and extracellular cAMP accumulation as well as the kinase activation for cAMP.[37] Atrazine, by this mechanism, exhibits sustained effects on variable signaling pathways since cAMP and the kinase are involved in the control of the transcriptional activity. These biological mechanisms alter the normal development and function of the fetus in utero and can interfere with the development of the hormonal, reproductive, immune, and nervous systems.

Epidemiologic Studies

Among the studies conducted examining the association between birth defects and pesticides, including atrazine, the findings have been mixed.

Kristensen et al. examined the prevalence of all birth defects, as well as specific birth defects among 4,565 cases in a case-control study, conducted from 1967 to 1991 in Norway.[38] The researchers had two indicators of pesticide exposure: the amount of money spent on pesticides on the farm (pesticide purchase), and tractor pesticide spraying equipment on the farm. Pesticide exposure, in particular in grain farming, was associated with limb reduction defects (Odds Ratio [OR] = 2.50, 95% Confidence Interval [CI]: 1.06 – 5.90). Moderate increases in risk for spina bifida and hydrocephaly were found, with the associations being the strongest for exposure to pesticides in orchards or greenhouses (spina bifida: 5 exposed cases, OR = 2.76, 95% CI: 1.07 – 7.13; hydrocephaly: 5 exposed cases, OR = 2.50, 95% CI: 1.34 – 9.09). Hypospadias, was associated with tractor spraying equipment, particularly when the parents cultivated grain. The authors also found the strongest associations for second-quarter conceptions and in grain farming where herbicides are used heavily in late spring. Although the large sample from a national population provides an opportunity to study the effects of exposure on certain defects, only crude proxies for true exposure were available. In addition, the study had misclassification bias of the exposure due to the cross-sectional nature of the agricultural census information.

A cohort study using the Ontario Farm Family Health Study conducted in Ontario, Canada between 1990 and 1993 by Weselak et al. examined the effect of parental pesticide exposures in the pre- and post-conception periods on the prevalence of birth defects in offspring.[39] Exposure of farm pesticide use was determined by the crop name, chemical name, reason for use, months of application, and the number of years of use. Additionally, maternal covariates were obtained, including age, education level, income, alcohol consumption and smoking during pregnancy, and season of conception. The authors found that pre-conception exposure to both cyanazine (OR = 4.99, 95% CI: 1.63 – 15.27) and dicamba (OR = 2.42, 95% CI: 1.06 – 5.53) were associated with increased risk of birth defects in male offspring. However, overall, the study did not find strong evidence for an association between parental pesticide exposure during the pre- and posts-conception periods and birth defects among the offspring. One limitation of this study, however, is the validity of the exposure assessment, including a number of unmeasured factors such as the quantity of the pesticides used, the time spent applying the pesticides, and the use of protective equipment which may have modified the actual exposures. Self-reporting of the birth defects, unmeasured confounders, and selection bias may have affected the results of this study.

Using a retrospective case-control study, Waller et al. used the Washington State Birth Certificate and US Geological Survey databases to determine if periconceptional exposure to agrichemicals was associated with the development of gastroschisis.[40] The authors used annual surface water concentrations by season of atrazine, nitrates, nitrites, and 2, 4-dichlorophenoxyacetic acid from established sites in Washington State and calculated the average concentration for each site between 2001 and 2006 to develop an exposure metric. The authors also used maternal covariates including age, race, smoking, county of residence at time of birth, years of education, occupation, and longitude and latitude of primary residence during pregnancy. The authors found that gastroschisis occurred more frequently among infants whose mothers lived less than 25 kilometers from a site of high surface water contamination with atrazine (OR = 1.6, 95% CI: 1.1 – 2.3). Mothers who resided within 25 – 50 kilometers of surface water sites with atrazine concentrations greater than 3 g/L showed an increased risk for gastroschisis as well (OR = 1.4, 95% CI: 1.2 – 1.7). A seasonal variation was observed in the prevalence of gastroschisis, with a peak occurring during spring conception (March – May; OR = 1.2, 95% CI: 1.1 – 1.5). This study demonstrated that maternal exposure to surface water atrazine is associated with fetal gastroschisis, particularly in spring conceptions, however, does not take into account exposure to such herbicides and pesticides from absorption and inhalation in addition to ingestion.

Agopian et al. conducted a case-control study using the Texas Birth Defects Registry from 1999 to 2008 to evaluate the relationship between estimated residential maternal exposure to atrazine during pregnancy and risk for male genital malformations.[41] Exposure to atrazine was determined using annual estimates of atrazine application levels for all Texas counties. Modest, but consistent associations were found between medium-low and/or medium levels of estimated periconceptional maternal residential atrazine exposure and every male genital malformation category evaluated. The adjusted odds ratio for medium compared to low atrazine levels and all male genital malformations was 1.2, with a 95% confidence interval of 1.1 – 1.3.

CHAPTER 3

ECOLOGICAL DESIGN WITH NEGATIVE BINOMIAL REGRESSION ANALYSIS

Methodology

Overview of Research Design

An ecological study was conducted using county level data to evaluate the association between prevalence rates of birth defects of infants in Kentucky and potential environmental exposures to atrazine herbicides. The study encompassed the years 2005-2014.

Sampling

The study included the years 2005 to 2014 for a total of 10 years. In the state of Kentucky, there are between 55,000 and 60,000 live births recorded each year.[42] All of the birth defect cases were obtained from the Kentucky Birth Surveillance Registry. Cases were included in the study if they were born between January 1, 2005 and December 31, 2014 and had a diagnosis including an ICD-9 code between 740.0 and 759.9 (congenital anomalies). All the cases were live births and the birth defects were either isolated or non-isolated. Cases with missing or no mother's county of residence information were excluded from the final unadjusted and adjusted analyses.

Initially, all birth defects were considered in the preliminary unadjusted and adjusted analyses. Pending adequate number of events, birth defects that are caused by endocrine disrupting chemicals were then examined: central nervous system defects; musculoskeletal birth defects; and genital defects. The ICD-9 codes included in the Kentucky Birth Surveillance Registry dataset were used to classify each subgroup of birth defects. Cases that had an ICD-9 code from 740.0 to 742.9 were grouped in the central nervous system birth defects group, cases that had an ICD-9 code from 754.0 to 756.9 were grouped in the musculoskeletal birth defects group, and cases that had an ICD-9 code from 752.0 to 752.9 were grouped in the genital birth defects group.

The University of Kentucky Institutional Review Board certified this study as exempt.

Data Collection Procedures/Data Sources

This study used several datasets for the data collection in this ecological design. A master database was constructed using the datasets with previously collected data. All of the data were collected at the county level, including aggregating the number of birth defects per county. There are 120 counties in Kentucky. The Kentucky State Data Center at the University of Louisville collects data on births in the state of Kentucky overall and by county annually. This data includes the number of births by county and in the entire state. The number of births obtained for the years 2005 through 2014 served as denominators to be used to calculate rates for each county and for the state of Kentucky.

The Kentucky Birth Surveillance Registry (KBSR) is a state mandated surveillance system designed to provide information on the incidence, prevalence, trends and patterns, and possible causes of birth defects, stillbirths, and disabling conditions. The KBSR is administered by the Department for Public Health in the Cabinet for Health and Human Services. It collects information on children from birth to age five who are diagnosed with any structural, functional, or biochemical abnormally. The system relies primarily on hospital reports, vital statistics, and laboratory reporting. The KBSR operates under the authority of KRS 211.651-670. This study used data from the KBSR to identify the numbers of cases available, their location in the state, and the type of birth defect.

The Kentucky Geological Survey (KGS) investigates and collects data on mineral, energy and water resources, and geologic hazards in Kentucky. This information and data is maintained by the KGS and is housed on the University of Kentucky campus. Data on groundwater (well and spring) contamination was gathered from the KGS. The KGS has a database of the analytical results of all ground water samples for atrazine and triazine herbicides since 1991. The atrazine water database included atrazine sampled at five types of sites: rivers, streams, wells, springs, and municipal water sources from 1998 to 2009.

The American Community Survey provides demographic data on population size, gender, age, and race, as well as other variables such as education level and median family income for each county and state in the United States. The 5-year estimates for the year 2010 were chosen since it falls in the middle of the study period. Variables obtained from the census data from the 5-year estimates served as covariates and were included in the master database by county. Covariates were obtained at the county level and included the percentage of people residing in a rural area, the median household income, the percentage who had a high school degree or higher, the percentage of females aged between 10-59, and the percentage of the female population that is of the white race.

The Census of Agriculture collects data on crop production at the state and county level. This data includes variables such as the number of farms, the number of acres planted, the bushels of crops produced, and the amount of production per acre. The Census of Agriculture collects agricultural data every five years. Variables of interest for this study included the number of acres of corn planted and bushels of corn harvested by county in 2007 and 2012. Since atrazine herbicides are most commonly applied to corn crops, these variables were used as an indirect metric to assess atrazine exposure.

Measurements

Exposure Metrics

For this study, no personal exposure or individual home measurements for herbicides were collected. Exposures, therefore, were estimated as aggregate annual county-level estimates from water quality data alone. To estimate environmental atrazine exposure in Kentucky, two overall metrics were used at the county level: atrazine water metrics and agricultural corn crop activity. The four exposure metrics were determined: the number of acres of corn planted within the county of residence, mean concentration of atrazine in county water samples, the number of county water samples above the maximum contaminant level (0.003 μg/mL), and the proportion of county water samples above the analytical limit of detection for atrazine.

The Census of Agriculture collects data on crop production by county every 5 years. Variables of interest for this study included the number of acres of corn planted and bushels of corn harvested by county in 2007 and 2012 from the Census of Agriculture. The average number of acres of corn planted and bushels of corn harvested for these two years were calculated to create one metric per variable for each county. Since atrazine herbicides are most commonly applied to corn crops, these variables were used as an indirect metric to assess potential atrazine exposure.

