HYPOSPADIAS AND PRENATAL EXPOSURE TO ATRAZINE VIA DRINKING WATER: A GEOGRAPHIC ANALYSIS

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Geography.

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ABSTRACT

Jennifer Winston: Hypospadias and Prenatal Exposure to Atrazine via Drinking Water: A Geographic Analysis
(Under the direction of Michael Emch)

This dissertation uses a disease ecology framework to investigate the etiology of hypospadias, a relatively common birth defect affecting the male genitourinary tract. It begins by considering the spatial distribution of hypospadias in North Carolina and whether that spatial distribution can be explained by either compositional or contextual risk factors. It then focuses on a potential contextual risk factor of interest: atrazine, one of the most widely used herbicides in the United States. An endocrine disruptor, atrazine breaks down slowly in soils and water, suggesting that mothers could be exposed to atrazine via contaminated drinking water.

This research uses data from the North Carolina Birth Defects Monitoring Program and the National Birth Defects Prevention Study. Three different methods are used to estimate maternal exposure to atrazine via drinking water: total atrazine applied to maternal county of residence; sampling data maintained by the United States Environmental Protection for compliance monitoring; and outputs from surface water and groundwater models from the United States Geological Service. After concluding that the surface and groundwater modeling metric is most appropriate for our dataset, this research concludes by incorporating maternal population and behavioral characteristics into analyses of hypospadias and maternal exposure to atrazine via drinking water.

Results indicate statistically significant spatial autocorrelation of hypospadias in eastern central North Carolina, which persists when controlling for compositional risk factors, and which suggests that contextual factors may influence the spatial distribution of hypospadias. Results further suggest possible role played by atrazine in a multi-factorial etiology of hypospadias. When controlling for maternal demographic and behavioral characteristics, hypospadias is found to be marginally significantly associated with daily maternal atrazine consumption during the critical window of genitourinary development (odds ratio = 1.03; $p = 0.054$). This reinforces the utility of a disease ecology framework in research of diseases of unknown or multifactorial etiology. It also suggests that further research is needed to evaluate the potential teratogenic properties of atrazine.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
CDC	Centers for Disease Control and Prevention
CHEEC	University of Iowa Center for Health Effects of Environmental Contamination
MRL	Maximum Residue Limits
NBDPS	National Birth Defects Prevention Study
NCBDMP	North Carolina Birth Defects Monitoring Program
OR	Odds Ratio
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WARP	Watershed Regressions for Pesticides

CHAPTER 1

INTRODUCTION

Background

Hypospadias is a relatively common congenital urinary tract defect affecting between 4 and 6 per 1,000 male infants (1). It is characterized by having the opening of the urethra located on the underside of the penis (2), and surgery is often needed to reposition the urethral opening. Left untreated, hypospadias can lead to difficulty in using a toilet, as well as sexual and fertility problems in adults (3). Hypospadias is believed to have a multifactorial etiology where both genetic susceptibility and environmental exposures may play a role (4).

This dissertation explores the spatial distribution of hypospadias in North Carolina, as well as the factors that might help explain this distribution. It then builds on this foundation by examining a possible association between hypospadias and atrazine, one of the most widely used agricultural herbicides in the United States. It does so by developing a novel approach for estimating environmental exposure to atrazine via drinking water, and comparing it to other previously used exposure estimation techniques. It is hoped that research may contribute to our understanding of a relatively common, but still poorly understood, birth defect, and thereby have the potential to improve birth outcomes. It is further hoped that the methodology developed in this study may also be applicable to other studies considering human health and atrazine exposure via drinking water.

Disease Ecology

Disease ecology is a medical geography theory that posits that population, behavior, and environment all play a role in determining disease outcomes (5). In fact, most risk factors for hypospadias can be organized into these three categories.

Population level risk factors for hypospadias include maternal characteristics including maternal health, parity, and genetics. Among maternal characteristics, untreated hypertension (6-8), thyroid disease (9), and diabetes (10) all seem to lead to higher risk. There is some evidence that maternal nutritional status may play a role, with certain vitamins (B12, choline, and methionine) perhaps reducing risk (11). There is conflicting evidence regarding maternal BMI, with Carmichael et al (12) finding greater risk amongst mothers with a BMI above 26, but with Adams et al (13) finding no evidence of an increased risk. Risk also increases with age (10, 14, 15), and is also highest among whites (10). First-borns are at a higher risk than higher-parity children (12, 16). Genetics also likely plays a role, as boys with a family history of hypospadias are also more likely to be born with the defect (17).

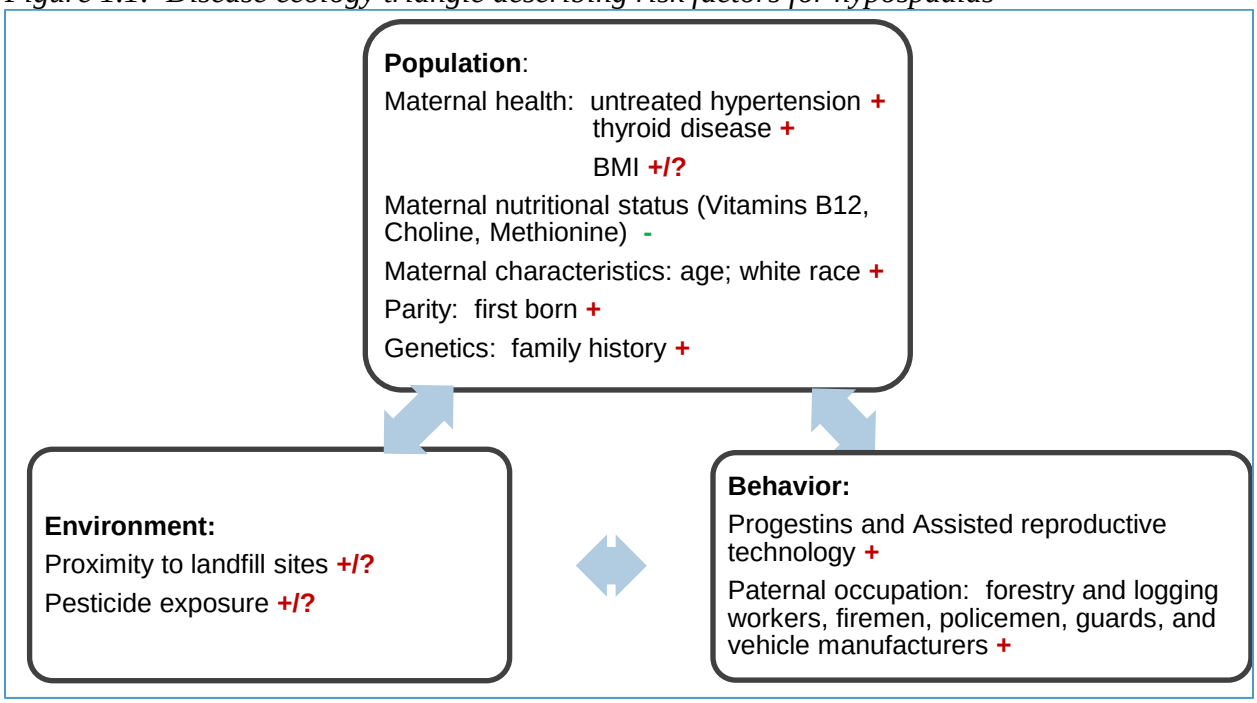
Behavioral factors include maternal use of progestins (18) and other assisted reproductive technology (17), which seem to increase risk. Fathers in certain occupations, including forestry and logging workers, firemen, policemen, guards, and vehicle manufacturers, may also be at an increased risk (19).

The role of environmental factors is the least well understood. There is some evidence to suggest that exposure to certain pesticides may be associated with urogenital defects. A potential mechanism for such defects may be interference with male genital development because of their ability to mimic or interfere with the function of certain hormones (20).

Supporting this hypothesis, Fernandez et al (21) and Giordano et al (22) find a relationship between urogenital defects and endocrine-disrupting chemicals, as well as to maternal occupational exposure to agriculture. Similarly, Brouwers et al (17) find that paternal exposure to pesticides increases hypospadias risk. The pathway for this exposure is not clear, but it may include maternal exposure via seminal fluid (1). Winchester et al. (23) find that 22 birth defects, including genital defects, are more likely to occur in live births with a last menstrual period during the time of peak annual agrichemical use. Rocheleau et al (24) also find evidence of a modest link between hypospadias and pesticide exposure, although a later study led by Rocheleau finds no association between occupational exposure to pesticides and hypospadias (25). There is also weak evidence of increased risk associated with proximity to landfill sites (26, 27).

Within a disease ecology framework, these risk factors can be summarized in a triangle, with population, behavioral and environmental risk factors each forming a vertex of the triangle. Each vertex then interacts with the others to describe the multi-factorial etiology of hypospadias. This mechanism is illustrated in Figure 1.1.

Figure 1.1: Disease ecology triangle describing risk factors for hypospadias



Theoretical Approach:

Neighborhoods and Health Framework

A neighborhoods and health framework suggests that the geographic distribution of a disease may be explained by the composition of the people who live in a place or by the context, or the unique environment, of a place (28, 29). Within disease ecology theory, this dissertation therefore uses a neighborhoods and health framework to investigate the spatial distribution of hypospadias, as well as the factors that might lead to that distribution. In the context of this dissertation, the population and behavioral risk factors for hypospadias identified above can be considered compositional effects, while the environmental risk factors can be considered contextual effects.

The first question examined by this dissertation therefore asks how hypospadias clusters in space. It then draws upon the neighborhoods and health framework to control, to the extent

possible for compositional effects. Any remaining unexplained variation will suggest that contextual effects are playing a role in the geographic distribution of hypospadias.

Watershed Modeling

To help identify what these contextual factors might be, this study uses a number of exposure estimation techniques, including watershed modeling. These models can be used to estimate contaminant concentrations in groundwater and streams when continuous monitoring data is unavailable. Root et al (31) was one of the first to incorporate watershed modeling into a medical geographical study of birth defects by considering whether gastroschisis risk was influenced by maternal residence downstream from textile mills. This dissertation builds upon that work via a novel adaptation of two hydrological models developed by the US Geological Survey (USGS) to estimate contaminant concentrations in groundwater and drinking water. It also compares the estimates provided by these models against water quality sampling conducted by the US Environmental Protection Agency (EPA), as well as data about pesticide use at the county level. It then compares the strengths and weaknesses of using these exposure estimation techniques to predict hypospadias risk.

This dissertation focuses on the potential environmental risk posed by exposure to atrazine via drinking water. Atrazine is one of the most widely used agricultural pesticides in the US, and is applied mainly to corn, sorghum, and sugarcane, both before and after planting (32). Bioaccumulation of this pesticide seems to be negligible (33, 34), but it is relatively mobile in soil and breaks down very slowly in water, with a half-life greater than 200 days in lakes and streams (35). Because atrazine remains for a long period time if washed into streams or

groundwater, exposure from contaminated wells or public drinking water supplies fed by these sources is possible.

Some animal studies suggest that atrazine may be associated with genitourinary malformations in frogs at concentrations as low as 0.1 parts per billion (33), although the US EPA concluded in 2007 that atrazine does not adversely affect amphibian gonadal development (32). In human studies, Meyer et al (36) find no statistically significant association between atrazine and hypospadias in their study of agricultural pesticides in eastern Arkansas. On the other hand, the Agency for Toxic Substances and Disease Registry (35) notes that maternal exposure to atrazine in drinking water has been associated with a number of adverse birth outcomes, including urinary system defects.

This dissertation will draw upon two watershed models in estimating maternal exposure to atrazine via drinking water: Stone et al's 2013 watershed regressions for pesticides (WARP) models for predicting stream concentrations of multiple pesticides (37) and Stackelberg et al's 2012 regression models for estimating concentrations of atrazine plus deethylatrazine in shallow groundwater in agricultural areas of the United States (38).

