A Linkage Study of Adverse Birth Outcomes With

Agricultural Land Use Practices in Missouri

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THESIS

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TABLE OF CONTENTS

LIST OF TABLES

<u>TABLE</u> <u>PAGE</u>

LIST OF FIGURES

LIST OF ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
EPHTN	Environmental Public Health Tracking Network
GEE	Generalized Estimating Equations
GM	Genetically Modified
NASS	National Agricultural Statistics Survey
PNC	Prenatal Care
RR	Relative Risk
SES	Socio-Economic Status
SGA	Small for Gestational Age
USDA	United States Department of Agriculture

SUMMARY

Agricultural land use, including pesticide application, is ubiquitous in the midwestern United States. Missouri is an agriculturally intensive state, growing primarily corn, soybeans, and wheat, with additional high-intensity rice and cotton farming in the southeastern counties in the state. Pesticides applied aerially or at the ground level can travel up to hundreds of meters from their intended application site, resulting in higher levels of pesticides in houses more proximal to pesticide-treated fields than those farther away. Further, families of agricultural workers as well as families that reside proximal to pesticide-treated fields may have increased risk of adverse birth outcomes including certain birth defects and adverse birth outcomes such as low birth weight and preterm births. Multiple pesticides are used on each crop and vary between crop species, with atrazine and glyphosate being the most commonly used chemicals on corn and soybeans, respectively. Atrazine has been specifically associated with adverse birth outcomes such as small for gestational age, lower birth weight and head circumference, and preterm births. There is some evidence that glyphosate may be genotoxic and disrupt endocrine function, but the literature is sparse. Agricultural land use practices have strong spatial and temporal trends, with intensive use of pesticides during specific months and on specific land within a county dedicated to a specific crop. As such, the aims of this study were (1) to evaluate the relationship between county-level measures of agricultural production and adverse birth outcomes in Missouri, and (2) to evaluate the importance of incorporating spatial and temporal information into the modeling of this data.

Corn, soybean, wheat, rice, and cotton crop densities were evaluated for their relationship with both low birth weight and preterm births in Missouri between 2004–2006. The covariates considered as potential confounders and effect modifiers in this study included gender, mother's race and ethnicity, mother's age at birth, quarter of birth, county median household income, and population density. County-level rates of maternal smoking and prenatal care status were also considered in the analyses. Three statistical approaches were taken to evaluate the relationship between each measure of crop density and each outcome, low birth weight and preterm births. The first method was Poisson regression, using the natural logs of full-term singleton births and all singletons births were used as the offsets for the low birth weight and preterm birth models, respectively. The second approach was generalized estimating equations, which accounts for both temporal and spatial correlations in the study observations. Finally, a distance decay random effects Poisson regression model which allowed for spatially varying random effect was used to account for the highly variable and non-random geographic distribution of crop production across each of the study states.

Strong positive associations between both rice and cotton density and both outcomes, low birth weight and preterm births, were found in Missouri across all three methodologies. Rice density was associated with significant increased risk of preterm births across all three models, as was cotton density with low birth weight births. Only eight out of 115 counties in Missouri produced rice between 2004 and 2006. There were five counties that produce cotton in Missouri, all of which also produced rice. Despite the geographic correlation between these counties, both rice and cotton densities remained significant predictors of low birth weight and preterm births in dual-exposure models with a slight weakening of the effect in the distance decay models. A positive, but only marginally significant association was observed between soybean density and low birth weight across all models. No associations between wheat or corn and either outcomes was observed.

SUMMARY (continued)

The strong associations between both rice and cotton and low birth weight and preterm births warrant further investigation. The limitations of the study include the ecological study design, limited covariates, and lack of chemical-specific exposure estimates. Despite these limitations, this study benefits from a large sample size and has the unique component of evaluating methodologies for linking land use measures with health outcome data.

I. INTRODUCTION

A. Background

More than 50% of land in the coterminous United States is dedicated to commercial agriculture (United States Department of Agriculture, 2002). In Missouri, the proportion of land dedicated to growing soybeans and corn within a county can be as high as 58% and 37% respectively (Figure 1). Most commercial agriculture relies on the application of large amounts of pesticides and fertilizers each year. For example, an estimated 821 and 857 million pounds of pesticides were used in the United States in 2006 and 2007 respectively (United States Environmental Protection Agency, 2011). While these pesticides and fertilizers increase crop yields, their use raises concerns about potential ecological and human health effects.