The KGS collects analytical data for groundwater and municipal water contamination from atrazine. Municipal water systems, as mandated by the EPA, must be sampled periodically for concentrations of certain agrichemicals, including atrazine and other triazine herbicides. The KGS also maintains a database of the analytical results of all ground water and surface water samples including springs, rivers, streams, wells, and municipal water sources, by county, for atrazine herbicides since 1991. These data were used to calculate the total number of samples collected in each county, the proportion of samples above the analytical limit of detection in each county, the highest concentration detected (maximum value) in each county, and the mean concentration level of the samples for each county. The atrazine samples were collected from 1998 to 2009.

The atrazine water database included atrazine samples at five types of sites: rivers, streams, wells, springs, and municipal water sources from 1998 to 2009. To assess the environmental burden of atrazine in surface water, rivers and streams water data were combined during the analysis. Wells and springs water data was combined with municipal water data to assess human exposure to atrazine in water by route of ingestion of drinking water. The majority of the population in Kentucky uses either springs and wells or municipal water for their drinking water.[43] The following three exposure metrics were used to examine both exposure by ingestion of drinking water (municipal, wells, and springs) and environmental exposures (rivers and streams).

Method 1 – Maximum Concentration Level

The maximum concentration level was the sample with the highest concentration level for the entire sampling period for each county.

For the river and streams data, the maximum concentration level was categorized into two groups based on the MCL of 0.003 μg/mL set by the EPA. The counties were categorized based on having any atrazine concentration level either at or above 0.003 μg/mL or having no samples that exceeded the 0.003 μg/mL.

To examine exposure by possible ingestion of atrazine in drinking water, a three-level ordinal variable was created by combining the data on the maximum concentration level for municipal, wells, and springs water sources. Maximum concentration levels were categorized if the MCL for all samples from municipal, wells, and springs water sources were measured to be above the MCL (high exposure), the maximum concentration level for either springs and wells, or municipal water sources were measured above the MCL (moderate exposure), and if the maximum concentration level for neither municipal or wells and springs water sources were measured above the MCL (low exposure).

Method 2 – Mean Concentration Level

The mean concentration level for each county was calculated by taking the arithmetic average of the samples taken through the entire study period. The mean concentration level was categorized into a three-level variable for the municipal, well, and spring data: 0 μg/mL; 0.00014 μg/mL – 0.098 μg/mL; and equal or above 0.098 μg/mL. For the river and streams data, the mean concentration level was categorized into a three-level variable: 0 μg/mL; 0.00000538 μg/mL – 0.4958 μg/mL; and equal or above 0.4958 μg/mL.

Method 3 – Limit of Detection

For this study, one-half of the lowest limit of detection of all the analytical methods was substituted as the measurement for all samples reported as below the analytical limit of detection for atrazine.[44] For example, if the lowest limit of detection is recorded at 0.003 μg/mL, then all values recorded as below the limit of detection will be substituted with 0.0015 μg/mL. The proportion of the samples above the limit of detection for each county was calculated by dividing the total number of samples taken that were above the limit of detection by the total number of samples collected for each county.

Exposure Assessment

A combined measure was created to assess the overall atrazine exposure. The data collected for rivers and streams, wells and springs, and municipal water sources were combined. Using the combined data, the three exposure metrics (maximum concentration level, mean concentration level, and limit of detection), along with the agricultural exposure metric (acres of corn), were used to examine the association of atrazine and birth defects. These combined exposures were used in the statistical analyses to estimate the atrazine exposure in this study. The overall mean concentration level was categorized into a three-level variable: low: 0 μg/mL, medium: 0.00000269 μg/mL – 0.50 μg/mL, and high: above 0.50 μg/mL. The cutoff points used in determining the mean concentration levels followed the methods of Maxwell et al.[45] The maximum concentration levels were categorized if the MCL for all samples from municipal, wells and springs, and river and streams water sources were measured to be above the MCL (3 samples), the maximum concentration level for either wells and springs and municipal, or wells and springs and river and streams, or municipal and river and streams water sources were measured above the MCL (2 samples), the maximum concentration level if only municipal, or wells and springs, or river and streams were measured above the MCL (1 sample), and if the maximum concentration level for neither municipal or wells and springs or river and streams water sources were measured above the MCL (0 samples). The average acres of corn planted were categorized into tertiles, with low being less than 1,656 acres of corn planted, moderate being 1,656 to 10,408 acres of corn planted, and high being more than 10,408 acres planted

Outcome Assessment

The primary outcome variable for this study was the birth defect prevalence rates. Birth defect cases were obtained from the KBSR for the years 2005-2014 with the mother's county of residence identified. The outcome variable first examined birth prevalence rate of all types of birth defects included in the KBSR and then examined the birth prevalence of the specific types of birth defects that are associated with endocrine disruptors (central nervous system birth defects, musculoskeletal birth defects, and genital birth defects).

Covariates

To account for other potential risk factors and confounders, county-level covariates known to be risk factors from the literature on birth defects[24,25,46-51] were included in this study. The county-level covariates from the American Community Survey 5-year estimates for 2010 included were the percentage of people residing in a rural area, the median household income, the percentage of females who had a high school degree or higher, the percentage of females aged between 10-59, and the percentage of the female population that is of the white race. The percentage of the county's population using public water was obtained from the KGS.

Data Management and Analysis

Data Management. A master database was constructed using the previously collected data from the datasets mentioned in the collection procedures and data sources section above.

Analysis. All data analyses were conducted using Statistical Analysis System (SAS) for Windows, version 9.4 (SAS Institute, Cary, North Carolina).

Birth Defect Prevalence Rates

Birth defect prevalence rates were calculated for each county from KBSR data for the study period 2005 to 2014 for all birth defects and for the specified endocrine disruptor birth defect groups (central nervous system, musculoskeletal, and genital) using the following formula:

$$\frac{number\ of\ cases}{total\ population\ of\ live\ births} \text{ x } 10{,}000$$

This formula provided the birth defect prevalence rate per 10,000 live births.

Ecological Statistical Analysis

For the estimation of the association between the four metrics of atrazine exposure and the prevalence rates of birth defects, a negative binomial regression model was used. The negative binomial regression model was used rather than the Poisson regression model because there was evidence of over-dispersion. The negative binomial regression method allows for a more flexible count data model with an additional variance parameter that can be used when the Poisson model does not fit well. The regression models were fit using the SAS PROC GENMOD procedure with a negative binomial distribution and the log function as the link. The offset included in the model was determined by the natural log of the total live births in each county for the ten year period and was used to calculate the prevalence rate ratios. The unadjusted and adjusted prevalence rate ratios, along with the 95% confidence intervals were reported for all birth defects and the three birth defects subgroups (central nervous system, musculoskeletal, and genital) and for each exposure metric. All statistical tests were evaluated using a significance level of 0.05.

There were forty models that were analyzed. The first twenty analyzed the unadjusted models of each of the four birth defect groups with each of the four atrazine exposure metrics separately. The second twenty models analyzed the adjusted models of each of the four birth defect groups with each of the four atrazine exposure metrics with the covariates that were listed above. Modeling each exposure metric separately was done to avoid potential collinearity.

Results

The number of atrazine samples ranged from 7 to 708 samples per county collected among all 120 Kentucky counties. The mean and median of all atrazine samples collected were 115 and 80, respectively. Descriptive statistics for the atrazine exposures metrics are presented in Tables 1 to 3. About 64% of counties had a least one sample that was above the MCL for wells, springs, and municipal water sources. Fifty-three counties had a moderate (0.00000269 μg/mL – 0.50 μg/mL) or high (≥ 0.50 μg/mL) mean concentration level from the river and streams water sources. The average acres of corn planted for the years 2007 and 2012 was 23,690,000 statewide.

Overall, there were a total of 18,743 cases of birth defects included in the KBSR from 2005 to 2014. The prevalence birth defect rate for the state of Kentucky from 2005 to 2014 for all birth defects was 329.91 per 10,000 live births. There were a total of 2,358 central nervous system birth defects, 6,999 musculoskeletal birth defects, and 5,246 genital birth defects. The prevalence birth defect rate for all birth defects, central nervous system birth defects, musculoskeletal birth defects, and genital birth defects are presented in Figure 2, graphed by year. The frequency and birth prevalence rates by year and each specific birth defect group are presented in Table 4.

Once the cases with missing counties were removed, the total number of birth defect cases was 16,070. There was a total of 1,381 cases included in the central nervous system birth defects group, 6,000 cases included in the musculoskeletal birth defects group, and 4,904 cases included in the genital birth defects group.

Tables 5 to 8 report the results of the unadjusted models for the rivers, streams, wells, springs, and municipal water sources combined with the prevalence rates for all birth defects, central nervous system birth defects, musculoskeletal birth defects, and genital birth defects. There was an inverse dose-response for maximum concentration levels exceeding the MCL for all four birth defect groups, with slight elevated risks in the low exposure category (1 sample above the MCL) and protective effects in the two highest exposure categories (2 and 3 samples above the MCL). Counties with high mean atrazine concentration had a 2.43 times higher prevalence of genital birth defects in comparison to low mean atrazine counties (p-value = 0.0089). The high exposure group for the mean concentration level had elevated risks in all birth defect groups except for the central nervous system birth defects group. Most of the unadjusted estimates were not statistically significant. Additionally, slightly protective unadjusted rate ratios were reported for the high category for acres of corn planted in all four models.

The results of the adjusted models are presented in Tables 9 to 12. The observed associations of the atrazine exposure metrics were similar to those reported in the unadjusted models after adjusting for potential confounders. The inverse dose-response that was observed in the unadjusted models for the maximum concentration levels was also observed in the adjusted models. Protective effects were reported for the highest exposure group (3 samples above the

MCL) for all birth defect groups. Protective effects were also reported for the second highest exposure group (2 samples above the MCL) for the central nervous system and musculoskeletal birth defects groups. For all four birth defects groups, the percent of the population with a high school degree or higher reported protective effects with the maximum concentration levels. Percent below the poverty level had slightly elevated risks for the musculoskeletal and genital birth defect groups.

In the adjusted model for the mean concentration level, both the all birth defects group and the genital birth defects group had elevated risk for both high and moderate exposures, whereas the central nervous system and musculoskeletal birth defects group had protective effects for the high exposure category. Counties with high mean atrazine concentration had a 1.43 times higher prevalence of genital birth defects in comparison to low mean atrazine concentration counties. The percentage of the population using public water had protective effects for all four birth defects groups.