This dissertation therefore uses these watershed modeling techniques to explore whether maternal exposure to atrazine via drinking water helps to explain any disease clustering that remains after controlling for composition. It also considers what similarities and or differences characterize the results of estimating maternal exposure to atrazine via drinking water via three different approaches: county-level atrazine use, US EPA water quality monitoring data, and output from USGS surface and groundwater models.

Conclusion

In conclusion, this dissertation seeks to understand the disease ecology of hypospadias, including the interaction of population (socioeconomic and biological), behavioral, and environmental factors in its etiology by analyzing birth defects data from North Carolina, Iowa, Arkansas and Texas. This dissertation is organized around three empirical papers. Chapter 2, "A geographic analysis of compositional and contextual risk factors for hypospadias births," begins by considering how hypospadias clusters in space. It then asks what compositional factors predict hypospadias risk throughout North Carolina, and whether any identified disease clusters remain after controlling (to the extent possible) for these effects. Chapter 4, "Comparison of exposure metrics for estimating maternal exposure to atrazine," describes three different exposure metrics for estimating exposure to atrazine via drinking water, and considers their strengths and limitations in estimating hypospadias risk. Finally, Chapter 6, "Hypospadias and maternal exposure to atrazine," returns to the disease ecology triangle and examines whether maternal exposure to atrazine via drinking water, either in isolation, or in combination with other factors, may help explain hypospadias risk.

CHAPTER 2

A GEOGRAPHIC ANALYSIS OF COMPOSITIONAL AND CONTEXTUAL RISK FACTORS FOR HYPOSPADIAS BIRTHS

Introduction

Hypospadias is a relatively common urinary tract defect affecting approximately 0.3 to 0.7% of live male births. It is characterized by a urethral opening on the underside of the penis, and can vary in degree according to the location of the urethral opening. Without surgery to repair the defect, it can result in urinary or sexual problems, particularly in more severe cases (1). It is believed to have a multifactorial etiology where population level and environmental level risk factors, as well as genetic influences, may play a role (4).

The present study seeks to identify spatial clustering of hypospadias in North Carolina from 2003 to 2005. Guided by the neighborhoods and health framework, it further seeks to disentangle risk factors contributing to that spatial clustering. Researchers of neighborhood effects on health have long noted variations in the spatial distribution of morbidity, mortality, and health behavior, and that these variations may be explained by either compositional or contextual effects. Neighborhood compositional effects result from differences among people who live in different places, while neighborhood contextual effects result from external environmental influences (28).

Within a neighborhoods and health framework, compositional risk factors for hypospadias may be broken into two categories: population factors and behavioral factors. Population factors include maternal health and other characteristics, parental genetics, and infant characteristics. Among maternal health factors, untreated hypertension (6-8), thyroid disease (9), and diabetes (10)

all seem to increase risk. There is conflicting evidence regarding maternal BMI, with some researchers finding greater risk among mothers with a BMI above 26 (12), but with others finding no evidence of an increased risk (13). Risk increases with maternal age (10, 14, 15), and is also highest among non-Hispanic whites (10, 39). Parental genetics also likely plays a role, as boys with a family history of hypospadias are also more likely to be born with the defect (17). Among infant characteristics, first-borns are at a higher risk than higher-parity children (12, 16), although this may be related to sub-fertility at the parental level (40).

Behavioral risk factors include maternal use of progestins (18) and other assisted reproductive technology (17), which seem to increase risk. There is some evidence that maternal diet may play a role, with certain dietary factors (B12, choline, and methionine) perhaps reducing risk (9). Some studies suggest that maternal smoking may be associated with decreased risk, especially for primiparous women (40), while others find no association between smoking and hypospadias (18). Infants born to fathers in certain occupations, including forestry and logging workers, firemen, policemen, guards, and vehicle manufacturers, may be at an increased risk (19).

The potential role played by contextual, or environmental, factors is less well understood. It has been hypothesized that endocrine-disrupting chemicals, including some pesticides, could interrupt normal urethral closure and lead to hypospadias. The evidence to support this hypothesis is mixed, however. A meta-analysis of studies conducted between 1966 and 2008 found a modest association between hypospadias and pesticide exposure (24), but another review of environmental and genetic contributors to hypospadias concluded that a clear association cannot be made between endocrine-disrupting exposures and hypospadias, and called for further study of environmental factors and hypospadias (1).

This study seeks to build on the current knowledge of population, behavioral, and environmental risk factors for hypospadias. Compositional risk factors for hypospadias considered by this study include maternal age, race/ethnicity, marital status, smoking, diabetes, socioeconomic

status, and parity. To help address the remaining ambiguity associated with endocrine-disrupting chemicals, including pesticides, the contextual risk factors included in this study focus on land use, including agriculture. We use an alternative approach to investigating the geographic distribution of hypospadias in North Carolina from 2003-2005 by examining clustering of residuals. To our knowledge, we are the first to examine compositional and contextual factors affecting the spatial distribution of birth defects using this novel approach.

Methods

Data were collected by the North Carolina Birth Defects Monitoring Program (NCBDMP), which is a population-based, active surveillance system. NCBDMP field staff review hospital medical records and discharge reports and regularly report malformations to the Registry. NCBDMP also links data about cases and controls to vital records to provide demographic information about both mother and infant and geocodes maternal address at birth.

This study population included all North Carolina resident women who delivered a live-born infant with hypospadias, and a 10% random sample of women who delivered a male infant without a known birth defect and who delivered in North Carolina in 2003-2005. Of these, 89% of cases and 93% of males without a known birth defect were successfully geocoded.

Hypospadias varies in severity – we included first, second, and third degree cases, or all successfully geocoded cases (n = 1,044), in this analysis. First-degree cases may be more prone to differing diagnoses by different doctors. However, hypospadias screening is a routine element of regular newborn assessments (41), which should reduce the number of overlooked cases. Further the mechanism by which environmental factors affect hypospadias risk may be subtle, so we wanted to include cases of all levels of severity. This is consistent with other authors studying environmental

effects on hypospadias risk (36, 42, 43). While we also had access to all successfully geocoded male births without a known birth defect, we randomly selected a 10% sample as controls (n = 16,477).

Compositional variables considered in this analysis were linked to cases and controls from vital records. These characteristics included age at delivery, race/ethnicity (classified for this study as non-Hispanic white, non-Hispanic black, Hispanic, and other), marital status, smoking, diabetes, parity, and two proxies for socioeconomic status (maternal education and month prenatal care began).

Contextual variables focused on land use characteristics and the total number of live births per block group during the study period. We used the 2006 National Land Cover Database from the US Geological Survey to classify the percent of each block group used by various land classes (developed land, crops, pasture, and forest). Due to the large number of block groups with no crops, we used the natural logarithm of this variable to normalize its distribution. We also aggregated the total number of live births per block group during the study period to control for the background birth population in geographic analyses. These contextual variables were created using ArcGIS v. 10.

To consider the geographic distribution of hypospadias, we estimated local Moran's I statistics for hypospadias cases to identify statistically significant clustering of high values (cases). We then mapped the location of "high-high" births, which signify hypospadias cases clustered near other hypospadias cases. The results of the local Moran's I analyses were validated with SaTScan's Bernoulli model, which uses a moving circular window of varying sizes to identify the location and size of disease clusters (44).

To consider the effect of compositional variables on hypospadias risk in North Carolina, we conducted backward stepwise logistic regression analyses in Stata 12.1. All available compositional characteristics from the NCBDMP were tested. In order to maximize prediction performance, we

retained variables significant at p-value ≤ 0.2. To estimate the remaining unexplained variation in hypospadias risk, we calculated standardized residuals for the final compositional regression model by dividing individual raw residuals by their standard deviation. We repeated local Moran's I statistics using the standardized residuals and mapped the location of remaining high-high values.

We then added contextual variables measuring land use and the number of male births per block group into the final regression model. Only statistically significant land-use variables were retained. We estimated a multilevel model to consider any potential nesting within block groups, but the group level effect was not significant, so we returned to the single-level model. Finally, we calculated standardized residuals on the final model, repeated local Moran's I statistics on the standardized residuals, and mapped the high-high values in order to consider whether inclusion of these contextual variables helped to better explain the spatial variation of hypospadias risk.

Results

Descriptive statistics suggest that, on average, mothers of hypospadias cases tend to be slightly older, more likely to be non-Hispanic white, lower parity, and have a higher socioeconomic status (as measured by educational attainment and prenatal care) (Table 2.1).

Table 2.1: Descriptive statistics for hypospadias cases and controls

	Cases		Controls		
Characteristic	**N**	**%**	**N**	**%**	**P-value**
Maternal age					0.01
<20	108	10.9	1,834	11.5	
20-24	219	22.0	4,206	26.3	
25-29	274	27.6	4,448	27.8	
30-34	249	25.1	3,608	22.6	
35+	144	14.5	1,907	11.9	
Maternal race/ethnicity					<0.01
Non-Hispanic white	700	70.4	9,560	59.7	
Non-Hispanic black	213	21.4	3,506	21.9	
Hispanic	56	5.6	2,254	14.1	
Other race	25	2.5	683	4.3	
Maternal education					<0.01
Less than high school	157	15.9	3,560	22.3	
High school	280	28.3	4,603	28.8	
More than high school	553	55.9	7,795	48.9	
Marital status					0.15
Married	656	66.0	10,195	63.7	
Unmarried	338	34.0	5,805	36.3	
Smoking					0.24
No	885	89.0	14,048	87.8	
Yes	109	11.0	1,955	12.2	
Diabetes					0.48
No	964	97.0	15,580	97.4	
Yes	30	3.0	423	2.6	
Received first trimester prenatal care					0.01

No	133	13.4	2,627	16.4	
Yes	861	86.6	13,376	83.6	
Previous live births					<0.01
No	490	49.3	6,674	41.7	
Yes	504	50.7	9,329	58.3	

Local Moran's I statistics for hypospadias cases and controls show significant high-high clustering in the eastern central portion of North Carolina (Figure 2.1, Panels A and B). SaTScan analyses (not shown) confirmed that the Census tract with 7 high-high cases also contained the center of the only statistically significant primary cluster (p-value 0.003), with 18 observed hypospadias cases falling within a 10.3 km radius where only 3.4 cases would have been expected. This Census tract is located in Johnston County. From a compositional standpoint, Johnston County has a very rapidly growing population, and a greater proportion of its population is white (80.1% vs. 71.9% statewide) and Hispanic (13.1% vs 8.7% statewide. From a contextual standpoint, Johnston County has historically been farmed.

Of the eight compositional variables included in the backward stepwise logistic regression model, five were retained (Table 2.2). Consistent with descriptive statistics, increased maternal age and non-Hispanic white race/ethnicity were associated with increased risk. Being married and at higher parity were associated with reduced risk. Smoking was retained in the model, with smokers at a decreased risk, but this variable was not statistically significant. Diabetes and SES (as measured by maternal education and month prenatal care began) were not associated with hypospadias risk in the compositional model. The local Moran's I of the standardized residuals from the compositional model showed a very similar spatial pattern (Figure 2.1, Panel C) to that of the hypospadias cases, with the greatest number of high-high cases remaining in Johnston County.

Table 2.2: Compositional risk factors retained by backward stepwise logistic regression model for hypospadias in North Carolina, 2003 – 2005.