Agricultural workers, including farmers and pesticide applicators, may be exposed to pesticides through direct handling of chemicals or contact with pesticide-laden crops. Agricultural use of pesticides, however, may result in exposures to communities surrounding agricultural fields in general. Pesticides applied aerially and at ground level have been detected hundreds of meters from their intended application sites, which indicates that residing near pesticide-treated fields may result in non-occupational pesticide exposures (Chester and Ward 1984; Rull et al., 2006). The take-home pathway is an important exposure route for families of agricultural workers, which have a higher exposure to these chemicals than non-agricultural workers and their families (Curl et al., 2002; Thompson et al., 2003; Curwin et al., 2005). For families without an occupational source of exposure, those who reside in houses proximal to pesticide-treated agricultural land had significantly higher levels of certain pesticides in their house dust than homes farther away (Lu et al., 2000; Fenske et al., 2002). This suggests that

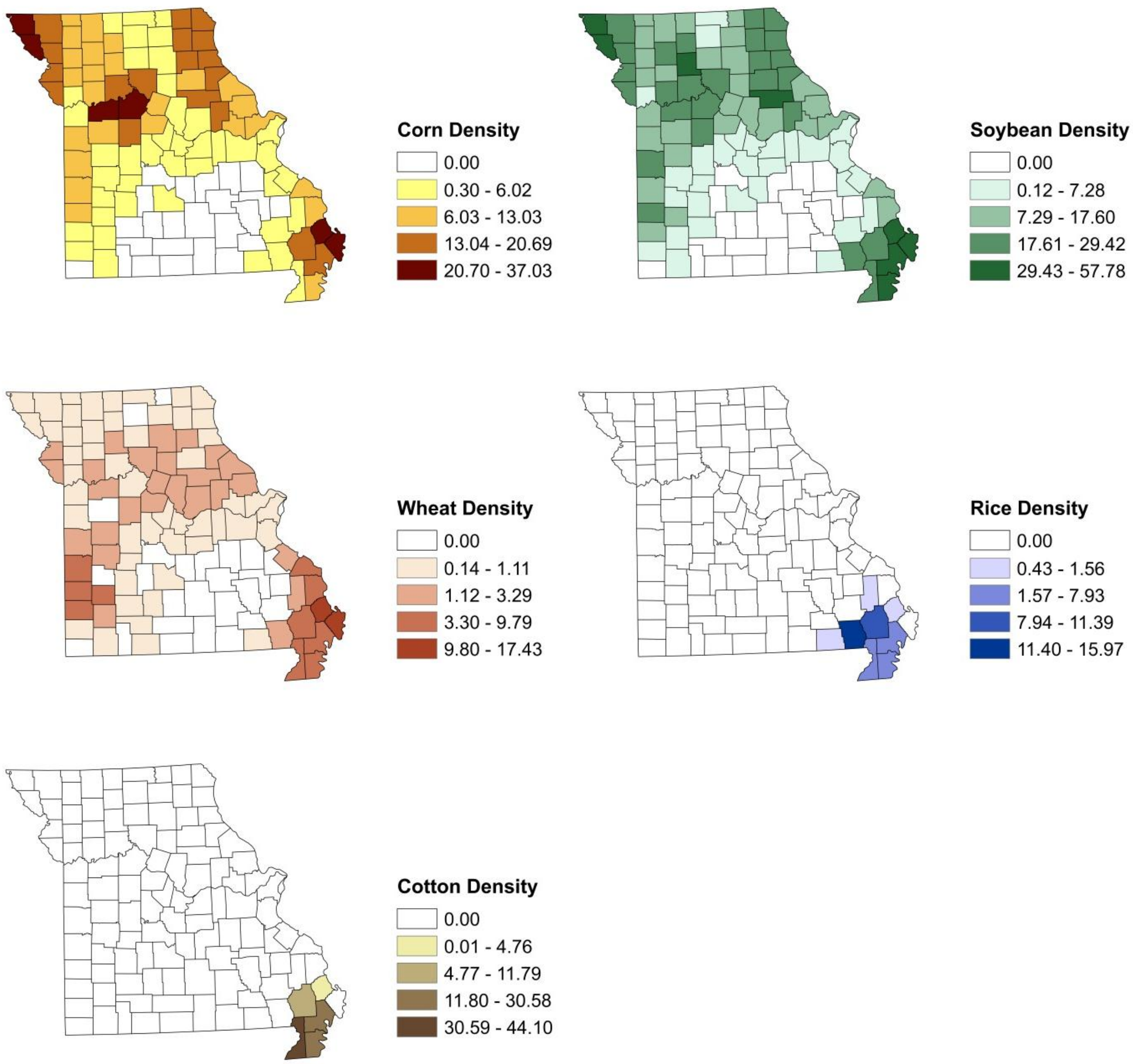

Figure 1. Crop coverage of corn, soybeans, wheat, rice, and cotton in Missouri, 2004–2006.
Data Source: United States Department of Agriculture, 2010.

there are pesticide exposure pathways beyond diet, water contamination, and residential pesticide use for those residing in and around agricultural regions.

Beyond higher exposures to pesticides, families of agricultural workers as well as families that reside proximal to pesticide treated fields have an increased risk of adverse birth outcomes including neural tube defects and limb reduction defects among infants (Schwartz et al., 1986; Shaw et al., 1999; Rull et al., 2006; Ochoa-Acuña and Carbajo 2009). Garry et al. (1996) found that rates for all birth anomalies were higher in the crop growing regions of Minnesota versus the non-agricultural regions from 1989–1992. Proximity to specific crop types has been evaluated for an association with adverse birth outcomes. Ochoa-Acuña and Carbajo (2009) found that limb birth defects were significantly positively associated with the amount of corn field acreage within a 500m buffer of mother's residence, but found no such association with soybean acreage. Using wheat acreage in a county as a surrogate for chlorophenoxy herbicide exposure, Schreinemachers (2003) saw an increase in birth malformations of the circulatory/respiratory and musculoskeletal/integumental systems in counties with higher versus lower acreages of wheat production. There is evidence of an association between agricultural pesticide exposure and preterm and/or low birth weight births, but results are not consistent across all studies (Shirangi et al., 2010). One study in Colorado found that low birth weight was associated with the total crop production, and specifically sugar beets and corn, within 300m of mother's residence (Xiang et al., 2000), while another found no association between proximity to agricultural land and low birth weight (Grether et al., 1987).