In the adjusted model for the percent of samples above the limit of detection, all birth defect groups except for the genital birth defects group had protective effects. There was a minute elevated risk for the percentage living below the poverty level for all groups except the central nervous system birth defects group.

Among all four birth defect groups, the moderate category of acres of corn planted showed a slight elevated risk whereas a protective effect was reported in the highest exposure category, except for the central nervous system birth defects group. Counties with a moderate number of acres of corn planted had 1.52 times higher prevalence of central nervous system birth defects compared to counties with the lowest number of acres of corn planted.

CHAPTER 4

SPATIAL ANALYSIS

Methodology

Overview of Spatial Analysis

Spatial analyses were conducted using county level data to examine the distribution of the atrazine metrics and birth defect prevalence rates in Kentucky and to evaluate clusters of birth defects initially and also with environmental exposures to atrazine herbicides. The study encompassed the years 2005-2010.

Data Collection Procedures/Data Sources

This study used several datasets for the data collection in this ecological design. The master database constructed (explained in Chapter 3) using the datasets with previously collected data was used to create the choropleth maps and conduct the geospatial analyses. The dataset contained information by each county, including the birth prevalence rates for all birth defects, central nervous birth defects, musculoskeletal birth defects, and genital birth defects, and the four atrazine exposure variables.

Data Management and Analysis

Data Management. The master database constructed using the previously collected data from the datasets mentioned in Chapter 3 was used.

Analysis. All maps were created using ArcGIS (ESRI 2011, Redlands, CA). All spatial analyses were conducted using Software for the Spatial, Temporal, and Space-Time Scan Statistics (SaTScan) v9.4.4 (Kulldorff 2011).

Choropleth Maps

Choropleth maps were created using ArcGIS to provide a visual presentation of the crude birth prevalence rates of the four birth defect groups and the atrazine exposure variables. A United States shapefile was obtained from the United States Census Bureau website.[52] Using the United States shapefile, the state of Kentucky was defined and extracted for use. The data obtained from Chapter 3 and the Kentucky map were joined by county in ArcGIS to create the choropleth maps. The birth prevalence rates for each of the four birth defect groups were mapped using the Jenks "Natural Breaks" technique to determine five categories for each. The prevalence rates were rounded to two decimal places. Each atrazine exposure variable was also mapped based on the categories explained in the methodology in Chapter 3. For all of the choropleth maps created, lighter colors indicate a lower prevalence rate or lower atrazine exposure, and darker colors indicate higher prevalence rates or higher atrazine exposure based on the specific metric.

Spatial Analysis

To examine if there were local spatial patterns of birth defects and concentrations of atrazine present, SatScan was used. The spatial scan statistic compares the rate of the birth defects within a scan window with the rate outside of it. The circular scan windows have continuously changing radii and are centered in each county's centroid. Larger clusters are formed when more county centroids are included from larger circular scan windows. The cluster includes neighboring, or nearby counties with centroids that fall within the circle. The rate of the cases for each birth defect groups in the population of each cluster of counties is compared with the rate in the rest of the state. Clusters of counties with significantly higher or lower rates than would be expected are identified using the Monte Carlo simulation and hypothesis testing. This hypothesis testing uses the null hypothesis to generate replicated patterns which is used to compare the observed spatial pattern of cases. Clusters of counties with significantly higher or lower prevalence rates of the birth defect groups than the rest of the state were identified in this study.

The master database mentioned above was used to implement the spatial scan statistic. The spatial scan statistic requires two sets of data, one describing the cases and one describing the population. For the cases file, the total number of cases per county obtained from the KBSR was used for each specific birth defect group. For the population file, the total live births per county that was obtained from the Kentucky State Data Center at the University of Louisville was used.

Purely spatial analyses (without regard to time) were performed, using a discrete Poisson model and the default settings in SaTScan: a maximum cluster size of 50%, a circular scan window, and 999 Monte Carlo replications. The high- and low-rate clusters were exported as shapefiles and overlaid on the corresponding choropleth map of prevalence for each birth defect.

Results

Figures 3 to 6 display the four atrazine exposure variables in Kentucky at the county level. Counties in the western and central regions had higher number of samples above the maximum contaminant level compared to the eastern region where almost all of the counties had zero samples above the maximum contaminant level, except for Pike county. Over half of the counties in Kentucky had a moderate (00000269 – 0.50 μg/mL) level of mean atrazine concentration, with 12 counties in the western region having a high (>0.50 μg/mL) level of mean atrazine concentration. In terms of the percentage of samples above the limit of detection, the majority of the counties in the eastern region had zero percent. The counties in the west, specifically the counties bordering the state of Tennessee, had higher percentages of samples above the limit of detection. The entire western region of the state had high (>10,408) acres of corn planted whereas the eastern region had low (<1,656) acres of corn planted.

Figures 7 to 10 display the clusters of high- and low-rates for all four birth defect groups with their respective crude birth prevalence rates by county from 2005 to 2014. For all birth defects, higher prevalence rates were located in the Bluegrass region of the state, with lower prevalence rates in many western counties, specifically those bordering the state of Tennessee. The largest high-rate cluster for all birth defects included 34 counties (relative risk = 1.41) and was located in the Bluegrass region, overlapping with two additional high-rate clusters. Most of the low-rate clusters for all birth defects were located in the western region of the state. Central nervous system birth defects had higher prevalence rates in counties in the central region, with most of the lower prevalence counties residing in the Bluegrass region. For central nervous system birth defects, one high-rate cluster was located in the central region and included 20 counties (relative risk = 1.89), and two low-rate clusters were located the west and north part of the state. The counties with the highest prevalence rates for musculoskeletal birth defects were located in the Bluegrass and central regions, and the lowest prevalence rates in the counties of the western region of the state. Musculoskeletal birth defects contained a low-rate cluster in the western region of the state (relative risk = 0.71) and a high-rate cluster composed of 18 counties in the Bluegrass region (relative risk = 1.48). The counties with the highest prevalence rates for genital birth defects were located in the Bluegrass region. The three counties with the highest prevalence rates for genital birth defects were Fulton, Bourbon, and Robertson. Many of the western counties bordering the Tennessee state line had lower prevalence rates for genital birth defects, similar to the map displaying the prevalence rates for all birth defects. The three high-rate clusters for genital birth defects were located in the northern region of the state, with relative risks of 1.22, 1.38, and 1.47, respectively. The western region of the state contained four of the five low-rate clusters for genital birth defects for Kentucky.

CHAPTER 5

IMPLICATIONS FOR PUBLIC HEALTH

Overall, the results of this study support the majority of previous research reporting some or mixed association between atrazine and birth defects. Counties with high mean atrazine exposure had higher rates of all birth defects and genital birth defects than counties with low mean atrazine exposure. This study examining the association of atrazine and birth defects reported mostly statistically insignificant results. There was no evidence of increasing strength of association when the atrazine exposure was categorized into increasing exposure levels for mean concentration level, samples above the maximum containment level, and acres of corn planted.

One concerning find during this study was the sharp decrease in the birth prevalence rates from 2008 to 2010 that was observed in Figure 2. During this time period within the KBSR, there were many programmatic and methodological changes that occurred, including the conditions abstracted, the type of coding used, the ICD-9 codes used for specific diagnoses, the data sources that were included in the registry, and areas of specific focus and interest within the program.[21] Further research, including sensitivity analyses, is needed to determine how this impacts the observed associations.

The high- and low-rate clusters for the central nervous system, musculoskeletal, and genital birth defects show agreement among the three maps, with the high-rate cluster in the central region and low-rate clusters in the western region. These high- and low-rate clusters may be due to the reporting of birth defects to the KBSR. Both the University of Kentucky Hospital in Lexington and the University of Louisville Hospital in Louisville have partnered to provide data on birth defects to the KBSR. These cities are the largest metro areas in the state and would capture all of the infants with birth defects that visit their facilities, causing the high-rate clusters.

The KBSR currently does not have a sharing agreement with the surrounding states. This may explain why there are a lot of low-rate clusters in the west, in the counties that are bordering Tennessee, as well as the small low-rate cluster in the northern region. Mothers from these counties may be traveling to Nashville in Tennessee or to Cincinnati in Ohio as those hospitals might be the closest to where the mothers are living.

Results of this study indicated lower prevalence rates for counties with high acres of corn planted compared to counties with moderate acres of corn planted, in which the prevalence rates were higher. This finding was consistent with another study conducted by Agopian et al., that used corn acreage and estimated atrazine application as the exposure assessment to analyze maternal residential atrazine exposure and male genital malformations.[41] The authors found that women with medium atrazine exposure had a significantly increased risk of having an infant with any male genital malformation (adjusted odds ratio = 1.20), whereas women who had high atrazine exposure had a decreased risk of having an infant with any male genital malformation (adjusted odds ratio = 0.96). The adjusted rate ratio in this study for the moderate acres of corn planted for all genital birth defects was 1.07, compared to the adjusted rate ratio for the high acres of corn planted for all genital birth defects, which was 0.80. These adjusted rate ratios are similar to the adjusted odds ratio in the comparison study.

In a study conducted by Winston et al., water supplies using surface water were used to estimate annual mean concentrations in streams and to estimate adjusted odds ratios for hypospadias.[53] In Texas, the adjusted odds ratio for the state level estimate for atrazine in the water supply for hypospadias was 1.22. This is similar to the findings in the adjusted model for the mean concentration level for all genital birth defects in this study, in which the adjusted rate ratio was 1.25. Although this study did not examine specific genital birth defects, it does include all hypospadias cases in the genital birth defects group.

The results of this study found that counties with moderate number of acres of corn planted had higher prevalence rates of central nervous system birth defects. Another study, conducted by Garry et al., found similar results. Garry et al. examined birth defects in the general population in Minnesota by crop region.[54] The state was divided into four general crop production areas. They found that that the odds ratio for central nervous system birth defects in regions with corn crops compared to non-crop regions was 1.56. This relates to the results of this study in which the counties that had moderate number of acres of corn planted had 1.52 times higher prevalence of central nervous system birth defects compared to the counties with the lowest number of acres planted. Additionally, the authors found that the odds ratio for all births with anomalies in regions with corn crops compared to non-crop regions was 1.16. Although this is slightly more than what was found in the current study, which was an odds ratio of 1.08, it still compares in that counties with moderate corn acreage have a higher prevalence rate for all birth defects compared to counties with low corn acreage.