Characteristic	Odds Ratio	95% Confidence Interval	P-value
Maternal age			
<20	1.0	Referent	
20-24	1.03	0.80 – 1.32	0.82
25-29	1.24	0.96 – 1.60	0.10
30-34	1.39	1.07 – 1.83	0.02
35+	1.54	0.15 – 2.06	<0.01
Maternal race/ethnicity			
Non-Hispanic white	1.00	Referent	
Non-Hispanic black	0.83	0.69 – 0.99	0.03
Hispanic	0.34	0.26 – 0.46	<0.01
Other race	0.49	0.33 – 0.74	<0.01
Marital status			
Unmarried	1.00	Referent	
Married	0.85	0.71 – 1.01	0.06
Smoking			
No	1.00	Referent	
Yes	0.82	0.66 – 1.01	0.07
Previous live births			
No	1.00	Referent	
Yes	0.71	0.62 – 0.82	<0.01
Constant	0.08	0.07 – 0.10	<0.01

When contextual variables were incorporated into the compositional model, the natural logarithm of the percent crop cover per block group was found to be significantly associated with hypospadias risk (Table 2.3). Other land use variables (developed land, pasture, and forest) were not significant and were excluded from the final model. The number of male births per block group was not statistically significant, but was retained in the final model to control for background population size. Overall model fit improved in the contextual model, with the Akaike information criterion (AIC) reducing from 7469.1 with the compositional model to 3828.3 with the contextual model. The local Moran's I of the standardized residuals from the final model shows somewhat less spatial clustering of unexplained risk in the eastern central portion of the state, although the primary cluster remains in Johnston County (Figure 2.1, Panel D).

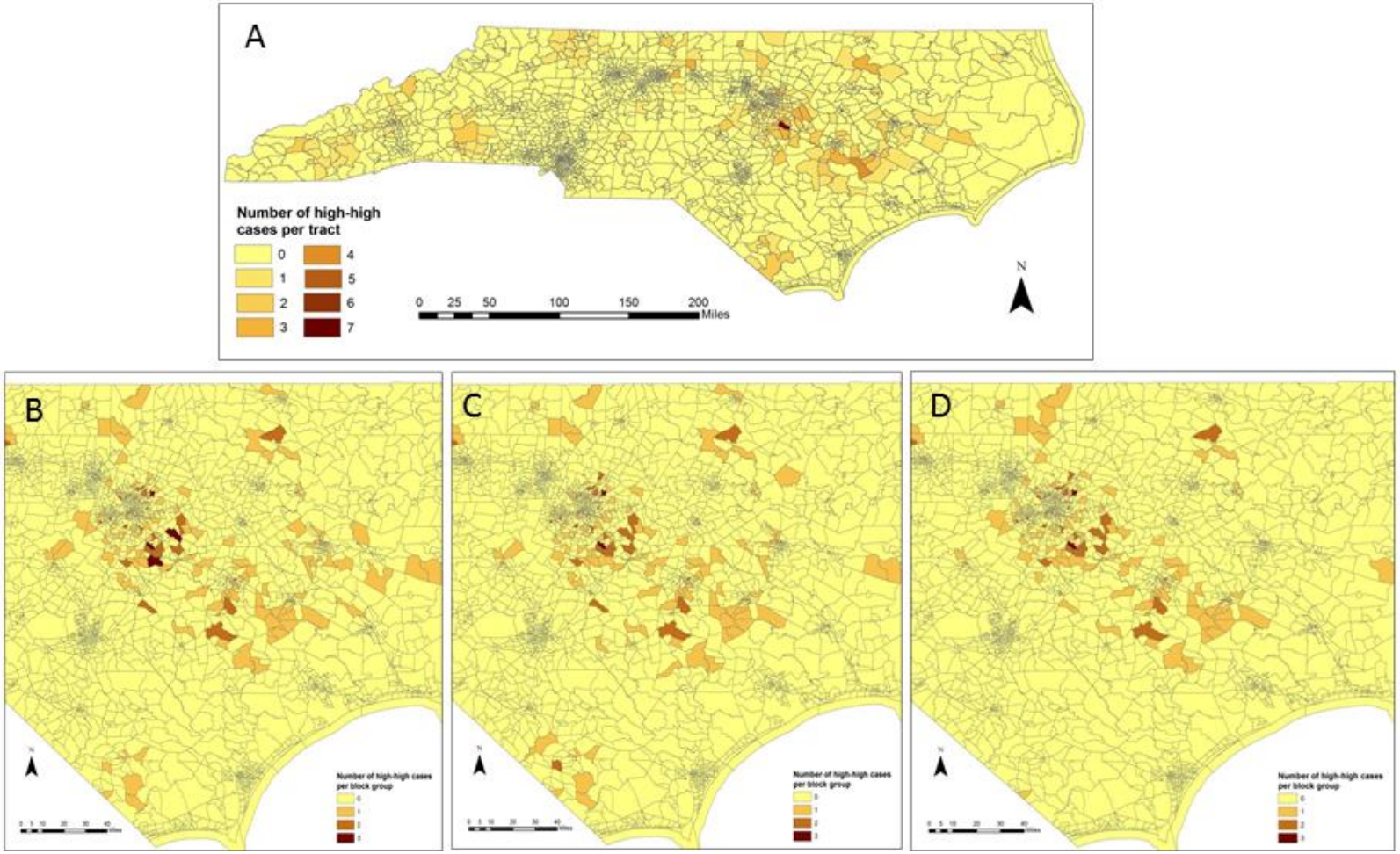
Table 2.3: Results of logistic regression model including compositional and contextual risk factors for hypospadias in North Carolina, 2003 – 2005.

Characteristic	**Odds Ratio**	**95% Confidence Interval**	**P-value**
Maternal age			
<20	1.0	Referent	
20-24	0.99	0.71 – 1.38	0.96
25-29	1.15	0.81 – 1.63	0.42
30-34	1.41	0.97 – 2.03	0.07
35+	1.42	0.94 – 2.13	0.10
Maternal race/ethnicity			
Non-Hispanic white	1.00	Referent	
Non-Hispanic black	0.78	0.61 – 1.02	0.07
Hispanic	0.23	0.13 – 0.38	<0.01
Other race	0.53	0.31 – 0.93	0.03

Marital status			
Unmarried	1.00	Referent	
Married	0.84	0.66 – 1.07	0.15
Smoking			
No	1.00	Referent	
Yes	0.77	0.58 – 1.02	0.07
Previous live births			
No	1.00	Referent	
Yes	0.84	0.66 – 0.98	0.03
Natural log of % of block group in crops	1.05	1.01 – 1.10	0.02
Number of male births per block group	1.00	0.99 – 1.00	0.29
Constant	0.09	0.07 – 0.12	<0.001

Figure 2.1: Local spatial autocorrelation of hypospadias cases and model residuals in North Carolina 2003-2005.

Panel A shows local spatial autocorrelation of hypospadias cases by Census tracts statewide. Panel B – D show spatial autocorrelation by Census block group in central North Carolina. Panel B shows local spatial autocorrelation of cases; Panel C shows local spatial autocorrelation of standardized residuals from the compositional model; and Panel D shows local spatial autocorrelation of standardized residuals from the compositional model.



Discussion

Moran's I analysis identified significant local spatial autocorrelation of hypospadias risk in North Carolina between 2003 and 2005. Backward stepwise logistic regression identified several important population-level factors contributing to hypospadias risk. However, local spatial autocorrelation remained even when controlling for these effects, which suggested that contextual, or environmental, factors might be playing a role in the distribution of hypospadias in this area.

Spatial autocorrelation of residuals was concentrated in eastern central North Carolina, which is known for its agricultural production, particularly hog farming, flue-cured tobacco, soybeans, and sweet potatoes (45). In fact, logistic regression indicated that the natural logarithm of percent of land cover in crops per block group was positively associated with hypospadias risk. Further, when crop cover and the number of live births per block group were included in the model, spatial clustering of the standardized residuals was somewhat diminished. This suggests that exposure to agriculture may be associated with hypospadias risk and lends indirect support to the somewhat conflicting evidence that exposure to pesticides may play a role.

This study only has access to information about maternal address at birth, not during the critical window of development. It also does not have information about place of work, which means that if mothers moved during their pregnancy, or if they spent significant amounts of time outside the home, we may not be accurately capturing contextual effects. Yet a study of exposure to air pollution using New York data found that only 16.5% of mothers moved during pregnancy, and most moved within such short distances that exposure assignments did not change substantially (46). Other studies have found much higher mobility during pregnancy – 33% of case and 31% of control mothers – but suggested that while maternal mobility may lead to exposure misclassification, any

such misclassification is likely to be non-differential, which would tend to bias the results toward the null (47).

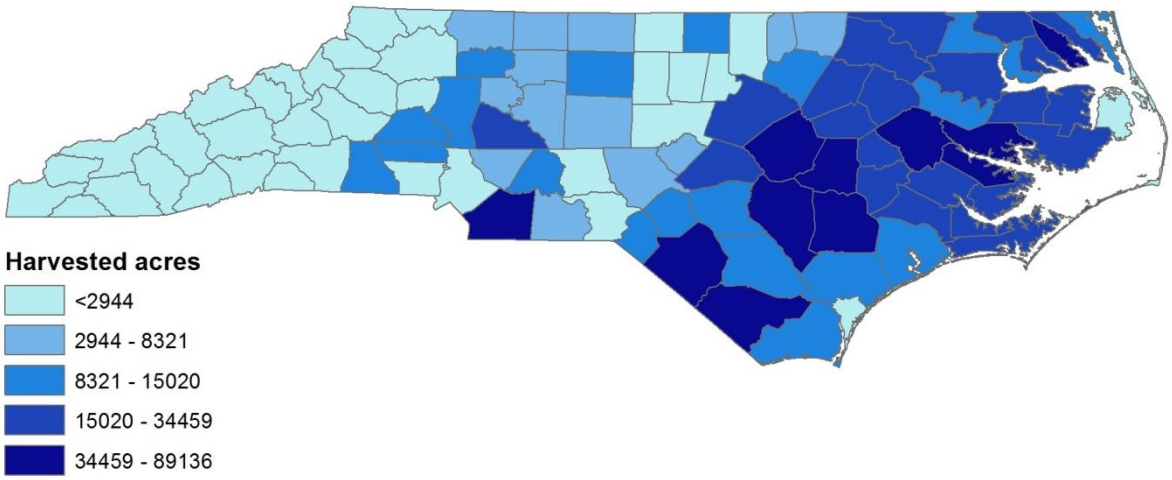
This study illustrates the potential contribution of mapping the spatial distribution of disease to generating hypotheses about disease etiology, and to investigating the relative contribution of contextual and compositional effects. The associations found with hypospadias in this analysis should not be interpreted as implying causality, and further research is needed to evaluate these findings. Future work will investigate the mechanism by which exposure to agriculture may be contributing to hypospadias risk in North Carolina. This ultimately might help inform policy interventions to help reduce hypospadias risk.

CHAPTER 3

USING GEOGRAPHIC CLUSTERING TO GENERATE HYPOTHESES ABOUT HYPOSPADIAS

The previous chapter uses geographic clustering methods to explore the geographic distribution of hypospadias and generate hypotheses about contextual factors that might play a role in hypospadias risk. As discussed, local Moran's I identified significant spatial autocorrelation of hypospadias in eastern central North Carolina. Backwards stepwise logistic regression found a number of compositional characteristics, including maternal race, maternal age, and parity that were associated with hypospadias. These factors could not, however, fully explain the spatial autocorrelation observed in eastern central North Carolina. This led to consideration of contextual factors unique to this area. As can be seen in Figure 3.1, agriculture, including soybean production, is an important feature of this part of the state (48). In fact, the natural logarithm of the percent of a block group in crops was found to be positively associated with hypospadias ($p < 0.01$). Further, spatial autocorrelation diminished somewhat when accounting for crop cover.