Typically, multiple pesticides are used on each crop and vary between crop species (Appendix A). Two of the most ubiquitous pesticides in the Midwest are atrazine and glyphosate, the active ingredient of RoundUp®. Atrazine is a pre-emergence broadleaf herbicide most commonly applied to corn. Between 66 and 82 million pounds of atrazine were applied in the coterminous United States each year between 1996 and 2007, and more than 80% of atrazine applied was used on corn fields (United States Geological Survey, 2010). Glyphosate is used for weed control on many genetically modified (GM) crops, but is used most intensively on soybean fields, where it was applied to roughly 90% of planted acreage in the United States between 2004–2006 (Appendix A, Table V). Glyphosate is also commonly applied to cotton (71% of planted acreage in 2006), and less commonly to corn (31% in 2005), wheat (47% in 2006), and rice (23% in 2006) (Appendix A, Tables IV–IX).

Atrazine has been specifically associated with adverse birth outcomes. Munger et al. (1997) found that Iowa communities with drinking water contaminated by atrazine, metolachlor, and cyanzine had significantly elevated risk of delivering small for gestational age (SGA) babies compared to non-contaminated adjacent counties. Chevrier et al. (2011) found that maternal exposure to atrazine during pregnancy was associated with lower birth weight, length, and head circumference. Ochoa-Acuña et al. (2009) reported that atrazine in drinking water during the third trimester and entire pregnancy was found to be significantly associated with an increased prevalence of SGA births in Indiana, but not with preterm births. Rinsky et al. (2012), observed a significantly increased risk of preterm births in Kentucky counties with the highest versus the lowest atrazine levels in drinking water between 2004 and 2006. Other studies, however, have shown no association between atrazine levels or pesticide-related activity during pregnancy and low birth weight or preterm births (Villanueva et al., 2005; Sathyanarayana et al., 2010).

While there is evidence that suggests glyphosate may be genotoxic and disrupt endocrine function in animal studies (de Castilhos and Cestari 2012; Romano et al., 2012), the literature is sparse. Savitz et al. (1997) found elevated, but not statistically significant, odds for preterm birth of infants whose fathers had been exposed to glyphosate-based pesticides prior to and at the time of conception. More recently, Gasnier et al. (2009) found glyphosate-based herbicides to be genotoxic and disrupt the endocrine system in human cells. However, the genotoxicity may be more dependent on the chemicals added to glyphosate-based herbicides than on the glyphosate itself. A recent industry sponsored review of the literature concluded that there is no evidence linking glyphosate to adverse reproductive outcomes and that the surfactants added to glyphosate formulations may be responsible for any observed toxicity (Williams et al., 2012).

Environmental exposures and health outcomes may have strong geographic and temporal patterns, and as such, traditional regression approaches that ignore spatial and temporal information may not be appropriate for their analysis. Geographic correlations in health outcomes may result from populations in different areas being exposed to greater risk or protective factors (Mike Jerrett, 2012, personal communication). Further, it is important to consider the non-independence of observations, if the unit of observation is a geographic unit such as county. Counties that are adjacent would be expected to be more similar in terms of confounders than counties farther away (Burnett et al.; 2001). For these reasons, incorporating spatial and temporal data into epidemiologic linkages of environmental data and health outcome data may be important.

B. **Study Aims**

The first aim of this study is to evaluate the relationship between county-level measures of agricultural production and adverse birth outcomes in the state of Missouri. Agricultural crop production, measured by the density of a specific crop acreage in each county, is used as a surrogate for community pesticide exposure. Two birth outcomes are explored: low birth weight in term births and preterm births in singleton births.

The second aim of this study was to evaluate the importance of incorporating spatial information in the modeling of this data, which was done by comparing the results of three statistical approaches with increasing spatial complexity. Spatial methods were employed to address two main methodological issues: (1) the exposure variables exhibit strong geographic patterns (Figure 1), and (2) adjacent counties are expected to be more similar than counties farther away on unmeasured covariates.

II. METHODS

A. Study Area

The study area was the state of Missouri, located in the midwestern United States. The predominant crop species across Missouri are corn, soybean, and wheat. Rice and cotton are intensively grown in the southeast corner of the state (Figure 1).

B. Birth Outcome Data

County-level counts of two adverse birth outcomes were provided by the Missouri Department of Health and Senior Services for the years 2004, 2005, and 2006. The outcomes examined in the present study were preterm births among live singleton births and low birth weight births among full term live singleton births. Preterm births were defined as infants born prior to 37 weeks gestation. Low birth weight births were defined as infants weighing less than 2,500 grams at or above 37 weeks gestation (i.e., full term). During the study period there were 140,329 live births in Missouri. All birth outcome data was stratified by race, ethnicity, gender, month of birth, year of birth, and county.