Strengths and Limitations

The primary strength of this study was the ten-year time period for which the birth defects data was available. This provided adequate numbers for each county since small sample sizes, low power, and rare birth defect outcomes are common in birth defect research. Additionally, this study included atrazine samples over a twelve year period (1998-2009) that overlapped with the study time period and was used to assess the atrazine exposure for each county. This is one of the few studies that has assessed exposure by measuring the amount of atrazine in water. The majority of the research has consisted of occupational studies in which the exposure was assessed through questionnaires. This study also uses three different methods to assess atrazine in the water as well as one agricultural method. The maximum concentration levels and mean concentration levels by county were used to create categorical variables to examine the increasing exposure of atrazine in the water samples. This allowed for dose-response relationships to be assessed when examining the association of atrazine and birth defects. The absence of multiple comparisons that may have reported associations based on chance alone, unlike previous studies, is also a strength.

Another strength of this study is the mapping and spatial analyses, and cluster identification that was conducted. Evaluating the clusters allowed an evaluation of the association between birth defects rates and the spatial distribution of atrazine in water levels. This is the first study to examine birth defect clusters independently as well as examine the clusters with the exposure for this time period in the state of Kentucky.

Although this ecological study allowed for county level data and trends to be analyzed, as well as it being advantageous due to the feasibility simply because obtaining individual-level exposure data may be expensive, difficult, and time-consuming, there are several limitations of this study that should be considered. The first limitation of this study was the missing data for four major subgroups of birth defects, including digestive tract defects, skin defects, chromosomal defects, and unspecified defects. In the KBSR, these four birth defect groups had about 30,000 cases. This leads to the overall prevalence rate for the state of Kentucky for all birth defects to be underestimated in this study. Including these cases in the prevalence rate for all birth defects, it would increase to 860.06 per 10,000 live births. By design, ecological studies examine the relationship between the outcome and the exposure at the population level instead of the individual level. Therefore, associations, or lack of associations, observed at the county in this study may not exist at the individual level (ecological fallacy). Since the data collected in this study for birth defects, atrazine, and covariates were all aggregated at the county level, confounding may not have been adequately controlled for.

The atrazine exposure metrics used in this study were not at the individual level, which is a limitation when assessing human atrazine consumption through wells, springs, and municipal water sources. The percent of the population by county served by public water sources was included in this study, however, this study did not have this information on the individual level. Additionally, the specific amount of atrazine consumed by the individuals living in each county is not available, as well as how much atrazine is absorbed and metabolized once it is ingested. Data on current occupation and occupational histories, including duration of exposure and frequency, of the individuals in each county was not available. It is advantageous to control for occupation since atrazine exposure is associated with certain occupations.[54-58] Other pesticides that may have been present in the water samples were unable to be controlled for in this study. Finally, the last limitation includes using the number of acres of corn planted as an indirect measure of atrazine exposure. This proxy of exposure may not accurately provide the amount of atrazine that contaminates rivers, streams, wells, springs, and municipal water sources even though the majority of atrazine is used on corn crops.

Future Directions

The design and results of this study will be useful in future studies examining the association between atrazine and birth defects. Ideally, individual exposure assessment of drinking water should be employed in future research rather than relying on ecologic exposure measures. An additional area for future research is to measure and evaluate effect modification by season and atrazine application. This research would provide more in-depth results to assess if there are differences on the association by the application season. Finally, a case-case study will be conducted in which specific birth defect groups will be examined. For this study design, the cases will be the infants with the specific birth defect that is being examined. The controls will also be cases included from the KBSR, but will be infants that have a birth defect that is not associated with atrazine, particularly chromosomal disorders.

Conclusions

Despite the limitations, the findings of this study are important and add to the existing literature on atrazine and birth defects. These findings will be useful in assisting public health departments, clinicians, and policymakers in reducing the risk of birth defects in infants that have been exposed to atrazine. Further research is needed to confirm these results and to develop exposure metrics at both the individual and county level that accurately represent atrazine exposure to explore this association.

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APPENDIX

Table 1. Descriptive Statistics of Atrazine Exposure Metrics for Wells, Springs, and Municipal Water Sources, 1998 – 2009 (N = 120 counties)

Characteristic	N (%)
Mean Concentration Level (μg/L)[1]	
High	15 (12.50)
Moderate	62 (51.67)
Low	43 (35.83)
Samples Above MCL[2]	
2	29 (24.17)
1	48 (40.00)
0	43 (24.17)

[1] Wells, Springs, and Municipal: High ≥0.098, Moderate 0.00014 – 0.098, Low 0

[2] Maximum Contaminant Level (MCL): 0.003 (μg/L)

Table 2. Descriptive Statistics of Atrazine Exposure Metrics for River and Stream Water Sources, 1998 – 2009 (N = 120 counties)

Characteristic	N (%)
Mean Concentration Level (μg/L)[1]	
High	14 (11.67)
Moderate	39 (32.50)
Low	67 (55.83)
Above MCL[2]	
Yes	50 (41.67)
No	70 (58.33)

[1] Rivers and Streams: High ≥0.4958, Moderate 0.00000538 – 0.4958, Low 0

[2] Maximum Contaminant Level (MCL): 0.003 (μg/L)

Table 3. Descriptive Statistics of Agricultural Exposure Metrics in Kentucky, Census of Agriculture, 2007 and 2012 (N = 120 counties)

Characteristic	Mean (SD)
Acres of Corn Planted (in thousands)	23.69 (40.58)
Bushels of Corn Harvested (in millions)	2.26 (4.20)

Table 4. Frequency and Prevalence of Birth Defects, Kentucky Birth Registry Surveillance, 2005-20014

Year	Total Live Births	All Birth Defects[1]		Genital Birth Defects		Musculoskeletal Birth Defects		Central Nervous System Birth Defects	
		Cases	Prevalence (per 10,000 live births)	Cases	Prevalence (per 10,000 live births)	Cases	Prevalence (per 10,000 live births)	Cases	Prevalence (per 10,000 live births)
2005	55990	1904	340.06	599	106.98	730	130.38	110	19.65
2006	57953	1874	323.37	520	89.73	732	126.31	164	28.30
2007	59297	1938	326.83	551	92.92	728	122.77	225	37.94
2008	58333	1819	311.83	515	88.29	624	106.97	241	41.31
2009	57532	1527	265.42	361	62.75	527	91.60	254	44.15
2010	55672	1169	209.98	230	41.31	421	75.62	264	47.42
2011	55370	2024	365.54	549	99.15	755	136.36	291	52.56
2012	55744	2150	385.69	628	112.66	822	147.46	280	50.23
2013	55699	2206	396.06	656	117.78	841	150.99	268	48.12
2014	56530	2132	377.14	637	112.68	819	144.88	261	46.17
Overall	568120	18743	329.91	5246	92.34	6999	123.20	2358	41.51

[1] This is excluding digestive tract defects, skin defects, chromosomal defects, and unspecified defects.

Table 5. Unadjusted Models of Atrazine Exposure Metrics and All Birth Defects

Measure	Unadjusted Estimate	Unadjusted Rate Ratio	95% CI	P-value
Samples Above MCL				
3	-0.5110	0.600	0.341-1.056	0.0763
2	-0.1152	0.891	0.587-1.352	0.5882
1	0.2009	1.223	0.809-1.846	0.3396
0	-	-	-	-
% above LOD	0.0016	1.002	0.994-1.009	0.665
Mean Concentration Level				
High	0.5068	1.660	0.937-2.939	0.9045
Moderate	-0.0233	0.977	0.667-1.430	0.0822
Low	-	-	-	-
Acres of Corn Planted				
High	-0.4080	0.665	0.447-0.989	0.0439
Moderate	-0.4842	0.616	0.414-0.917	0.0169
Low	-	-	-	-

Table 6. Unadjusted Models of Atrazine Exposure Metrics and Central Nervous System Birth Defects

Measure	Unadjusted Estimate	Unadjusted Rate Ratio	95% CI	P-value
Samples Above MCL				
3	-0.6190	0.538	0.255-1.135	0.1038
2	-0.0657	0.936	0.551-1.590	0.8079
1	0.0290	1.029	0.606-1.749	0.9146
0	-	-	-	-
% above LOD	-0.0034	0.997	0.987-1.007	0.610
Mean Concentration Level				
High	-0.2715	0.762	0.346-1.678	0.5001
Moderate	-0.1227	0.885	0.547-1.431	0.6170
Low	-	-	-	-
Acres of Corn Planted				
High	-0.2426	0.978	0.577-1.658	0.9338
Moderate	0.0228	1.023	0.605-1.731	0.9322
Low	-	-	-	-

Table 7. Unadjusted Models of Atrazine Exposure Metrics and Musculoskeletal Birth Defects

Measure	Unadjusted Estimate	Unadjusted Rate Ratio	95% CI	P-value
Samples Above MCL				
3	-0.4120	0.662	0.367-1.193	0.1698
2	-0.1157	0.891	0.578-1.374	0.6005
1	0.0756	1.079	0.703-1.655	0.7294
0	-	-	-	-
% above LOD	-0.0021	0.998	0.990-1.006	0.610
Mean Concentration Level				
High	0.2293	1.258	0.685-2.308	0.4591
Moderate	-0.1126	0.894	0.603-1.324	0.5747
Low	-	-	-	-
Acres of Corn Planted				
High	-0.2426	0.785	0.517-1.190	0.2541
Moderate	-0.3280	0.720	0.475-1.092	0.1222
Low	-	-	-	-

Table 8. Unadjusted Models of Atrazine Exposure Metrics and Genital Birth Defects