Figure 3.1: Distribution of soybean production in North Carolina, 2007



The remaining chapters of this dissertation will explore a potential mechanism for this correlation between hypospadias and crop cover. It will focus on atrazine, one of the most widely used commercial herbicides in the United States. Although atrazine is metabolized fairly quickly, it can remain in soils and groundwater for a long time, meaning that people might become exposed to atrazine via drinking water. Chapter 4 will therefore consider three different metrics for estimating maternal exposure to atrazine via drinking water. Chapter 6 will then use one of these metrics to investigate a possible association between hypospadias and exposure to atrazine.

CHAPTER 4

COMPARISON OF EXPOSURE METRICS FOR ESTIMATING MATERNAL EXPOSURE TO ATRAZINE

Introduction

Hypospadias is one of the most common birth defects in the United States, affecting approximately 1 in 125 live male births. It is characterized by a urethral opening located on the ventral side of the penis, and is hypothesized to have a multifactorial etiology, where genetic susceptibility may combine with endocrine disrupting chemicals to lead to an increased risk (4).

One endocrine disrupting chemical that has been examined for a possible association with hypospadias is atrazine, which is one of the most widely used herbicides in the United States. It has been studied for potential teratogenic effects because it may disrupt normal functioning of the endocrine system and because it remains in the environment for long periods of time (35). Atrazine is commonly found in groundwater and surface water in the United States, (49) exposing humans via contaminated drinking water (35).

Although there is some evidence to support a link between atrazine and urogenital defects, ambiguity about this relationship remains, in part because of the difficulty in measuring prenatal exposure. Laboratory studies involving male genital malformations in rats (50) and amphibians (33, 51-53) have allowed researchers to carefully quantify exposure to atrazine. However, to our knowledge, only one study has directly measured atrazine and its metabolites via urinalysis in a human study of atrazine and congenital defects. That study, conducted by

Chevrier *et al*, suggested a weak association between atrazine or atrazine metabolites and male genital anomalies, but the finding was not statistically significant, possibly due to the small sample size (54).

Because of the expense associated with both urinalysis and prospective study design, and to ensure sufficient sample size, most other studies examining a possible relationship between atrazine and adverse birth outcomes have relied on a retrospective ecological exposure assessment. Several of these studies have assigned mothers an atrazine concentration based on monitoring samples from their public water utility (55-58). Other studies estimated exposure by assigning mothers the estimated amount of atrazine applied to their county of residence (43, 59, 60).

Chevrier *et al* found atrazine metabolites in urine more frequently amongst women living in rural areas and amongst women living in municipalities with the highest level of atrazine contaminated tap water (54). It is unclear, however, whether either of the commonly used ecological approaches to atrazine exposure assessment (water utility monitoring data or county-level estimates) would accurately estimate atrazine or its metabolites in urine, or how well they would correspond to one another. While this study does not have access to urinalysis data, it seeks to compare the two most common exposure assessment techniques, along with a third, novel, technique in order to consider the effects of different exposure assessment metrics on hypospadias risk estimates.

Methods

Data:

This study used data from the North Carolina Birth Defects Monitoring Program (NCBDMP), which collects data on infants born with congenital abnormalities in North Carolina. Trained field staff collect birth defect data from hospital medical records. NCBDMP combines these data with other administrative data from hospital discharge data, vital records, and Medicaid claims, including geocoded maternal residential address at birth.

Hypospadias is classified by severity, based on the location of the urethral opening. First degree cases are the mildest and most common, with severity increasing in second and third degree cases (4). Because any potential role played by endocrine disrupting chemicals may be subtle, we included all levels of severity in this study. Cases therefore included all successfully geocoded first-, second-, and third-degree hypospadias cases (n=1,172) born in North Carolina between 2003 and 2005. Controls (n=17,635) consisted of a 10% random sample of all male births in North Carolina during the same time period.

Exposure assessment metrics:

Maternal exposure was estimated using three different methods in order to compare two exposure estimation techniques used by other studies of atrazine and birth defects, as well as a third exposure estimation technique. All three methods used residential address at birth and year of conception, estimated by subtracting estimated gestational age from date of birth. Because we did not have access to water quality monitoring data beyond public water supplies, women using private wells were excluded from this analysis.

Exposure estimation using county-use data:

The first exposure estimation technique, referred to hereafter as the county metric, used the "Annual county atrazine use estimates for agriculture, 1992-2007" dataset from the US Geological Survey (USGS) (61). This dataset was created by combining proprietary data from the DRMKynetic AgroTrak database on the total mass of atrazine applied annually to crops with data on harvested crop acreage from the US Department of Agriculture Censuses of Agriculture and the National Agriculture Statistics Service.

We assigned mothers to a county by overlaying maternal residential address with polygons of North Carolina counties using ArcGIS version 10. Mothers were assigned an exposure based on the estimated number of tons of atrazine per square mile applied to their county of residence during their estimated year of conception.

The county metric was then divided into four categories based on the exposure distribution of the controls. Because we had non-normally distributed data, we set cut points to maximize the interval between groups. The first category (referent) was equal to the first decile of exposure in the controls; the second category was equal to the second through fifth deciles; the third category was equal to the sixth through eighth deciles; and the fourth category was equal to the ninth and tenth deciles.

Exposure estimation using US Environmental Protection Agency monitoring data:

The second exposure estimation technique, referred to hereafter as the monitoring metric, used data from the US Environmental Protection Agency's (EPA) Six-Year Review Contaminant Occurrence Data (1998-2005) (62). These data are based on monitoring data collected from public water supplies for compliance with Safer Drinking Water Act requirements for atrazine.

Samples collected with values less than or equal to EPA's maximum residue limit (MRL) for atrazine were recorded as the MRL, or 0.1 micrograms per liter (μg/L). Samples exceeding the MRL were recorded as the actual recorded concentration.

We assigned mothers to a water utility by overlaying maternal residential address with public water system service area polygons from the North Carolina Center for Geographic Information and Analysis (63). Mothers were assigned an atrazine concentration based on the mean value of all available monitoring samples for their water supply for the calendar year of their conception.

Because of the high number of samples recorded at or below the MRL, the monitoring metric was categorized as a binary variable, with mothers at or below the MRL categorized as unexposed, and mothers above the MRL categorized as exposed.

Exposure estimation using US Geological Survey atrazine models:

The third method, referred to hereafter as the watershed modeling metric, combined estimates from USGS models estimating atrazine concentrations in streams and in groundwater. For streams, we used the estimated annual mean atrazine concentration predicted by the Watershed Regressions for Pesticides (WARP) model, which estimates stream concentrations of atrazine in stream reaches throughout the US. The model is a function of watershed atrazine use intensity, as estimated by annual agricultural atrazine use in the watershed divided by watershed area; the percentage of the watershed agricultural land with a soil-restrictive layer within the top 25 cm of the soil surface; total precipitation during May and June of the sampling year; the rainfall erosivity factor from the Universal Soil Loss Equation; and the percentage of total streamflow caused by precipitation on saturated soil (Dunne overland flow) (37).

For groundwater estimates, we used site-variable model predictions from the "Regression Models for Estimating Concentrations of Atrazine plus Deethylatrazine in Shallow Groundwater in Agricultural Areas of the United States." This model is derived from a residence-time indicator, atrazine use intensities, artificial drainage, depth to the seasonally high water-table, organic matter content of the uppermost soil layer, permeability of the least permeable soil layer, rate of recharge, and well depth. (38).

We then used geographic coordinates for surface and groundwater intakes from the North Carolina Division of Environmental Health to link atrazine concentrations to public water utilities (64). For surface water intakes, we used WARP estimates from the nearest stream reach to assign an annual mean atrazine concentration to the intake. For groundwater intakes, we used gridded atrazine predictions from the USGS groundwater model and bilinear interpolation to estimate atrazine concentrations based on the grid cell where the intake was located and the adjacent grid cells. Each public water utility was then assigned an atrazine concentration equal to the mean of the predicted atrazine concentrations for all of the intakes for that utility. Mothers were assigned an atrazine concentration using public water supply polygons as outlined for the monitoring metric.

The watershed modeling metric was categorized according to the distribution of the controls. The distribution was non-normally distributed, and cut points were selected to maximize the interval between groups. For the watershed metric, the first category (referent) was equal to the first decile of exposure in the controls; the second category was equal to the second through fourth deciles; the third category was equal to the fifth through ninth deciles; and the fourth category was equal to the tenth decile.

Comparison of exposure metrics:

The three exposure metrics were compared both as continuous and as categorical variables to consider the differences between each exposure assessment metric. The categorized county and watershed modeling metrics and the binary EPA metric were first mapped to visualize the geographic distribution and degree of missingness for the three metrics. Continuous metrics were then compared using Pearson correlation coefficients.

Categorized and binary metrics were compared using weighted kappa statistics. The kappa statistic measure of agreement is 0 when the amount of agreement is what would be expected due to chance and 1 when there is perfect agreement. The weighted kappa statistic is used for comparing ranked categories. It assigns categorizations that agree completely a weight of 1; categorizations that are near one another a weight closer to 1; and categorizations further apart a weight closer to 0. For example, if a mother was placed in the third category by the county metric and in the fourth category by the watershed modeling metric, she would receive a weight of 0.667, but if she was placed in the second category by the county metric and in the fourth category by the watershed modeling metric, she would receive a weight of 0.333.

We also calculated risk estimates for hypospadias using both the continuous and categorized metrics using logistic regression. We then compared how different exposure metrics influenced risk estimates for hypospadias. The purpose of these analyses was not to estimate the risk associated with exposure to atrazine, per se, but rather to examine if and how the exposure assessment metrics performed differently in epidemiologic models. We performed all geographic analyses using ArcGIS version 10 and all statistical analyses using Stata version 13.1.

Results

Figure 4.1 illustrates the geographic distribution of each of the three metrics. The county metric has the most comprehensive geographic distribution. While the monitoring and watershed modeling metric both display a large degree of missingness, there is at least some geographic overlap between the watershed modeling metric and the county metric and between the watershed modeling metric and the monitoring metric. The water utilities classified as highly exposed generally seem to fall in counties classified in the third and fourth categories of exposure. Similarly, where data is available, the watershed modeling metric seems to identify as more highly exposed water utilities that are classified as exposed in the monitoring metric.

Figure 4.1: Map of exposure metrics in North Carolina. Panel A illustrates the county metric; Panel B illustrates the monitoring metric; Panel C illustrates the watershed modeling metric.