C. Agricultural Land Use Measurements

Agricultural land use, as measured by the percentage of county land dedicated to the production of specific crops, was used as an indicator of the potential for community exposure to agricultural chemicals. The specific crops of interest were corn, soybeans, wheat, rice, and cotton. The acreage of planted and harvested in each county were obtained from the United States Department of Agriculture's (USDA) National Agricultural Statistics Service (USDA, 2010). For each county, crop density was calculated by dividing the area of land planted with a specific crop by the total land area of the county (United States Department of Commerce, 2011). In epidemiologic analyses, only those crops that comprised at least 10% of total agricultural land were considered. In addition to corn, soybeans, and wheat, Missouri includes counties with high rice and cotton production, therefore rice and cotton densities were examined as well.

D. **Data for Other Risk Factors**

County-level covariates considered as potential confounders and effect modifiers in this study included mother's race and ethnicity, mother's age at birth, quarter of birth, county median household income, and population density. County-level rates of maternal smoking and prenatal care status were also considered in the analyses.

Variables including race, ethnicity, gender, and month and year of birth were provided with the health outcomes data from the Missouri Department of Health and Senior Services along with the birth outcome data. The race categories used in this analysis were White, Black, and Other. The ethnic categories used were Hispanic, non-Hispanic, and unknown. Quarter of birth was defined as (1) January through March, (2) April through June, (3) July through September, and (4) October through December.

Maternal age group was obtained from data provided by the Centers for Disease Control Environmental Public Health Tracking Network (EPHTN). The percentage of births born to mothers in each age group was calculated from data on the number of full term singleton live births born to each maternal age group per county divided by the total number of full term singleton live births per county. Maternal age at birth was divided into four categories: 10–19, 20–29, 30–39, and 40–54 years of age.

The median household income in each county for years 2004, 2005, and 2006 was used as an indicator of socio-economic status (SES) and was obtained from the U.S. Census Bureau's American Community Survey (United States Department of Commerce, 2010). Population density of each county was used as an indicator of urbanicity. Annual county-level rates of maternal smoking and access to prenatal care were obtained from the Missouri Department of Health and Senior Services (http://health.mo.gov/data/CommunityDataProfiles/).

Consistent with other studies (Schreinemachers 2003; Rinsky et al., 2012), we excluded counties with large metropolitan centers (population greater than 300,000) to reduce potential confounding by unmeasured confounders. Although more urban counties have little to no exposure as classified in this study, they may have other factors that affect rates of preterm or low birth weight births. We assumed that the county of conception and of mother's residency during the pregnancy is the same as the county of residency at birth.

E. **Statistical Methods**

The present study employed an ecologic study design to link existing data sources on environmental exposures and birth outcomes. Descriptive statistics included: Pearson and Spearman correlations, chi-square statistics, and mapping in ArcGIS. To address the second aim of this study, three different multivariate approaches of increasing statistical complexity were used to evaluate the association between crop density and low birth weight and preterm births.

1. **<u>Method 1</u>**

Poisson regression was used to evaluate the association between crop production patterns and either low birth weight or preterm births. The natural logs of full-term singleton births and all singletons births were used as the offsets for the low birth weight and preterm birth models, respectively. Poisson regression models were performed using PROC GENMOD in SAS 9.2.

2. **<u>Method 2</u>**

To account for the correlation between county rates across the years, we used generalized estimating equations (GEE) using the Poisson distribution to estimate risk of low birth weight or preterm births with each exposure measure. This model structure accounts for the correlation between repeated county measures across the three study years. All GEE Poisson regression models were performed using PROC GENMOD in SAS 9.2.

3. **<u>Method 3</u>**

A distance decay random effects Poisson model that allowed for spatially varying random effect was used to account for the highly variable and non-random geographic distribution of crop production across each of the study states. The adjacency matrix used assigned each pair of counties in the entire study area with a 0 or 1 value, 1 indicating adjacency. County was assigned as a random effect in these models. Distance decay models were performed in R using the GAMEPHIT software (Burnett et al., 2001).

Each method was performed for each combination of outcome and exposure measure. Confounders and effect modifiers were examined in each analysis, including maternal race, maternal ethnicity, infant gender, county rate of maternal age groups, county median income, county population density, rate of maternal smoking and access to prenatal care, season of birth, and year. An interaction term between the exposure variables and season of birth was examined in an attempt to detect seasonal differences in the exposure-outcome relationship.

III. RESULTS

A. Descriptive Results

The densities of corn, soybeans, and wheat production in each county are illustrated in Figure 1. Although corn and soybeans are the most commonly grown crops in the study area, the extent to which they are grown varies widely between counties. Missouri grew more soybeans than corn, with a median soybean density of approximately 14% (62% maximum), compared to a median corn density of 6% (37% maximum). Missouri grew considerably less wheat, with median values between 0.25%–2%, although in Missouri some counties had more than 20% wheat density during the study years. Rice and cotton were grown only in five to eight counties in Missouri. However, in counties that did grow cotton, the median density was 26.8% (See Appendix B for full crop statistics).