Measure	Unadjusted Estimate	Unadjusted Rate Ratio	95% CI	P-value
Samples Above MCL				
3	-0.7358	0.479	0.254-0.904	0.1038
2	-0.1212	0.886	0.560-1.402	0.8079
1	0.2816	1.325	0.839-2.093	0.9146
0	-	-	-	-
% above LOD	0.0050	1.005	0.997-1.013	0.2309
Mean Concentration Level				
High	0.8514	2.343	1.238-4.435	0.0089
Moderate	-0.0254	0.975	0.640-1.485	0.9059
Low	-	-	-	-
Acres of Corn Planted				
High	-0.5243	0.592	0.378-0.926	0.0217
Moderate	-0.5957	0.551	0.352-0.862	0.0091
Low	-	-	-	-

Table 9. Adjusted Model: Number of Samples above the Maximum Contaminant Level

	All Birth Defects				Genital Birth Defects				Musculoskeletal Birth Defects				Central Nervous System Birth Defects			
Characteristic	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**
Samples Above MCL																
3	-0.3111	0.7326	0.428-1.255	0.257	-0.3888	0.6779	0.369-1.245	0.210	-0.2360	0.790	0.442-1.409	0.424	-0.4699	0.625	0.300-1.304	0.210
2	0.0511	1.0524	0.689-1.607	0.813	0.1012	1.107	0.687-1.782	0.677	-0.0172	0.983	0.624-1.550	0.941	-0.0052	0.995	0.558-1.775	0.986
1	0.3439	1.4105	0.971-2.048	0.071	0.3698	1.448	0.955-2.194	0.082	0.2739	1.315	0.878-1.970	0.184	0.1106	1.117	0.667-1.87	0.674
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High School Degree or Higher	-0.0447	0.9563	0.921-0.993	0.019	-0.0623	0.939	0.900-0.981	0.004	-0.0298	0.971	0.932-1.010	0.146	-0.0624	0.939	0.889-0.992	0.025
Median Household Income	0.00002	1.0000	0.9999-1.0001	0.298	0.00004	1.000	1.000-1.0001	0.059	0.00002	1.000	0.999-1.0001	0.284	0.00001	1.000	0.999-1.0001	0.583
% Below the Poverty Level	-0.0008	0.9992	0.962-1.038	0.966	0.0068	1.007	0.965-1.051	0.755	0.0134	1.013	0.972-1.057	0.529	-0.0272	0.973	0.923-1.027	0.319
% Using Public Water	-0.0049	0.9951	0.986-1.005	0.320	-0.0070	0.993	0.983-1.004	0.199	-0.0056	0.994	0.984-1.005	0.293	0.0020	1.002	0.988-1.017	0.787
% of Females Age between 10 - 59	-0.1243	0.8831	0.839-0.929	<0.0001	-0.1341	0.875	0.825-0.927	<0.0001	-0.1377	0.871	0.826-0.919	<0.0001	-0.1511	0.860	0.801-0.922	<0.0001
% of Female Population White	-0.2639	0.7680	0.689-0.857	<0.0001	-0.2959	0.744	0.662-0.836	<0.0001	-0.2332	0.792	0.703-0.892	0.0001	-0.2322	0.793	0.686-0.916	0.002

Table 10. Adjusted Model: Mean Concentration Level

	All Birth Defects				Genital Birth Defects				Musculoskeletal Birth Defects				Central Nervous System Birth Defects			
Characteristic	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**
Samples																
High	0.1137	1.120	0.644-1.948	0.687	0.3555	1.427	0.764-2.67	0.265	-0.0710	0.931	0.518-1.676	0.813	-0.5450	0.580	0.273-1.232	0.156
Moderate	0.2008	1.222	0.856-1.747	0.270	0.2020	1.252	0.843-1.861	0.265	0.1198	1.127	0.771-1.649	0.537	-0.0258	0.975	0.603-1.576	0.916
Low	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High School Degree or Higher	-0.0429	0.958	0.921-0.997	0.033	-0.0589	0.943	0.901-0.986	0.010	-0.0294	0.971	0.931-1.012	0.165	-0.0681	0.934	0.885-0.987	0.015
Median Household Income	0.00002	1.0000	0.9999-1.0001	0.342	0.00003	1.000	0.999-1.0001	0.095	0.00002	1.000	0.999-1.0001	0.255	0.00001	1.000	0.999-1.0001	0.4322
% Below the Poverty Level	0.0062	1.006	0.968-1.046	0.752	0.0162	1.016	0.973-1.061	0.462	0.0189	1.019	0.978-1.062	0.374	-0.0284	0.972	0.922-1.025	0.293
% Using Public Water	-0.0053	0.995	0.985-1.005	0.301	-0.0066	0.993	0.983-1.004	0.235	-0.0064	0.994	0.983-1.004	0.244	-0.0016	0.998	0.984-1.013	0.830
% of Females Age between 10 - 59	-0.1161	0.890	0.845-0.938	<0.0001	-0.1234	0.884	0.833-0.938	<0.0001	-0.1327	0.876	0.8304-0.924	<0.0001	-0.1604	0.852	0.795-0.913	<0.0001
% of Female Population White	-0.2720	0.762	0.679-0.855	<0.0001	-0.2891	0.749	0.661-0.848	<0.0001	-0.2443	0.783	0.694-0.884	<0.0001	-0.2386	0.788	0.682-0.910	0.001

Table 11. Adjusted Model: Percent of Samples Above Limit of Detection

	All Birth Defects				Genital Birth Defects				Musculoskeletal Birth Defects				Central Nervous System Birth Defects			
Characteristic	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**
% of Samples Above LOD	-0.0020	0.998	0.991-1.005	0.561	0.0015	1.00	0.994-1.009	0.705	-0.0028	0.997	0.990-1.004	0.446	-0.0049	0.995	0.986-1.005	0.320
High School Degree or Higher	-0.0437	0.957	0.921-0.995	0.028	-0.0619	0.940	0.899-0.983	0.006	-0.0288	0.972	0.933-1.012	0.170	-0.0670	0.935	0.885-0.988	0.017
Median Household Income	0.00002	1.000	0.999-1.0001	0.231	0.00004	1.000	1.000-1.0001	0.044	0.00002	1.000	0.999-1.0001	0.213	0.00002	1.000	0.999-1.0001	0.447
% Below the Poverty Level	0.0040	1.00	0.966-1.044	0.841	0.0145	1.015	0.971-1.060	0.517	0.0172	1.017	0.976-1.061	0.421	-0.0276	0.973	0.922-1.026	0.311
% Using Public Water	-0.0031	0.997	0.981-1.007	0.544	-0.0067	0.993	0.982-1.004	0.231	-0.0039	0.996	0.986-1.007	0.462	0.0025	1.003	0.988-1.017	0.732
% of Females Age between 10 - 59	-0.1245	0.883	0.840-0.928	<0.0001	-0.1353	0.873	0.826-0.924	<0.0001	-0.1344	0.874	0.831-0.920	<0.0001	-0.1528	0.858	0.802-0.918	<0.0001
% of Female Population White	-0.2683	0.765	0.683-0.856	<0.0001	-0.2912	0.747	0.661-0.845	<0.0001	-0.2393	0.787	0.698-0.888	<0.0001	-0.2401	0.786	0.679-0.910	0.001

Table 12. Adjusted Model: Acres of Corn Planted

	All Birth Defects				Genital Birth Defects				Musculoskeletal Birth Defects				Central Nervous System Birth Defects			
Characteristic	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**	**Estimate**	**Adjusted Rate Ratio**	**95% CI**	**P-value**
Samples of Corn																
High	-0.2372	0.7889	0.508-1.224	0.290	-0.2215	0.801	0.492-1.306	0.374	-0.1891	0.828	0.521-1.316	0.424	0.0507	1.052	0.583-1.899	0.866
Moderate	0.0782	1.081	0.735-1.591	0.692	0.0639	1.066	0.694-1.638	0.771	0.1219	1.130	0.752-1.696	0.556	0.4200	1.522	0.900-2.573	0.117
Low	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High School Degree or Higher	-0.0421	0.959	0.922-0.997	0.034	-0.0604	0.941	0.901-0.984	0.008	-0.0278	0.973	0.934-1.013	0.182	-0.0629	0.939	-.889-0.992	0.024
Median Household Income	0.00001	1.000	0.999-1.0001	0.394	0.00004	1.000	1.000-1.0001	0.066	0.00002	1.000	0.999-1.0001	0.365	0.0000	1.000	0.999-1.0001	0.758
% Below the Poverty Level	-0.0037	0.996	0.957-1.037	0.855	0.00579	1.006	0.962-1.052	0.800	0.0113	1.011	0.969-1.056	0.609	-0.0249	0.975	0.923-1.031	0.378
% Using Public Water	-0.0050	0.995	0.985-1.005	0.315	-0.0073	0.993	0.982-1.004	0.186	-0.0061	0.994	0.984-1.004	0.251	-0.0026	0.997	0.983-1.012	0.729
% of Females Age between 10 - 59	-0.1387	0.871	0.826-0.917	<0.0001	-0.1543	0.857	0.807-0.911	<0.0001	-0.1466	0.864	0.818-0.912	<0.0001	-0.1555	0.856	0.795-0.921	<0.0001
% of Female Population White	-0.2548	0.775	0.693-0.867	<0.0001	-0.2904	0.748	0.662-0.845	<0.0001	-0.230	0.795	0.705-0.896	0.0002	-0.2399	0-787	0.680-0.910	0.0012

Figure 1. Birth Prevalence Rates for All Birth Defects, Genital, Musculoskeletal, and Central Nervous System Birth Defects, Kentucky, 2005 – 2014 (N=18,743)