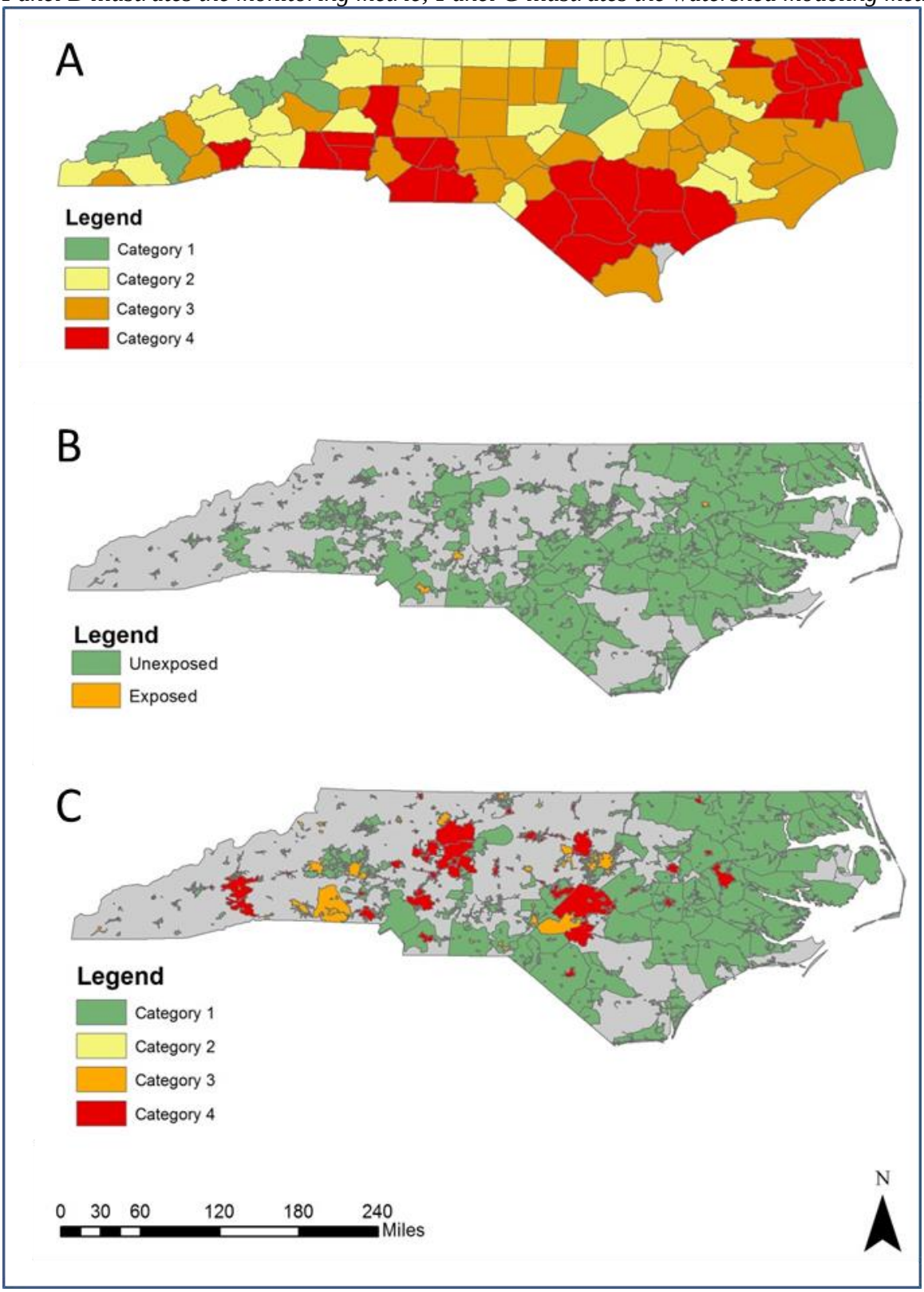


Table 4.1 presents the distribution of each of the continuous exposure metrics. The mean and median values for the county metric are 12.9 and 6.8 lbs/mi^2, respectively (interquartile range = 2.7-14.8). The mean and median values for the watershed modeling metric are 0.05 and 0.04 µg/L, respectively (interquartile range (0.03-0.05). The distribution for the monitoring metric is highly skewed, however, with the minimum, mean, median, and 75th percentile values all equal to 0.1.

Table 4.1: Distribution of continuous exposure metrics

Exposure metric	Mean	Median	Interquartile range	Min, Max values
County metric (in lbs/mi^2)	12.9	6.8	2.7, 14.8	0.04, 287.0
Monitoring metric (in µg/L)	0.1	0.1	0.1, 0.1	0.1, 1.2
Watershed modeling metric (in µg/L)	0.05	0.04	0.03, 0.05	0.01, 0.5

Table 4.2 presents Pearson correlation coefficients between the three continuous exposure metrics. Correlation coefficients range from 0.23 to 0.62, with the county and monitoring metrics least correlated with each other and the monitoring and watershed modeling metrics most correlated with one another.

Table 4.2: Pearson correlation coefficients between continuous exposure metrics assigned to women who delivered a baby in North Carolina (N)

Exposure metrics compared	N	Pearson Correlation
County and monitoring	6,933	0.23
Monitoring and watershed modeling	2,930	0.62
County and watershed modeling	6,313	0.58

Comparisons between categorized exposure assessment metrics are presented in Tables 4.3 - 4.5. Weighted kappa values are all significantly different from 0. Agreement is lower when comparing the monitoring metric to either the watershed modeling metric or county metrics, with weighted kappa values of 0.01 and agreement at between 47% and 56% (Tables 4.3-4.4). Agreement is slightly higher when comparing the watershed modeling and county metrics, with a weighted kappa value of 0.29, and 77% agreement (Table 4.5).

Table 4.3: Comparison of categorized watershed modeling metric and binary monitoring metric

	Watershed modeling category				
	1	2	3	4	Total
Monitoring (unexposed)	462	1163	878	248	2751
Monitoring (exposed)	0	10	102	66	178
Total	462	1173	980	314	2929
Weighted kappa = 0.01 (p <0.01). Percent agreement = 55.65%					

Table 4.4: Comparison of categorized county metric and binary monitoring metric

	County category				
	1	2	3	4	Total
Monitoring (unexposed)	1236	2146	1591	1781	6754
Monitoring (exposed)	0	72	87	20	179
Total	1172	3089	1713	959	6933
Weighted kappa = 0.01 (p< 0.01). Percent agreement = 47.95%					

Table 4.5: Comparison of categorized watershed modeling and county metrics

	Watershed modeling category				
	1	2	3	4	Total

County category 1	198	200	0	0	598
County category 2	215	520	1013	23	1771
County category 3	54	690	1332	260	2336
County category 4	65	411	798	333	1307
Total	732	1821	3143	616	6312
Weighted kappa = 0.29 (p < 0.01). Percent agreement = 77.0%					

Table 4.6 compares crude risk estimates for hypospadias for the county and watershed modeling exposure metrics on a continuous scale. Crude risk estimates could not be computed for the monitoring metric due to the lack of variability as described in Table 4.1. The odds ratio from the county level metric is 0.97, and the odds ratio from the watershed modeling metric is 2.29. The odds ratio greater than one from the watershed modeling metric suggests a positive relationship, while the odds ratio near one for the county level metric suggests no association; however, neither of the estimates is statistically significant.

Table 4.6: Comparison of crude risk estimates from continuous scale exposure assessment metrics.

Exposure Metric	**N (cases)**	**OR**	**95% Confidence Interval**
County level metric[a]	17,536 (1,046)	0.995	0.95 – 1.06
Watershed modeling model metric[b]	6,313 (362)	1.02	0.97 – 1.06

[a] *increment of change = 12.1 lb/mi^2 IQR*

[b] *increment of chang e= 0.02 µg/L IQR*

Table 4.7 presents crude risk estimates for hypospadias from the categorized county and watershed modeling exposure metrics. The monitoring metric is again omitted due to lack of variation in exposure estimates. Both the county metric and watershed modeling metric suggest

a slightly elevated risk in the highest categories of exposure, with odds ratios of 1.14 and 1.23 respectively, but again, neither of the estimates is statistically significant.

Table 4.7: Comparison of risk estimates from categorized exposure assessment metrics

Exposure Metric	Estimated Atrazine Level	N (cases)	Odds Ratio	95% Confidence Interval
County metric	Category 1 (<1.6 lbs/mi^2)	1,940 (102)	1.0	Referent
	Category 2 (1.6 – 5.2 lbs/mi^2)	4,964 (291)	1.12	0.89 – 1.41
	Category 3 (5.2 – 14.1 lbs/mi^2)	5,362 (340)	1.22	0.97 – 1.53
	Category 4 (>14.1 lbs/mi^2)	5,270 (313)	1.14	0.90 – 1.43
Watershed modeling metric	Category 1 (0.006 – 0.017 µg/L)	732 (43)	1.0	Referent
	Category 2 (0.017 – 0.033 µg/L)	1,821 (92)	0.85	0.59 – 1.24
	Category 3 (0.033 – 0.074 µg/L)	3,143 (183)	0.99	0.70 – 1.40
	Category 4 (0.076 – 0.50 µg/L)	606 (44)	1.23	0.80 – 1.90

Discussion

A number of studies have attempted to estimate prenatal exposure to atrazine via drinking water in order to evaluate possible teratogenic effects of atrazine (43, 55-60). These studies have generally used residential address to either assign mothers to counties or to drinking water supplies and then to estimate exposure based on the amount of atrazine applied in a county or sampled via monitoring. In the absence of urinalysis, however, it is impossible to know whether these studies are accurately estimating prenatal exposure.

This study considers how different exposure metrics might influence estimated exposure to atrazine by comparing two metrics used by other studies – the county metric and the monitoring metric – as well as a third metric which uses USGS model outputs to estimate atrazine in drinking water. The three metrics are compared using maps, Pearson correlation coefficients and kappa statistics. The county metric and the watershed modeling metric are also compared using and unadjusted odds ratios of hypospadias risk.

The maps and Pearson correlation coefficients found the watershed modeling metric to be more similar to the county and monitoring metrics, with the monitoring metric to be less similar to the county metric. The kappa statistic found the watershed modeling and county exposure metrics to be more similar than either the monitoring metric and county metric or the monitoring metric and watershed modeling metric. Because the watershed modeling metric includes atrazine use at the county level, and estimates exposure for water utilities, it makes sense that the watershed modeling metric has more in common with the county metric and the monitoring metric than county metric and monitoring metric have in common with one another.

We estimated odds ratios for hypospadias for the county metric and the watershed modeling metric to consider the effect of different exposure estimates on risk estimates. There was not enough variation in the monitoring metric to include it in this portion of the analysis. The continuous county level metric suggested no association between atrazine exposure and hypospadias. The continuous watershed modeling metric, the categorical watershed modeling metric, and the categorical county metric suggest a small, but statistically non-significant, increase in risk at higher levels of exposure. These odds ratios are not adjusted for any potential confounders, so should not be interpreted as representing the actual risk between atrazine and

hypospadias. However, they do illustrate the differences in odds ratios produced by different exposure metrics.

Although it is impossible to know whether any of the exposure metrics explored in this study approximate the "true" level of atrazine exposure, there are trade-offs associated with each of the exposure metrics. Because the vast majority of monitoring samples at public water utilities in North Carolina during the study period were at or below the MRL, and because North Carolina does not report data about atrazine concentrations at or below the MRL, the monitoring metric provided very little variation in estimated exposure, which prevented us from estimating odds ratios for hypospadias using this metric. Some states do report values for concentrations below the MRL, so it is possible that these data might provide more useful estimates of exposure in other states. For North Carolina, however, a lack of data prevents us from considering effects that might occur below the 0.1 µg/L threshold set by EPA.

The county metric provides exposure estimates to a much larger proportion of the study population and provides greater variation in estimated exposure. It may also be more prone to the ecological fallacy, however, because average pesticide use at the county level may not be an accurate indicator of individual exposure.

The watershed modeling metric attempts to address some of these shortcomings by providing a wider range of estimated exposure, and by incorporating the way that surface water flows through a watershed or groundwater moves through soils. On the other hand, the USGS models are not designed to address residence time in reservoirs or lakes, so therefore may not be accurately estimating atrazine concentrations in public water utilities.

Kappa statistics showed very little correlation between any of the three exposure metrics, which suggests that each of the metrics is capturing something different. This could indicate that

only one of the metrics is a good surrogate for estimating atrazine exposure, although it is impossible to confirm which one is the "good" one without knowledge of the actual amount of atrazine to which women were exposed. On the other hand, it could also indicate that none of the metrics is a good surrogate for estimating atrazine exposure, but simply that each is measuring something different.

A limitation of all of the metrics explored in this study is the use of a calendar year cut-off to assign exposure. Because the exposure metrics relied on annual mean atrazine estimates, the exposure estimate for a baby conceived in December would be based on mean estimates for the prior year, while the exposure estimate for a baby conceived in January would be based on mean estimates for the coming year. This may lead to exposure misclassification, particularly for winter births.