There were a total of 140,329 live births in Missouri between 2004 and 2006 (Table I). A large majority of mothers were white (93.35%) and non-Hispanic (95.62%). There were slightly more males born than females (51.39% versus 48.61%). The proportion of births across the three study years was roughly equal, as was the proportion of births in each quarter of the year. Preterm births comprised nearly 13% of all live singleton births. Roughly 2.5% of live full-term singleton births in the study area were classified as low birth weight.

Missouri had publicly available county-level information on maternal smoking and prenatal care. The median percentage of mothers who smoked during pregnancy was 26% (range: 10.30–65.40), and the median percentage of women who received no prenatal care during pregnancy was 13.10% (range: 0.20–96.70).

TABLE I

MATERNAL AND INFANT CHARACTERISTICS FOR BIRTHS (N=140,329) OCCURING FROM 2004 TO 2006 IN RURAL MISSOURI COUNTIES

Variable	**N**	**Percent**
Births		
Live births	140,329	100.00
Live singletons	136,057	96.96
Live term singletons	120,751	86.05
Maternal Race		
White	130,998	93.35
Black	5,462	3.89
Other/Unknown	3,869	2.76
Maternal Ethnicity		
Non-Hispanic	134,185	95.62
Hispanic	5,900	4.20
Unknown	244	0.17
Maternal Age		
Median % of mothers aged <19	-	12.67
Median % of mothers aged 40-54	-	1.30
Gender		
Male	72,116	51.39
Female	68,213	48.61
Season of Birth		
Jan–Mar	33,610	23.95
Apr–Jun	34,730	24.75
Jul–Sep	36,942	26.33
Oct–Dec	35,047	24.97
Year		
2004	45,451	32.39
2005	46,629	33.23
2006	48,249	34.38
Median % of maternal smoking	-	26.00
Median % of mothers who received no PNC[a]	-	13.10
Percentage of mothers enrolled in WIC	-	52.90

MATERNAL AND INFANT CHARACTERISTICS FOR BIRTHS (N=140,329) OCCURING FROM 2004 TO 2006 IN RURAL MISSOURI COUNTIES

Variable		**Percent**
Outcomes		
Preterm	17,630	12.96
Low birth weight	3,007	2.49

[a] Prenatal care (PNC)
Data sources: Missouri Department of Health and Senior Services and CDC Environmental Public Health Tracking Network.

B. **Regression Analyses**

Multivariable models of low birth weight and preterm births for Missouri are presented in Tables II and III. All relative risks have been scaled to reflect a 30% increase in crop density.

Cotton density was strongly associated with increased risk of low birth weight births across all three methodological approaches (Method 1 RR [95%CI]: 1.50 [1.25–1.80]; Method 2 RR [95%CI]: 1.50 [1.31–1.71]; Method 3 RR [95%CI]: 1.76 [1.32–2.33]). Similarly, rice density was strongly associated with an increase in risk of low birth weight births in both the Poisson and GEE models (Method 1 RR [95%CI]: 1.85 [1.18–2.90]; Method 2 RR [95%CI]: 1.85 [1.22–2.82]), but was no longer significantly associated in the distance decay random effect models (Method 3 RR [95%CI]: 1.74 [0.96–3.15]). A marginally significant positive association was observed between soybean density and low birth weight births. No association was observed between corn or wheat density and low birth weight births.

Similar to the low birth weight results, rice and cotton densities were strongly associated with premature births in the Poisson and GEE models. This association remained significant for rice density and preterm births in distance decay models (RR [95%CI]: 1.66 [1.15–2.40]) (Table II). There was a reduced risk of preterm births observed in association with increasing corn and soybean densities in Missouri. However, this association was only significant in Poisson models, and was weakened and non-significant in the GEE and distance decay models.

TABLE II

ADJUSTED[a] SINGLE EXPOSURE REGRESSION RESULTS FOR PRETERM AND LOW BIRTH WEIGHT BIRTHS AND THEIR ASSOCIATIONS WITH CORN DENSITY, SOYBEAN DENSITY, AND WHEAT DENSITY IN MISSOURI, 2004–2006, ACROSS THREE MODELING APPROACHES

		Poisson *(Method 1)*	**GEE** *(Method 2)*	**Distance Decay** *(Method 3)*
Outcome	**Exposure Variable**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Low birth weight	Corn	1.03 (0.86–1.24)	1.03 (0.86–1.24)	1.05 (0.77–1.42)
	Soybean	1.10 (0.97–1.24)	1.10 (0.98–1.24)	1.11 (0.91–1.34)
	Wheat	1.14 (0.78–1.68)	1.14 (0.85–1.54)	0.97 (0.51–1.84)
	Rice	**1.85 (1.18–2.90)**	**1.85 (1.22–2.82)**	1.74 (0.96–3.15)
	Cotton	**1.50 (1.25–1.80)**	**1.50 (1.31–1.71)**	**1.76 (1.32–2.33)**
Preterm births	Corn	0.84 (0.78–0.91)	0.84 (0.75–0.94)	0.91 (0.76–1.08)
	Soybean	0.95 (0.90–1.00)	0.95 (0.88–1.03)	1.00 (0.89–1.13)
	Wheat	0.91 (0.77–1.08)	0.91 (0.73–1.13)	0.94 (0.66–1.33)
	Rice	**1.77 (1.46–2.14)**	**1.77 (1.49–2.09)**	**1.66 (1.15–2.40)**
	Cotton	**1.19 (1.09–1.29)**	**1.19 (1.09–1.29)**	1.19 (0.98–1.44)

[a] Models adjusted for maternal race, maternal ethnicity, gender, mother's age group, maternal smoking, access to prenatal care, prenatal WIC use, county median income, county population density, season of birth, and year.