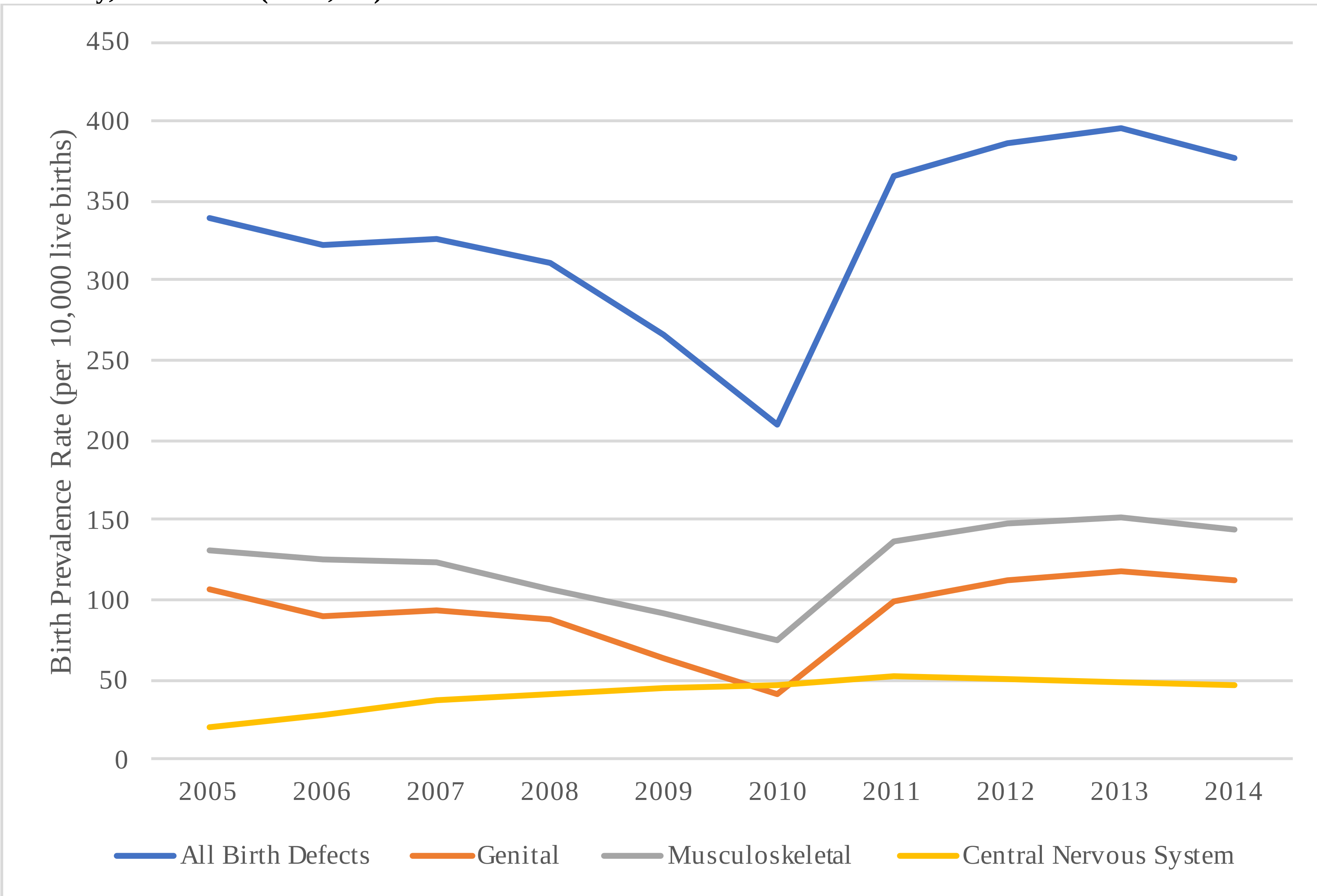

Figure 2. Mean Concentration Levels of Atrazine in Kentucky, 1998 – 2009

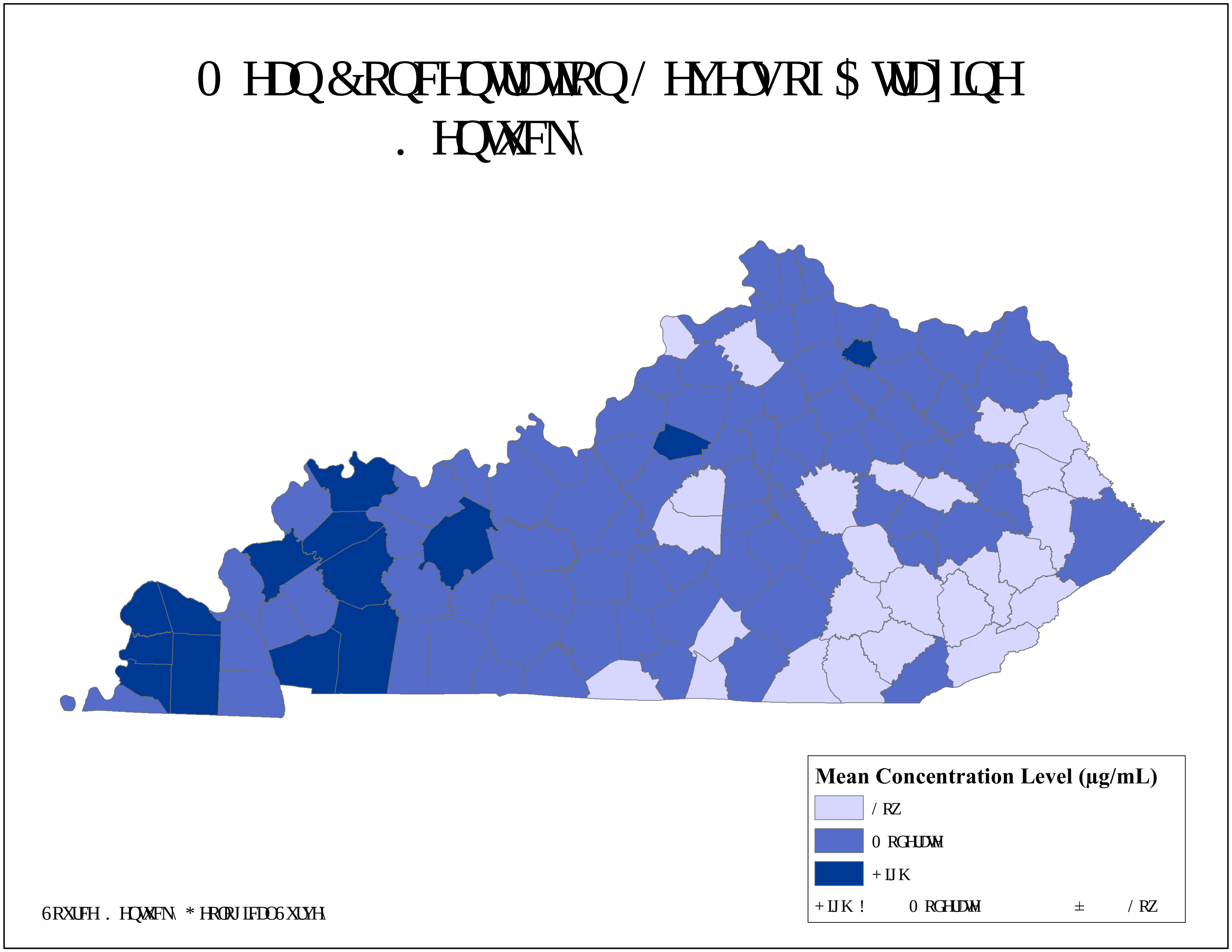

Figure 3. Number of Samples Above the Maximum Contaminant Level for Atrazine in Kentucky, 1998 – 2009

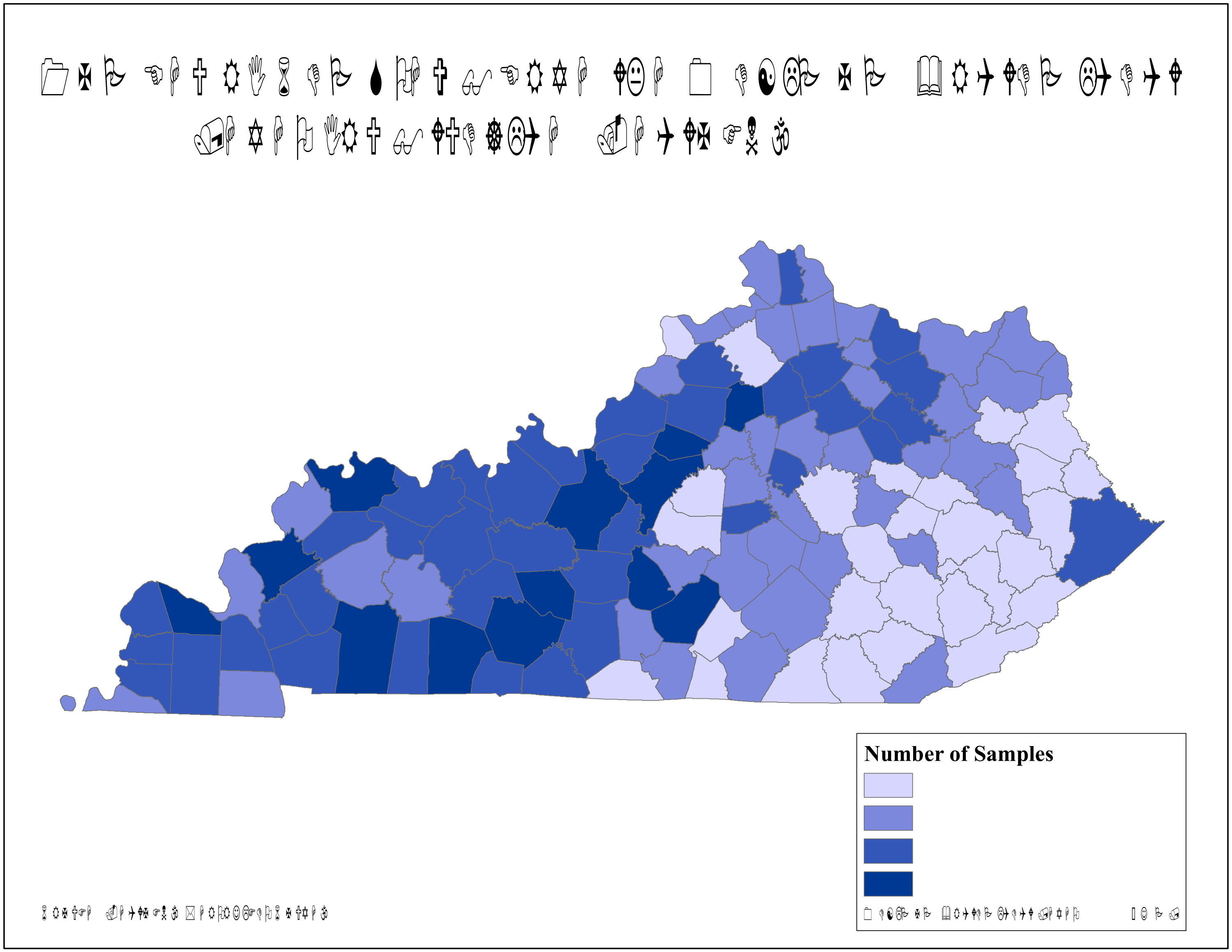

Figure 4. Percent of Samples Above the Limit of Detection for Atrazine in Kentucky, 1998 – 2009