A further limitation of all of the metrics is the lack of information about maternal water consumption. If hypospadias risk increases when genetic susceptibility combines with endocrine disrupting chemicals exceeding some threshold, it would be important to know both the concentrations the chemicals of interest in drinking water, as well as the amount of drinking water consumed in estimating a threshold.

On the other hand, given the expense associated with prospective studies of birth outcomes including urinalysis for a given exposure, ecological exposure estimation techniques may be useful for identifying potential teratogens for further study. Difference in the magnitude and direction of odds ratios the importance of exposure measurement in estimating disease risk. Further research is needed to better understand the potential role played by atrazine in hypospadias risk.

CHAPTER 5

SELECTING AN EXPOSURE METRIC TO EXAMINE A RELATIONSHIP BETWEEN ATRAZINE AND HYPOSPADIAS

The previous chapter compared three different metrics for estimating maternal exposure to atrazine via drinking water: the county metric, which uses the amount of atrazine applied to a mother's county of residence; the monitoring metric, which uses water quality samples collected for compliance monitoring; and the watershed modeling metric, which uses output from USGS surface water and groundwater models to estimate atrazine concentrations in public water utilities. Although there are limitations associated with each of these metrics, the county metric does not provide an estimate of concentrations in drinking water, and may be more subject to the ecological fallacy than the other metrics. Further, the monitoring metric does not provide information about atrazine concentrations below EPA's MRL. The watershed modeling metric overcomes the shortcomings associated with the other two metrics because it is able to estimate concentrations in individual water supplies, and at a full range of exposures. The next chapter will therefore use the watershed modeling metric to examine a possible association between atrazine and hypospadias risk.

The previous chapters used data from the North Carolina Birth Defect Monitoring Program (NCBDMP) in order to explore the etiology of hypospadias and to compare different exposure estimation techniques. Although NCBDMP data contains information about demographic characteristics, it does not have information about maternal behavior or about

residential address throughout pregnancy. In order to consider a possible association between atrazine and hypospadias within a disease ecology framework, the next chapter will use data from the National Birth Defects Prevention Study (NBDPS), which includes behavioral covariates and detailed information about residence throughout pregnancy. This will provide us the unique opportunity to incorporate maternal water consumption and residence during the critical window of exposure for genitourinary development into our estimates of exposure to atrazine. It will also allow us to incorporate maternal demographic and behavioral characteristics in a multi-factorial consideration of atrazine and hypospadias.

CHAPTER 6

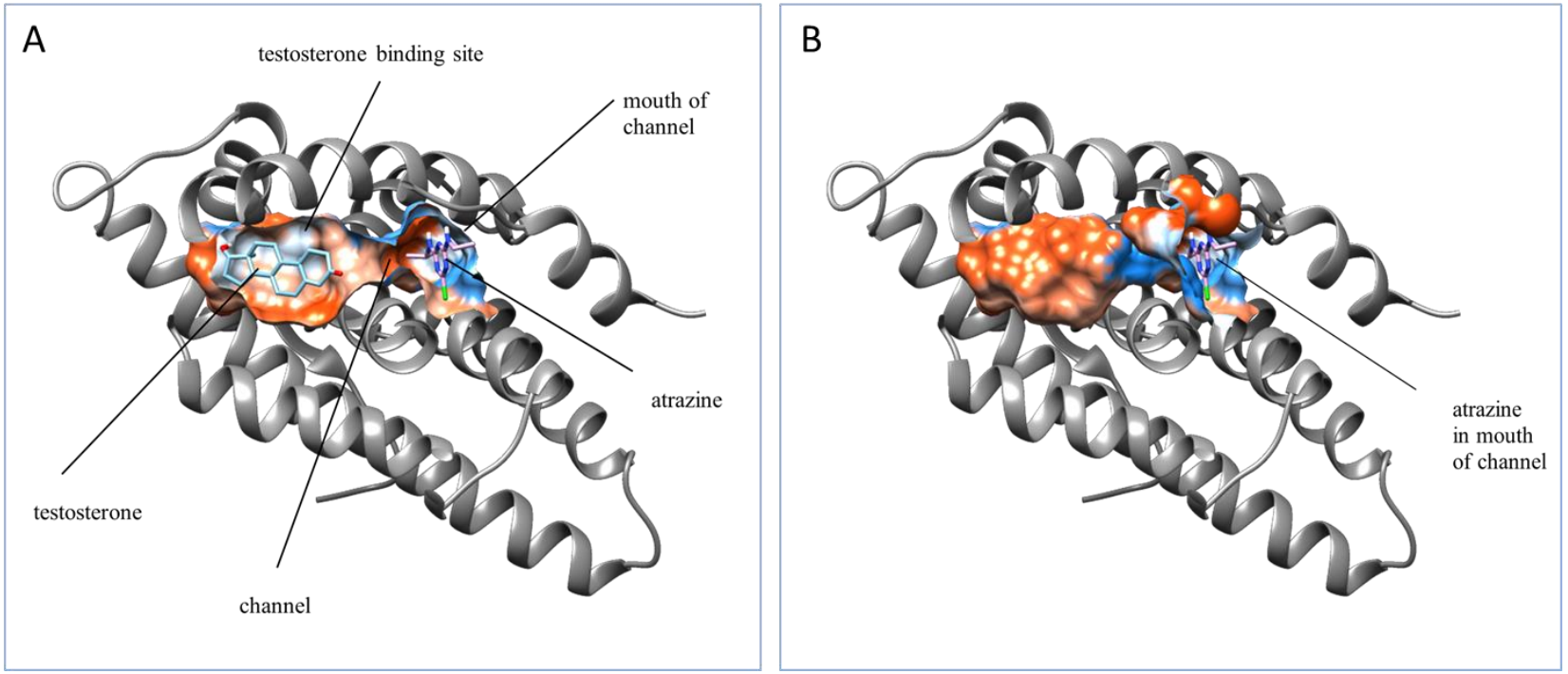
HYPOSPADIAS AND MATERNAL EXPOSURE TO ATRAZINE

Introduction

Hypospadias is a relatively common birth defect of the male urinary tract, affecting between 4 and 6 out of every 1,000 male births. It occurs as a result of abnormal urethral closure during gestational weeks 8-14, and manifests with a urethral opening on the underside of the penis (1). It has a significant public health impact, as surgical repair is often needed to allow for normal urinary and sexual function, and even after correction, hypospadias may result in psychosocial and sexual problems later in life (3).

Normal urethral closure during fetal development depends upon binding of testosterone to the androgen receptor and subsequent androgen receptor. It has therefore been suggested that endocrine disrupting chemicals might increase hypospadias risk (1). One potential endocrine disrupting chemical that has been examined for an association with genitourinary malformations is atrazine, one of the most widely used agricultural herbicides in the United States (38). A possible mechanism for atrazine to disrupt genitourinary development would be if atrazine could block the channel to the testosterone binding site on the androgen receptor. This would prevent testosterone from traveling through the necessary channel to bind to the androgen receptor and could thereby prevent complete urethral closure (See Figure 6.1).

Figure 6.1: Illustration of possible mechanism for atrazine to prevent testosterone from binding to the active site of androgen receptor. Panel A illustrates a cross-section of the androgen receptor including the mouth and channel leading to the testosterone binding site. Panel B illustrates atrazine blocking the mouth of the channel leading to the active site. (65)



There is experimental evidence to support a link between atrazine and genitourinary malformations in both rats (50) and amphibians (33, 51-53). The evidence to document a specific link between hypospadias and atrazine in humans is somewhat equivocal, however. Winchester *et al* found an elevated prevalence of "other urogenital anomalies," but not of "malformed genitalia" among infants conceived during months of the highest concentrations of atrazine and other chemicals measured by the US Geological Survey's National Water Quality Assessment Program (23). Chevrier *et al* examined urinary biomarkers of atrazine and general male genital anomalies. They found a non-significant increase in male genital anomalies amongst mothers with quantifiable atrazine or atrazine metabolites in urine, but their sample size was small (5 cases exposed and 18 case unexposed) (54). To our knowledge, only two studies have looked specifically at atrazine and hypospadias in humans, with conflicting results. The first study, by Meyer et al, 2006, assigned maternal exposure to several agricultural pesticides (including atrazine) by estimating the amount of pesticides applied within a 500 meter buffer of the mother's home. They did not find evidence of an association between hypospadias and atrazine (36). The second study, by Agopian et al, 2013, assigned atrazine levels to mothers based on their county of residence at birth. They found some evidence of an increased risk of hypospadias for mothers in the 25^{th}-75^{th} percentiles of exposure and for the 75^{th}-90^{th} percentiles of exposure, but suggested that further research was needed to confirm the mechanism for an association between hypospadias and county level atrazine use (43).

A number of studies have suggested that hypospadias has a multifactorial etiology (4, 8, 17). This would suggest that any role played by environmental exposures such as atrazine would

combine with other maternal demographic and behavioral characteristics to influence hypospadias risk. A number of maternal characteristics have previously been linked to hypospadias risk. From a demographic standpoint, risk increases with increasing maternal age (10, 14) and is higher amongst non-Hispanic white mothers (10, 39). There is conflicting evidence about an association with maternal education (12, 39, 66), but evidence of an inverse association with maternal parity (12, 67, 68) and of a positive association with plurality (7, 12, 69). Amongst behavioral characteristics, maternal diet may influence risk, with higher dietary intake of choline, methionine, and vitamin B12 associated with lower risk (11). Use of fertility medications and procedures, on the other hand, seems to increase risk (17, 69, 70).

In this study, we seek to build on existing research examining the potential relationship between atrazine and hypospadias by incorporating information about maternal water consumption, as well as other known demographic and behavioral risk factors. We use a novel technique to estimate maternal exposure to atrazine in drinking water, and take advantage of unique data that includes information about behavioral covariates and maternal residential address throughout pregnancy. This allows us to investigate the role of atrazine in conjunction with other factors that may contribute to the multi-factorial etiology of hypospadias.

Methods

Data from this study comes from the National Birth Defects Prevention Study (NBDPS), which is a population-based case-control study conducted in ten states with the Centers for Disease Control and Prevention. NBDPS identifies second- and third-degree hypospadias cases, which are considered moderate to severe (1), from birth defect surveillance registries and randomly selects controls from birth certificates or birth hospital records. NBDPS does not

include first-degree, or mild, hypospadias cases due to variable medical documentation. NBDPS also collects data on a wide number of covariates via computer-assisted telephone interview. Covariates include information about water consumption, maternal address throughout pregnancy, and a number of known risk factors for hypospadias, including maternal age, maternal race/ethnicity, parity, plurality, maternal choline intake, and use of fertility medications (71).

The study included interviewed hypospadias cases (n= 343) and male controls (n=1,422) from North Carolina, Iowa, Arkansas, and Texas with estimated due dates between 1998 and 2005. These states were selected from the NBDPS study sites because atrazine concentrations in streams were predicted to be higher than in other study sites (72). These years were selected because they were the years for which data were collected about water consumption.

Mothers using a public water supply were assigned a water utility for each reported residential address by the University of Iowa Center for Health Effects of Environmental Contamination (CHEEC), using public water supply service area polygons where available, and Census place names and borders where service area polygons were unavailable.