There is strong geographic overlap among Missouri counties that produce rice and cotton. Dual-exposure models were examined to determine if the associations observed in single-exposure models persisted when both rice and cotton densities were in a model together. In these models, rice and cotton remained positively associated with increased risk of low birth weight births (Table III; RR_{COTTON} 1.17–1.43; RR_{RICE} 1.36–1.71). The effect of cotton was attenuated in the distance decay model, but the association between rice and low birth weight was strengthened. Both rice and cotton were associated with preterm birth in dual-exposure models, but the magnitude of effect was much larger for rice (Table III; RR_{COTTON} 1.10–1.70; RR_{RICE} 1.57–1.65). A slight attenuation of the rice density–preterm birth association and slight strengthening of the association with cotton was observed in distance decay models.

TABLE III

ADJUSTED[a] DUAL-EXPOSURE REGRESSION MODEL RESULTS FOR PRETERM AND LOW BIRTH WEIGHT BIRTHS AND THEIR ASSOCIATIONS WITH COTTON AND RICE DENSITY IN MISSOURI, 2004–2006, ACROSS THREE MODELING APPROACHES

		Poisson *(Method 1)*	**GEE** *(Method 2)*	**Distance Decay** *(Method 3)*
Outcome	**Exposure Variable**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Low birth weight	Rice	1.36 (0.82–2.67)	**1.36 (1.05–1.77)**	**1.61 (1.12–2.33)**
	Cotton	**1.43 (1.18–1.75)**	**1.43 (1.24–1.65)**	1.17 (0.97–1.40)
Preterm births	Rice	**1.65 (1.34–2.03)**	**1.65 (1.36–2.03)**	1.57 (0.90–2.73)
	Cotton	**1.10 (1.00–1.21)**	**1.10 (1.01–1.21)**	**1.70 (1.28–2.24)**

[a] Models included both rice and cotton density and adjusted for maternal race, maternal ethnicity, gender, mother's age group, maternal smoking, access to prenatal care, prenatal WIC use, county median income, county population density, season of birth, and year.

IV. **DISCUSSION**

Strong positive associations between both rice and cotton density and both outcomes, low birth weight and preterm births, were found in Missouri. Rice density was associated with significant increased risk of preterm births across all 3 models, as was cotton density with low birth weight births. Only eight out of 115 counties in Missouri produced rice between 2004 and 2006. There were five counties that produce cotton in Missouri, all of which also produced rice. Despite the geographic correlation between these counties, both rice and cotton densities remained significant predictors of low birth weight and preterm births in dual-exposure models with a slight weakening of the effect in the distance decay models. The five most commonly used pesticides on rice fields are clomazone, propanil, azoxystrobin, quinclorac, and glyphosate (Appendix A, Table VIII), although 35 pesticides were used on rice crops in the United States in 2006 (USDA, 2007). Glysophate, ethephon, mepiquat chloride, trifluralin, and acephate were the most commonly applied pesticides on cotton fields in the United States (Appendix 1, Table VII), but over 80 chemicals were used on cotton fields in the United States in 2005 (USDA, 2006). There is a paucity of literature on these chemicals and their toxicity to humans. There is some evidence that trifluralin may be associated with increased cancer incidence in pesticide applicators (Kang et al., 2008), but few epidemiologic studies have been conducted.

A consistent positive trend between corn and soybean density and low birth weight births was observed in rural counties in Missouri, although this association was not statistically significant in any of the three models. Conversely, there was negative trend of association between corn and soybean densities and preterm births. This reduction in risk of preterm births was significant in single-exposure Poisson models (Method 1) and generalized estimating equation models (Method 2) of corn and preterm births. From these results, there is no clear evidence of an

association between either corn or soybean density and either outcome of interest. Soybean density approaches significance in the low birth weight models.

There was no consistent association observed between wheat density in a county and low birth weight or preterm births in this study (Table II). This is consistent with Schreinemachers (2003), who found no effect of wheat coverage and either of these outcomes. Although grown in Missouri, wheat is not grown on the scale of corn or soybean (Figure 1). Therefore, before concluding that there is no association between wheat density and these birth outcomes, this research question should be explored further in high wheat-producing states such as North and South Dakota, Nebraska, Kansas, and Oklahoma (USDA, 2012).