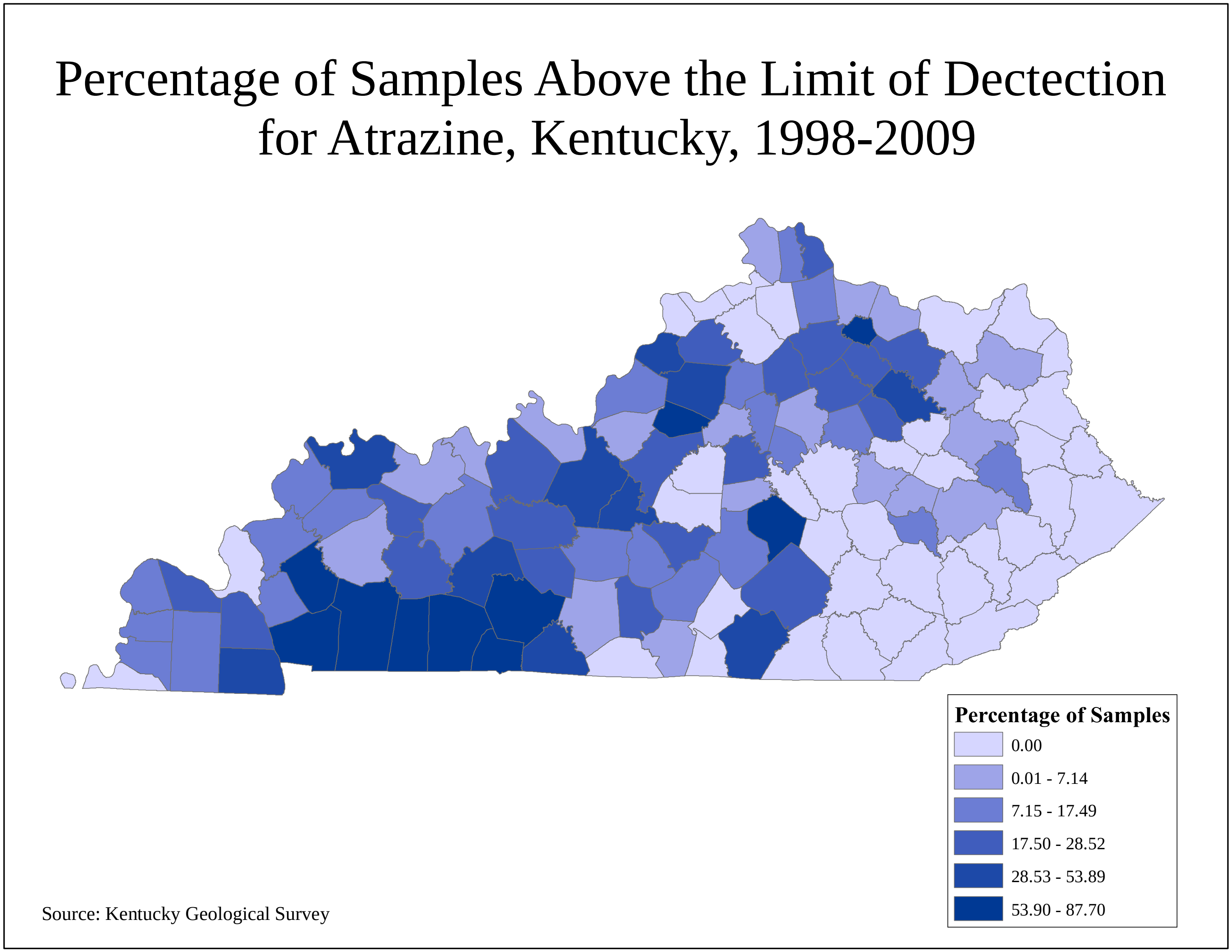

Figure 5. Average Acres of Corn Planted in Kentucky by Tertile: 2007 and 2012

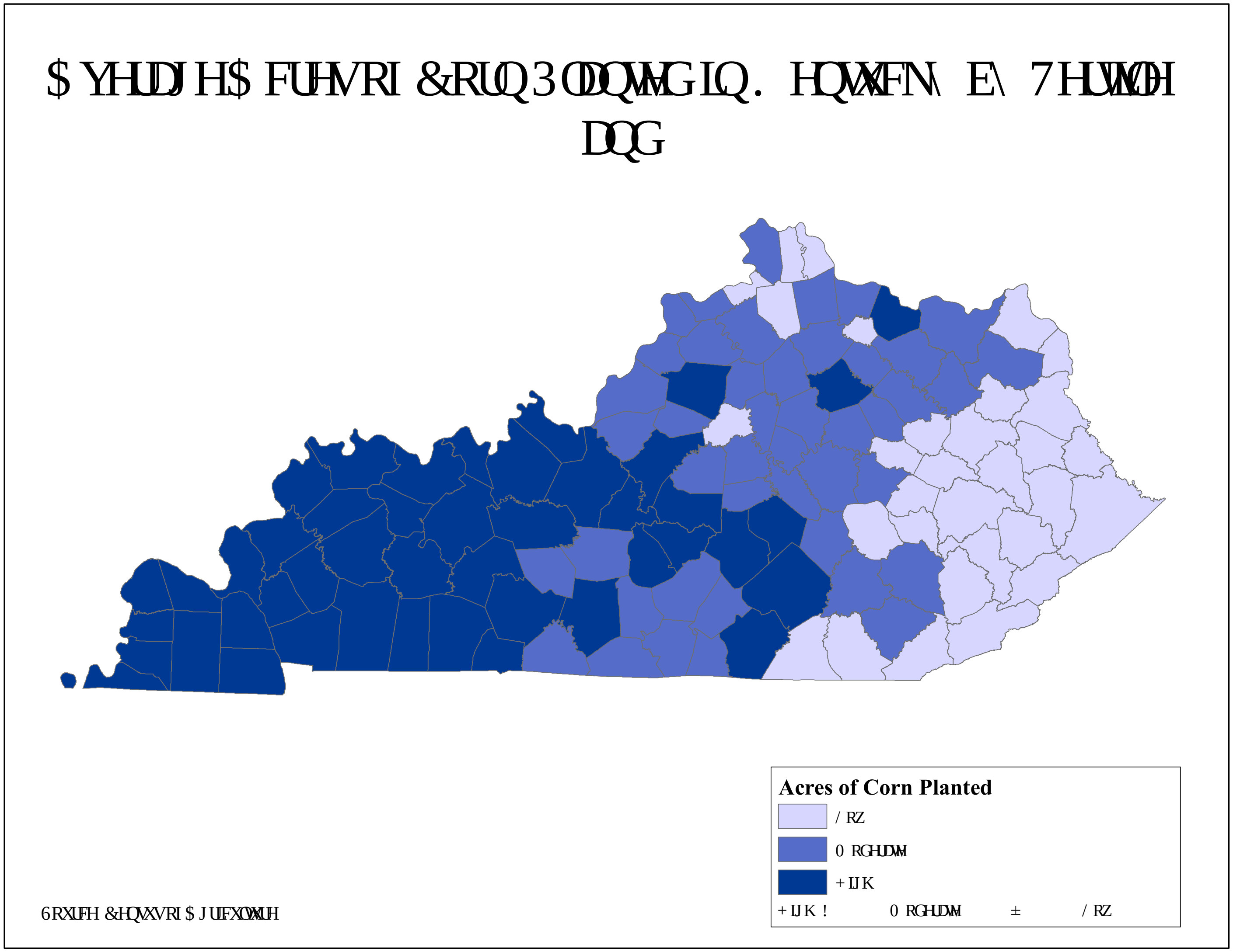

Figure 6. Clusters of High- and Low-Rates of All Birth Defects Prevalence

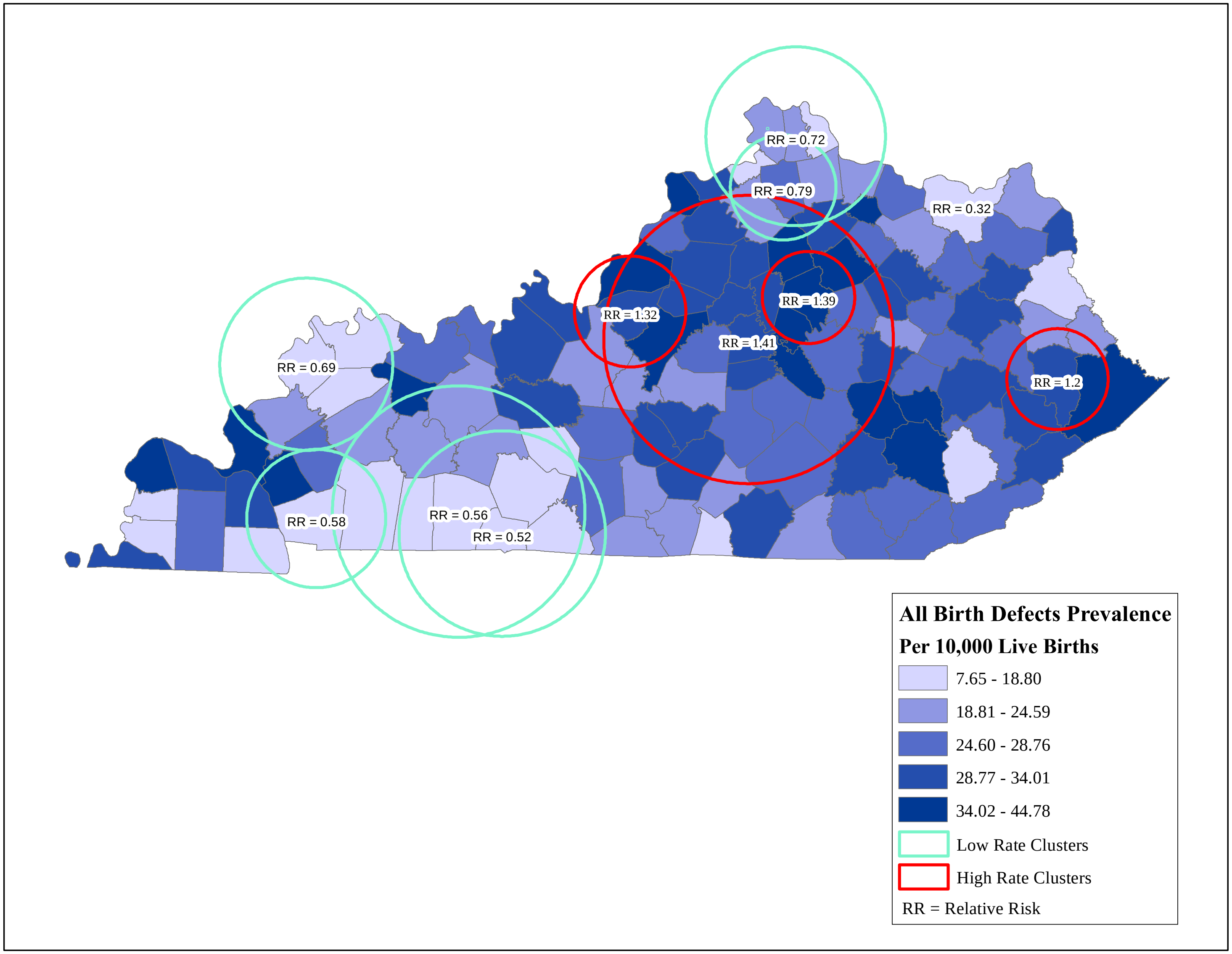