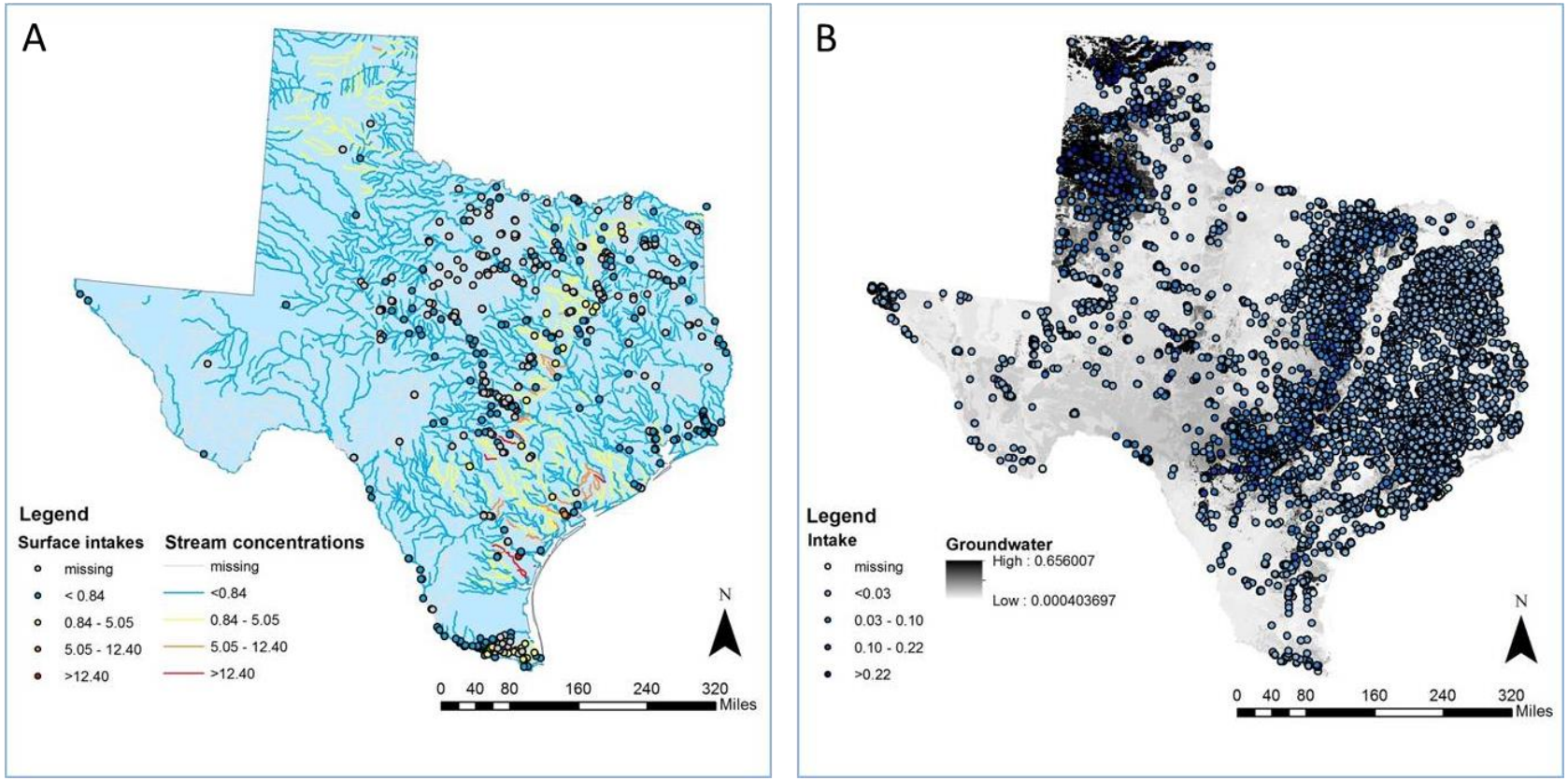
We estimated atrazine concentrations using two US Geological Survey (USGS) models. For water supplies using surface water, we used estimated annual mean atrazine concentrations in streams predicted by the Watershed Regressions for Pesticides (WARP) model. WARP uses estimated watershed-level atrazine use, the percentage of the watershed's agricultural land with a soil restrictive layer near the surface, total precipitation during May and June of the sampling year, rainfall erosivity for the watershed, and streamflow caused by precipitation on saturated soil in order to generate nationwide estimates of atrazine concentrations in streams (37). For

water supplies using groundwater, we used site-variable model predictions from the "Regression Models for Estimating Concentrations of Atrazine plus Deethylatrazine in Shallow Groundwater in Agricultural Areas of the United States." This model uses groundwater residence time, atrazine use intensity, artificial drainage practices, depth to the seasonally high water-table, content of the uppermost soil content, soil permeability, groundwater recharge rates, and well depth to provide gridded estimates of atrazine concentrations in shallow groundwater (38).

We assigned atrazine concentrations to public water supplies based on the type and location of the water intakes for each utility. Geographic coordinates of surface and groundwater intakes for public water utilities were available for Iowa, Texas, and North Carolina (64, 73-75). For Arkansas, we used Google Earth to geocode water intakes using descriptions of intake locations available from the Arkansas Department of Health (76).

For surface water intakes, we used WARP estimates from the nearest stream reach to assign an annual mean atrazine concentration to the intake. For groundwater intakes, and for mothers in NBDPS using private wells, we used gridded atrazine predictions from the USGS groundwater model and bilinear interpolation to estimate atrazine concentrations based on the grid cell where the intake was located and the adjacent grid cells. (See Figure 6.2). Each public water utility was then assigned an atrazine concentration equal to the mean of the predicted atrazine concentrations for all of the intakes for that utility.

Figure 6.2: Illustration of assignment of atrazine concentrations to water intakes for public water utilities in Texas. Panel A illustrates WARP stream estimates and concentrations assigned to surface water intakes. Panel B illustrates USGS groundwater estimates and concentrations assigned to groundwater intakes.



We based our exposure assessment on maternal residential addresses during gestational weeks 6-16. This window encompass the critical weeks for urethral development during gestational weeks 8-14 (42). Mothers using public water were assigned an atrazine exposure based on the estimated atrazine concentration in the public water utility assignments from CHEEC. Mothers using well water were assigned an atrazine value based on bilinear interpolation of gridded atrazine predictions from the USGS groundwater model. This assignment was conducted using ArcGIS version 10. Mothers with more than one residential address during the critical exposure period were assigned a weighted value based on the atrazine concentration and the number of weeks at each address. We excluded mothers without a full residential history, mothers using public water who were not successfully matched to a public water utility, and mothers using a utility that was not successfully assigned an atrazine concentration by one of the two USGS models. This reduced our sample size to 123 cases and 415 controls. We conducted a sensitivity analysis to help characterize any selection bias that might be introduced by this loss of sample size.

We then estimated the daily amount of atrazine consumed via drinking water by a mother by multiplying the estimated atrazine concentration in a mother's drinking water by the self-reported number of glasses of water drunk daily by the mother. The self-reported number of glasses consumed ranged from 0 to 24 glasses of water daily. Because it is unlikely that pregnant women are consuming no water, we converted the women who reported drinking 0 glasses to missing prior to multiplying by estimated atrazine concentration.

We tabulated the distributions of maternal socioeconomic, demographic, and behavioral characteristics and hypospadias cases and controls and compared them using chi-squared tests.

We then built unadjusted and adjusted logistic regression models for two predictors of interest: estimated concentration of atrazine in a mother's drinking water supply; and for estimated daily maternal atrazine consumption. We estimated crude and adjusted odds ratios for hypospadias for both predictors. Covariates used for adjustment were selected based on existing literature, and included private well use, state of residence, maternal age, maternal race/ethnicity, plurality, parity, maternal education, choline use, and use of artificial reproductive technology as possible confounders. Analyses were performed using Stata version 13.1.

Results

We present the distributions of socioeconomic, demographic, and behavioral characteristics for mothers of hypospadias cases (n = 123) and male controls (n = 415) in Table 6.1. Significant differences in distribution between cases and controls were observed for state of residence, maternal race or ethnicity, maternal education, previous pregnancies, plurality, and use of fertility medications or procedures. While controls were fairly evenly distributed amongst the four states, 79.6% of cases lived in Arkansas and North Carolina. Mothers of cases were more likely to be non-Hispanic white, more highly educated, and to have used fertility medications or procedures, and infants were more likely to be a result of a first pregnancy or a multiple birth. Mothers of cases were slightly more likely to report drinking 5 or more glasses of water a day, but this was only marginally significant ($p = 0.09$). Significant differences in distributions were not observed for reported use of a private well, maternal age, or maternal choline intake.

Table 6.1: Characteristics of NBDPS hypospadias cases and controls with estimated atrazine exposure, 1998-2005

	Cases		Controls		
Characteristic	N	%	N	%	P-value
State of residence					<0.01
Arkansas	49	39.8	85	20.5	
Iowa	17	13.8	103	24.8	
Texas	8	6.5	101	24.3	
North Carolina	49	39.8	126	30.4	
Private well use					0.72
No	85	70.3	292	71.9	
Yes	36	29.7	114	28.1	
Reported water consumption					0.09
0 glasses	3	2.4	22	5.3	
1-4 glasses	77	62.6	217	52.3	
5 or more glasses	43	35.0	176	42.4	
Maternal age					0.23
<20	10	8.1	43	10.4	
20-24	27	22.0	89	21.5	
25-29	25	20.3	118	28.4	
30-34	42	34.2	105	25.3	
≥35	19	15.5	60	14.5	
Maternal race/ethnicity					<0.01
Non-Hispanic white	94	76.4	242	58.3	
Non-Hispanic black	16	13.0	32	7.7	
Hispanic	8	6.5	106	25.5	
Other	5	4.1	35	8.4	
Maternal education					<0.01
<High school	5	4.1	86	20.7	

High school	30	24.4	111	26.8	
>High school	88	71.5	218	52.5	
Previous pregnancies					<0.01
No	55	44.7	122	29.4	
Yes	68	55.3	293	70.6	
Plurality					0.01
No	114	92.7	405	97.6	
Yes	9	7.3	10	2.4	
Maternal choline intake*					0.15
<187.4 mg	27	22.0	72	17.4	
187.4 – 249.6 mg	30	24.4	85	20.5	
249.7 – 336.3 mg	34	27.6	104	25.1	
>336.4 mg	32	26.0	154	37.1	
Fertility medications or procedures					0.02
No	111	90.2	393	95.6	
Yes	12	9.8	18	4.4	

*Categories for maternal choline intake from Carmichael *et al* (11)

We present raw distributions for estimated concentrations of atrazine for a mother's drinking supply and for estimated daily atrazine consumption in Table 6.2. Mean and median concentrations are higher for controls than for cases for both estimated atrazine in water supply and estimated atrazine consumption. In addition, the mean is greater than the median for all estimates. The difference is greater for the estimated atrazine consumption because a small number of mothers consumed a large amount of water, increasing the skew of the data.

Table 6.2: Distribution of estimated atrazine in water supply and estimated atrazine consumption

	Cases				**Controls**			
	Mean	Median	IQR	Min, Max	Mean	Median	IQR	Min, Max
Estimated atrazine in water supply (µg/L)	0.091	0.018	0.001 – 0.04	0.0001, 2.0	0.17	0.019	0.002 – 0.051	0.0001, 4.0
Estimated atrazine consumption (µg/L)	0.491	0.066	0.004 – 0.16	0.0002, 15.8	0.61	0.073	0.010 – 0.274	0.0003, 19.7

We present crude and adjusted odds ratios for hypospadias for the estimated concentration of atrazine in a mother's drinking supply and for a mother's estimated daily atrazine consumption in Table 6.3. Crude odds ratios fall below 1.0 for estimated concentrations of atrazine and for estimated atrazine consumption, but are not significant. After adjustment for private well use, state of residence, maternal age, maternal race/ethnicity, plurality, parity, maternal education, choline use, and use of reproductive medications or procedures, both odds ratios exceed 1.0. The adjusted odds ratio for estimated daily atrazine consumption is marginally significant ($p = 0.054$).