Due to the geographic distribution of the exposure variables and the between-year correlation of the observations in the study, three statistical approaches were used in this study to better understand which methodology is most appropriate for these types of data. For each crop type, Methods 1 and 2, Poisson regression, and GEE Poisson regression, yielded similar estimates of relative risk (Table I). Application of Method 3, which allowed for spatially varying random effects, yielded different risk estimates than Methods 1 and 2, but the pattern of change was not consistent. For example, in single-exposure models, Method 3 attenuated the association between rice density and low birth weight births, but increased the magnitude of association between cotton density and low birth weight births (Table II). In dual-exposure models, the pattern of change was reversed and method 3 strengthened the association between rice and low birth weight births, while attenuating the risk of cotton. The rationale for using this model is that one would expect adjacent counties to be more alike on unmeasured confounders than counties farther away from each other. To this extent, Method 3 which allows for spatially varying random effects may be most appropriate for future linkage studies that employ aggregated data from geographically dispersed populations.

The use of an ecological study design presents a significant limitation to the interpretation of these results: The absence of individual-level confounders related to birth outcomes and exposure potential prevents the evaluation of causal associations. The ecologic study design was useful, however, in examining these associations across large populations that are geographically dispersed and for identifying associations worthy of further investigation.

Although the current study had seasonal outcome measures, the exposure variables were annual measures and therefore the timing of exposure in relation to the outcomes is uncertain. A season by exposure interaction term was used to test the effect of seasonal pesticide application on low birth weight and preterm births; however these coefficients were not statistically significant. Therefore, the results of this study are unable to generate hypotheses about critical windows of exposures for either outcome, though crop production and the time windows for agricultural exposures related to adverse birth outcomes may be seasonal. Schreinemahcers (2003), for example, observed an increase in birth malformations in infants conceived between April and June which are the application months of the largest class of pesticides used on wheat. The third trimester has been suggested as the time period during which fetal weight gain is most susceptible to effects of pesticide exposure, although there is some evidence that the first trimester is important as well (Sathyanarayana et al., 2010). The first trimester may be of particular importance for preterm birth risk as chromosomal abnormalities generally occur within the first few weeks of pregnancy and have been shown to increase the odds of preterm birth (Dolan et al., 2007).

The lack of a significant seasonal interaction term may reflect the challenges of exposure ascertainment. While the amount of land planted annually with a specific crop may be a good surrogate for the amount of pesticides applied, it does not include temporally refined exposure metrics. Bell et al. (2001) found that while proximity to agricultural pesticide application was a risk factor for fetal death, the risk varied and was elevated for different pesticides depending on the timing of in utero exposure.

Finally, the current study included limited confounders and may leave residual confounding by unknown variables. County-level linkage studies are a priority for the Centers for Disease Control and Prevention's (CDC) Environmental Public Health Tracking Network, and as such, reliably and consistently collected county-level risk factors and covariates are necessary for effective linkage studies. While important risk factors like maternal cigarette use and prenatal care access were available for this study, several other important factors such as maternal alcohol use and maternal residency during pregnancy were not. Furthermore, these risk factors were only available at the county level.

Despite these limitations, this study had several strengths. First, the study had a large sample size for all of the outcomes of interest (Table I). The robust sample size allows for greater statistical power to detect small risks. The study also included states with great variability in exposure and outcome. Finally, this research is aimed at developing new methodologies for incorporating geospatial data into a health linkage study. These methodologies can be used to address other linkages of health and environmental data.

V. CONCLUSIONS

This study is one of the first of its kind to explore the relationship between specific measures of agricultural land use and low birth weight and preterm births employing multiple analytic approaches. The observed associations between soybean density and low birth weight births suggest more future research on glyphosate-based herbicides warrant further research. Similarly, the observed increase in risk of preterm and low birth weight births in counties with high cotton and rice densities in warrants further investigation. These crops are ubiquitous in many counties throughout the Midwest and future epidemiologic research should focus on the main active ingredients in the most commonly applies pesticides. Further epidemiologic studies, performed on a temporally and geographically refined scale, are needed to better understand the associations found in this study. Studies of this kind would benefit greatly from improved exposure assessment and publicly available county-level risk factor data.

APPENDICES

APPENDIX A

Agricultural chemical application data for top five active ingredients applied to corn, soybeans, wheat, rice, and cotton in the United States 2004–2006. Tables were adapted from data published in the Agricultural Chemical Usage Field Crop Summaries from 2004–2006, USDA NASS.

TABLE IV

AGRICULTURAL CHEMICAL APPLICATION OF TOP FIVE ACTIVE INGREDIENTS ON CORN FIELDS IN SELECTED STATES[a] IN THE UNITED STATES, 2005

Active Ingredients	Area Applied (%)	Applications (No.)	Rate per Application (lbs/acre)
Atrazine	66	1.1	1.028
Glyphosate iso salt (isopropylamine salt)	31	1.3	0.727
Acetochlor	23	1.0	1.645
S-Metolachlor	23	1.0	1.323
Mesotrione	20	1.0	0.116

[a] Data inlcuded from the following states: CO, GA, IL, IN, IA, KS, KY, MI, MN, MO, NE, NY, NC, ND, OH, PA, SD, TX, and WI.

Data source: Agricultural chemical usage 2005 field crop summary. National Agricultural Statistic Services, USDA.