Figure 7. Clusters of High- and Low-Rates of Central Nervous System Birth Defects Prevalence

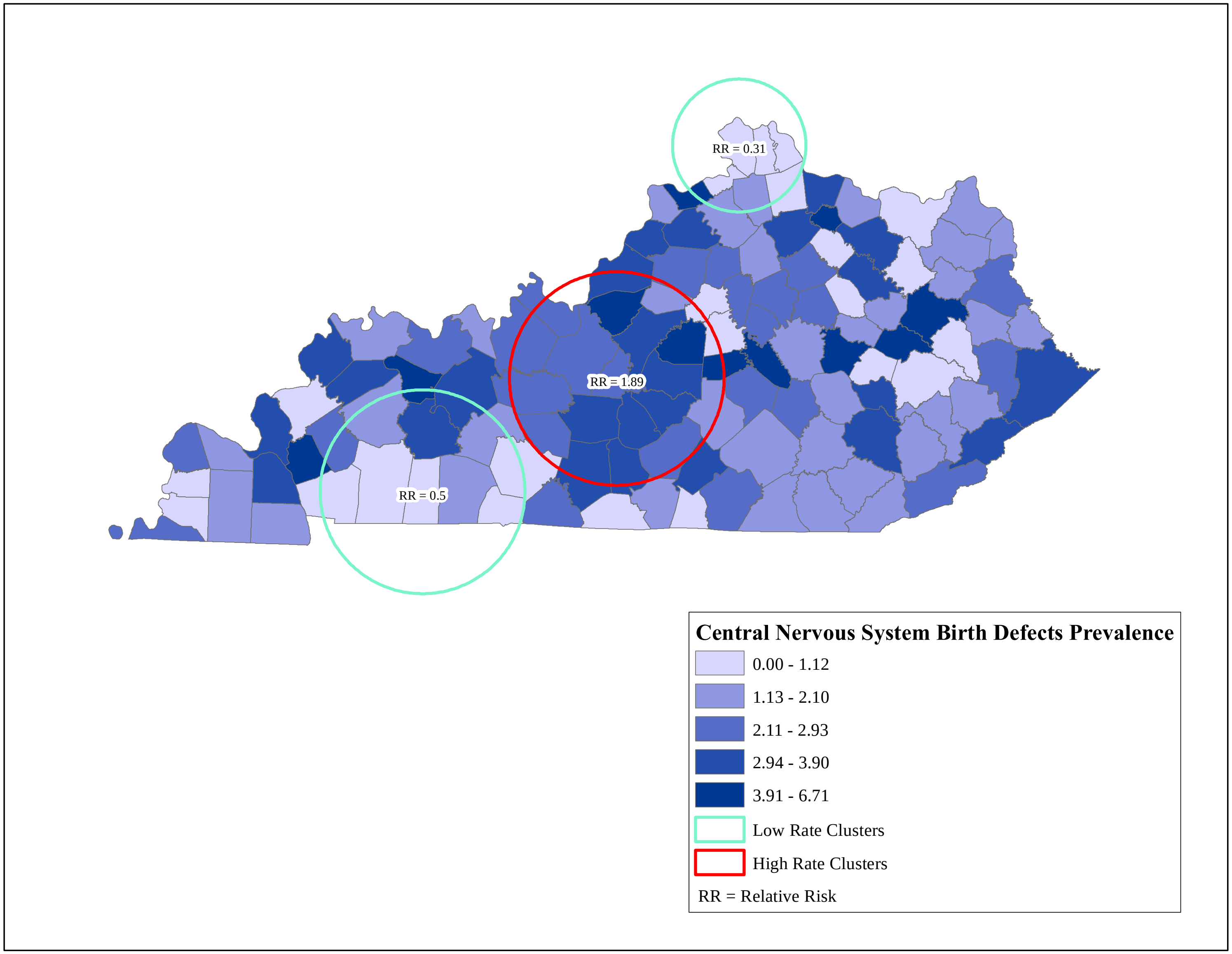

Figure 8. Clusters of High- and Low-Rates of Musculoskeletal Birth Defects Prevalence

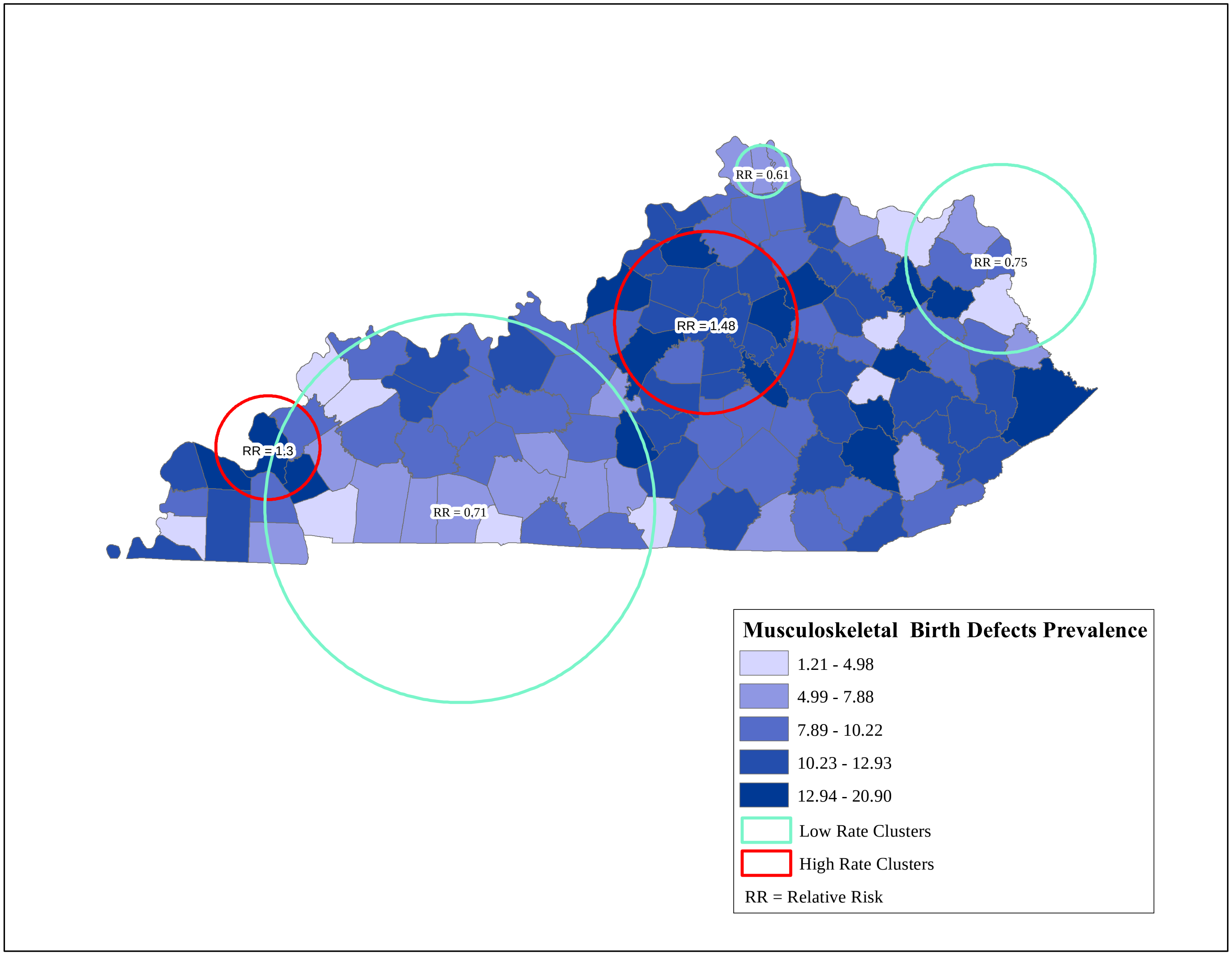

Figure 9. Clusters of High- and Low-Rates of Genital Birth Defects Prevalence