Table 6.3: Association between atrazine and hypospadias in the National Birth Defects Prevention Study, 1998-2005

	N (cases)	Crude OR	p-value	Adjusted OR[b]	p-value
Estimated atrazine in water supply[a]	538 (123)	0.97 (0.94, 1.00)	0.07	1.01 (0.98, 1.04)	0.57
Estimated atrazine consumption[a]	513 (120)	0.99(0.96, 1.02)	0.53	1.03 (0.998, 1.06)	0.054

[a] OR for interquartile range of 0.03 µg/L for estimated atrazine in water supply and 0.15 µg/L for estimated atrazine consumption.
[b] ORs adjusted by private well use, state of residence, maternal age, maternal race/ethnicity, plurality, parity, maternal education, choline use, and use of artificial reproductive technology.

Table 6.4 presents odds ratios for the final adjusted logistic regression model for hypospadias for daily estimated atrazine consumption. When accounting for atrazine consumption, as well as other demographic and behavioral characteristics, residents of Arkansas are statistically significantly more likely to have a hypospadias birth than residents of Iowa or Texas. In addition, more highly educated women, nulliparous women, and women with plural births have a statistically significantly greater likelihood of a hypospadias birth.

Table 6.4: Final logistic regression model for estimated maternal atrazine consumption via drinking water and hypospadias, including covariates.

Characteristic	Odds Ratio	95% Confidence Interval	P-value
Estimated atrazine consumption[a]	1.03	0.99 – 1.29	0.054
State of residence			
Arkansas	1.00	Referent	
Iowa	0.21	0.10 – 0.43	<0.01
Texas	0.16	0.05 – 0.54	<0.01
North Carolina	0.62	0.36 – 1.07	0.09
Private well use			
No	1.00	Referent	
Yes	1.58	0.92 – 2.71	0.10
Maternal age			
<20	1.00	Referent	
20-24	1.01	0.38 – 2.71	0.98
25-29	0.43	0.15 – 1.23	0.12
30-34	0.88	0.31 – 2.51	0.81
≥35	0.73	0.24 – 2.27	0.59
Maternal race/ethnicity			
Non-Hispanic white	1.00	Referent	
Non-Hispanic black	1.15	0.54 – 2.45	0.72
Hispanic	0.49	0.14 – 1.66	0.25
Other	0.31	0.10 – 1.01	0.05
Maternal education			
<High school	1.00	Referent	
High school	3.60	1.18 – 10.96	0.02

>High school	4.97	1.58 – 15.63	<0.01
Previous pregnancies			
No	1.00	Referent	
Yes	0.76	0.63 – 0.92	<0.01
Plurality			
No	1.00	Referent	
Yes	7.36	2.06 – 26.38	<0.01
Maternal choline intake*			
<187.4 mg	1.00	Referent	
187.4 – 249.6 mg	1.22	0.62 – 2.42	0.57
249.7 – 336.3 mg	1.41	0.72 – 2.79	0.32
>336.4 mg	0.91	0.46 – 1.83	0.80
Fertility medications or procedures			
No	1.00	Referent	
Yes	1.88	0.74 – 4.78	0.19
Constant	0.23	0.65 – 0.80	0.02

[a] OR for interquartile range of 0.03 µ/L for estimated atrazine in water supply and 0.15 µ/L for estimated atrazine consumption

We present a sensitivity analysis comparing characteristics of women who were successfully assigned an atrazine exposure and women who were not successfully assigned an atrazine exposure in Table 6.2. Mothers who were included in the USGS metric are more likely than mothers who were excluded to be mothers of hypospadias cases; to use private wells; to live in North Carolina; to be over age 30; and to be a race or ethnicity other than non-Hispanic white, non-Hispanic black, or Hispanic.

Table 6.5: Characteristics of women successfully assigned an atrazine exposure and women who were not successfully assigned an atrazine exposure.

	Included in USGS metric (N = 513)	Excluded from USGS metric (N = 1,252)	P-value
Hypospadias			<0.01
Controls	393 (76.6%)	1,029 (82.2%)	
Cases	120 (23.4%)	223 (17.8%)	
Private well use			<0.01
No	367 (71.5%)	993 (98.5%)	
Yes	146 (28.5%)	15 (1.5%)	
State of Residence			<0.01
Arkansas	131 (25.5%)	459 (36.7%)	
Iowa	106 (20.7%)	353 (28.2%)	
Texas	105 (20.5%)	324 (25.9%)	
North Carolina	171 (33.3%)	116 (9.3%)	
Maternal age			<0.01
<20	50 (9.8%)	203 (16.2%)	
20-24	112 (21.8%)	313 (25.0%)	
25-29	134 (26.1%)	370 (29.6%)	
30-34	139 (27.1%)	252 (20.1%)	
≥35	78 (15.2%)	114 (9.1%)	
Maternal race/ethnicity			<0.01
Non-Hispanic white	318 (62.0%)	810 (64.8%)	
Non-Hispanic black	47 (9.2%)	138 (11.0%)	
Hispanic	110 (21.4%)	262 (20.9%)	
Other race/ethnicity	38 (7.4%)	41 (3.3%)	
Maternal education			0.17
Less than high school	87 (17.0%)	215 (17.8%)	
High school	135 (26.3%)	364 (30.2%)	
More than high school	291 (56.7%)	627 (52.0%)	
Previous pregnancies			0.50
No	167 (32.6%)	426 (34.3%)	

Yes	349 (67.4%)	826 (65.7%)

Discussion

After adjusting for maternal socioeconomic, demographic, and behavioral characteristics, we observed a modest, and marginally significant, association between hypospadias and maternal consumption of atrazine via drinking water during gestational weeks 6-16. This association was not observed in crude odds ratios, or when not accounting for the total amount of drinking water consumed. This lends support to the hypothesis that hypospadias has a multi-factorial etiology, wherein genetics, maternal characteristics, and environmental factors may interact to contribute to hypospadias risk. It further provides some evidence to suggest that, when combined with other risk factors including state of residence, private well use, maternal education, parity, and plurality, atrazine may be associated with hypospadias.

Certain limitations should be considered when interpreting these results. While the USGS models that we employed allowed us to estimate atrazine concentrations in drinking water, we cannot be sure that they accurately predict maternal exposure to atrazine without a validated, repeated measure of atrazine in maternal urine during pregnancy. We did consider other exposure estimation techniques, however, first using monitoring data from the US Environmental Protection Agency, and then using the amount of atrazine applied at the county level (see Chapter 4). Both of these alternatives proved problematic. Monitoring data was not available for atrazine concentrations below the US Environmental Protection Agency's Minimum Risk Level, which prevented us from considering associations between hypospadias and lower levels of atrazine. Further, the total amount of atrazine applied at the county level would not

have allowed us to consider the interaction between atrazine concentrations in drinking water and maternal water consumption.

Another limitation was our inability to assign atrazine concentrations to many of the NBDPS women. Sensitivity analyses revealed that women who were not successfully assigned an atrazine concentration were likely to be older and to use private wells, which were characteristics associated with increased risk study. They were also more likely to be a race or ethnicity other than non-Hispanic white, non-Hispanic black, or Hispanic, and to live in North Carolina, which were characteristics associated with decreased hypospadias risk in this study. It is therefore unclear how exclusion of these women may have influenced our results.

This study also had several strengths. Our selected modeled exposure estimates allowed us to consider a specific mechanism for a possible association between atrazine and hypospadias. It also took advantage of the unique water consumption and other covariate data available through the National Birth Defects Prevention Study (NBDPS), which allowed us to consider atrazine as part of a multifactorial etiology of hypospadias. While we were only able to successfully assign an exposure to 123 cases and 415 controls, the marginally significant association that we found suggests that when behavioral and demographic traits interact with exposure, the effect is strong enough to detect a signal even with a small sample size.

This study provides additional support for the body of evidence suggesting that atrazine may be associated with male genitourinary malformations in humans. Further research including larger sample size and urinalysis for model validation is warranted in order to provide a clearer picture of this multifactorial disease. This may help inform future birth defect prevention efforts.

CHAPTER 7

CONCLUSION

This research uses disease ecology theory to shed light on the multifactorial etiology of hypospadias. It also lends support to a possible association between this relatively common birth defect and agricultural pesticide use. Chapter 1 discusses existing research about population, behavioral, and environmental risk factors for hypospadias. Chapter 2 considers the spatial distribution of hypospadias in North Carolina in order to explore contextual factors that might play a role in hypospadias risk. It illustrates that, even when controlling to the extent possible for compositional factors, spatial autocorrelation of disease incidence persists, which suggests a role being played by contextual factors. Because this persistent spatial autocorrelation occurs in a part of North Carolina, where agriculture plays an important role, crop cover is then investigated as one potential contextual factor of interest. The percentage of a block group in agriculture is, in fact, found to be significantly associated with hypospadias, and does explain a small amount of the remaining spatial autocorrelation of hypospadias in North Carolina.

The remaining chapters then build on this framework by focusing on one potential mechanism for an association between hypospadias and proximity to crop cover: maternal exposure to the herbicide atrazine via drinking water. Chapter 4 examines three different metrics for estimating maternal exposure to atrazine via drinking water. It finds that different exposure assessment techniques yield different crude estimates of hypospadias risk. After considering strengths and weaknesses of each of the exposure metrics, Chapter 5 concludes that using USGS

groundwater and surface water models to estimate atrazine concentrations in drinking water are the most appropriate for our dataset because they produce concentration estimates for individual water supplies (instead of use at the county level). They also produce estimates at a range of concentrations, including those falling below the EPA's MRL.

Chapter 6 then uses the watershed modeling metric, in conjunction with behavioral and demographic data collected by the National Birth Defects Prevention Study, to examine a possible association between hypospadias and maternal exposure to atrazine via drinking water. To our knowledge, this is the first study to consider atrazine in conjunction with survey data on water consumption and maternal address throughout pregnancy. It is also the first to use USGS surface and groundwater models in a birth defects study.

Consistent with the disease ecology framework, our research emphasizes the importance of incorporating behavioral and other maternal characteristics in the study of diseases. As illustrated in Chapter 6, crude odds ratios do not find a significant association between atrazine and hypospadias, but logistic regression incorporating water consumption, maternal age, maternal race/ethnicity, maternal education, parity, plurality, maternal choline intake, and use of fertility procedures and medications yield an adjusted odds ratio of 1.14 ($p = 0.054$) for a 1 µg/L increase in daily maternal atrazine consumption during the critical window of exposure for genitourinary development. This illustrates the utility of incorporating disease ecology theory into the study of hypospadias and other diseases with a multi-factorial etiology. It also provides additional evidence for a role played by environmental factors in hypospadias births.

Study Limitations:

This research has a number of limitations, as described in the preceding chapters, which are generally a result of missing data. The studies using NCBDMP data only have information about maternal address at birth. If mothers moved during pregnancy, using maternal address at birth may lead to exposure misclassification, especially since the critical window for genitourinary development is relatively early in pregnancy, during gestational weeks 8-14. NCBDMP data also does not include information about maternal behavioral characteristics, which meant that we could not include potentially important risk factors such as use of fertility medications or procedures, maternal diet, and maternal drinking water consumption, in the compositional risk factors that we considered in our analysis of the geographic distribution of hypospadias in North Carolina.

In addition, in the absence of repeated urinalysis during early pregnancy, we do not know the "true" level of maternal exposure to atrazine. Thus, while we are able to compare the three exposure metrics described in Chapter 4, we cannot evaluate which is the most accurate in estimating maternal exposure.

Directions for further research:

The significant association between hypospadias and crop cover, as found in Chapter 1, as well as the marginally significant adjusted association between hypospadias and daily maternal atrazine consumption, as found in Chapter 6, suggests that further research on possible teratogenic effects of atrazine is warranted. Such research should include urinalysis of pregnant women, which would enable validation of one or more exposure estimation techniques, and allow further research into the potential association between atrazine exposure and genitourinary

malformations. Further research is also needed consider other endocrine disrupting chemicals which might function in the same way.

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