APPENDIX A (continued)

TABLE V

AGRICULTURAL CHEMICAL APPLICATION OF TOP FIVE ACTIVE INGREDIENTS ON SOYBEAN FIELDS IN SELECTED STATES IN THE UNITED STATES, 2004–2006

Year	Active Ingredients	Area Applied (%)	Applications (No.)	Rate per Application (lbs/acre)
2004[a]	Glyphosate	87	1.5	0.730
	Chlorimuron-ethyl	7	1.0	0.020
	Sulfentrazone	6	1.1	0.110
	Trifluralin	5	1.0	0.830
	Pendimethalin	4	1.0	0.860
2005[b]	Glyphosate iso salt	88	1.5	0.755
	Lambda-cyhalothrin	6	1.0	0.023
	Chlorpyrifos	5	1.0	0.477
	Trifluralin	4	1.0	0.782
	2,4-D, 2-EHE	4	1.0	0.440
2006[c]	Glyphosate iso salt	92	1.7	0.802
	2,4-D, 2-EHE	7	1.0	0.493
	Lambda-cyhalothrin	6	1.1	0.020
	Chlorpyrifos	5	1.1	0.454
	Glyphosate	4	1.7	0.630

[a] Data included from the following states: AR, IL, IN, IA, KS, MN, MO, NE, ND, OH, and SD.
[b] Data included from the following states: AR, IL, IN, IA, KS, KY, LA, MI, MN, MS, MO, NE, NC, OH, SD, TN, and VA.
[c] Data included from the following states: AR, IL, IN, IA, KS, KY, LA, MI, MN, MS, MO, NE, NC, ND, OH, SD, TN, VA, and WI.
Data sources: Agricultural chemical usage field crop summary, 2004–2006. National Agricultural Statistic Services, USDA.

APPENDIX A (continued)

TABLE VI

AGRICULTURAL CHEMICAL APPLICATION OF TOP FIVE ACTIVE INGREDIENTS ON WHEAT FIELDS IN SELECTED STATES[a] IN THE UNITED STATES, 2004 AND 2006

Year	Active Ingredients	Area Applied (%)	Applications (No.)	Rate per Application (lbs/acre)
2004	Fenoxaprop-p-ethyl	48	1.1	0.050
	Glyphosate	46	1.1	0.410
	MCPA	45	1.1	0.280
	2,4-D	36	1.0	0.370
	Dicamba	23	1.3	0.070
2006	Glyphosate iso salt	47	1.0	0.381
	Fenoxaprop-p-ethyl	37	1.0	0.052
	MCPA, 2-ethylhexyl	34	1.0	0.268
	2,4-D, 2-EHE	29	1.0	0.344
	Clodinafop-propargil	26	1.0	0.042

[a] Data inlcuded from the following states: MT and ND, 2004 and 2006.

Data sources: Agricultural chemical usage field crop summary, 2004 and 2006. National Agricultural Statistic Services, USDA.

APPENDIX A (continued)

TABLE VII

AGRICULTURAL CHEMICAL APPLICATION OF TOP FIVE ACTIVE INGREDIENTS ON UPLAND COTTON FIELDS IN SELECTED STATES[a] IN THE UNITED STATES, 2005

Active Ingredients	**Area Applied (%)**	**Applications (No.)**	**Rate per Application (lbs/acre)**
Glyphosate iso salt	71	2.2	0.713
Ethephon	58	1.1	1.055
Mepiquat chloride	33	2.1	0.030
Trifluralin	32	1.0	0.862
Acephate	27	2.1	0.412

[a] Data inlcuded from the following states: AL, AR, CA, GA, LA, MS, NC, TN, and TX.

APPENDIX A (continued)

TABLE VIII

AGRICULTURAL CHEMICAL APPLICATION OF TOP FIVE ACTIVE INGREDIENTS ON RICE FIELDS IN SELECTED STATES[a] IN THE UNITED STATES, 2006

Active Ingredients	Area Applied (%)	Applications (no.)	Rate per Application (lbs/acre)
Clomazone	50	1.0	0.427
Propanil	46	1.1	3.194
Azoxystrobin	27	1.1	0.140
Quinclorac	24	1.1	0.280
Glyphosate iso salt	23	1.2	0.773

[a] Data inlcuded from the following states: AR, CA, LA, MS, MO, and TX.

Data source: Agricultural chemical usage 2006 field crop summary. National Agricultural Statistic Services, USDA.

APPENDIX B

TABLE IX

MEDIAN AND RANGE VALUES OF CROP DENSITY[a] ACROSS ALL RURAL COUNTIES IN MISSOURI AND WITHIN SPECIFIC CROP GROWING COUNTIES, 2004–2006

	State-wide			Crop-growing Subset			
Crop	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	**No. of Counties**
Corn	6.73	0.00	37.14	7.31	0.23	37.14	78–87
Cotton	0.00	0.00	46.61	26.79	3.42	46.61	5
Rice	0.00	0.00	16.42	6.95	0.43	16.42	5–8
Soybeans	14.37	0.00	62.11	14.49	0.12	62.11	85–89
Wheat	1.53	0.00	21.83	1.58	0.12	21.83	82–88

[a] Crop densities calculated by dividing the total annual acreage of a crop planted in a county by the total county area, multiplied by 100. Data provided by USDA NASS.

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