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Pesticide exposure and risk of hypospadias: assessment and the adequacy of exposure measurements

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PESTICIDE EXPOSURE AND RISK OF HYPOSPADIAS: ASSESSMENT AND THE ADEQUACY OF EXPOSURE MEASUREMENTS

by

Carissa Marie Rocheleau

An Abstract

Of a thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Epidemiology in the Graduate College of The University of Iowa

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Thesis Supervisor: Associate Professor Paul A. Romitti

ABSTRACT

Hypospadias is a congenital malformation that occurs in 0.3-1% of live births, in which the meatus (the urethral opening) is dorsally malpositioned. Uncorrected hypospadias can cause difficulties in urination, abnormal sexual function, and adverse psychological consequences; surgical correction, though generally successful, constitutes an economic burden for families. Several common classes of pesticides have demonstrated potential to disrupt normal endocrine hormones that regulate fetal genitourinary development. Past epidemiologic studies of pesticide exposure and risk of hypospadias have been limited by limited available data, small sample sizes, or poor ascertainment of pesticide exposure.

The objective of this study was to examine the relationship between parental occupational pesticide exposure and risk of hypospadias in their offspring; and further, to assess whether addition of residential pesticide exposure data is feasible and contributes to overall pesticide exposure. We began by conducting a meta-analysis of the current literature, in which summary measures of occupation (such as census occupation code) had been used to assign pesticide exposure. We found elevated but marginally significant risks of hypospadias were associated with maternal occupational exposure (PRR of 1.36, CI = 1.04-1.77), and paternal occupational exposure (PRR of 1.19, CI= 1.00-1.41) in the previously published literature.

We then used industrial hygienist review of occupational histories to estimate the relationship between pesticide exposure and risk of hypospadias. We found that maternal occupational exposure to any pesticides (yes/no) was not associated with an increased risk of hypospadias (OR = 0.83, 95% CI = 0.6-1.1), cumulative insecticide (OR = 1.09;

95% CI = 0.9- 1.3), herbicide (OR = 1.05; 95% CI = 0.9- 1.2), or fungicide (OR = 0.91; 95% CI = 0.7-1.2) exposure. These negative findings might be explained by a lack of relationship at the low levels of exposure observed in this study population, in which case another farm exposure could be related to hypospadias; or this negative finding may be due to exposure misclassification.

Finally, we evaluated the feasibility and relevance of collecting residential pesticide exposure and direct reports of occupational exposure from fathers. Residential pesticide use during the six months prior to pregnancy and during pregnancy was common among control mothers: 45% reported that their home had been treated for insect or rodent pests; 47% reported that their lawn or garden had been treated for weeds or insect pests; 16% used a lawn service; 26% reported that a pet had been treated for fleas, ticks, or mites (including flea and tick preventives); 17% reported community-wide sprayings for pests; and 16% reported that their workplaces were treated for pests. Case mothers were more likely to report that their home had been treated of insect or rodent pests (50%) or that a pet had been treated for fleas, ticks, or mites (36.5%). Our results suggest that collection of information on residential pesticide use is feasible, and the impact of residential pesticide use on birth defects risk should be assessed in future studies.

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PH.D. THESIS

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To Thomas E. Nelson (1970 – 2005)

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LIST OF ABBREVIATIONS

ATF3	Activating Transcription Factor 3 gene
B6	The six months prior to conception
BMI	Body-Mass Index
CI	Confidence Interval
CTGF	Connective Tissue Growth Factor gene
CYR61	Cysteine-Rich Angiogenic Inducer 61 gene
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
DOB	Date of birth or pregnancy end date
HOEPS	Home and Occupational Exposure to Pesticides Study
IH	Industrial Hygienist
IRB	Institutional Review Board
JEM	Job-Exposure Matrix
MeSH	Medical Subject Headings
NAICS	North American Industry Classification System
NBDPS	National Birth Defects Prevention Study
OR	Odds Ratio
P1-P9	1st month of pregnancy through the ninth month of pregnancy
PPE	Personal Protective Equipment
PRR	Pooled Risk Ratio
SOC-2000	Standard Occupational Classification 2000
T1	The first trimester of pregnancy
T2	The second trimester of pregnancy
T3	The third trimester of pregnancy

CHAPTER I

AN INTRODUCTION TO THE STUDY OF PESTICIDE EXPOSURE AND HYPOSPADIAS

Public Health and Clinical Significance of Hypospadias

Hypospadias is a congenital malformation of males in which the meatus, the urethral opening, is malpositioned dorsally. In the U.S., hypospadias occurs in 0.3-1% of live births[1-3]. The severity of hypospadias varies and is classified as 1st degree (anterior), 2nd degree (middle), or 3rd degree (posterior) depending on the position of the meatus; 3rd degree hypospadias is the most severe and difficult to correct[4]. Hypospadias is often accompanied by dorsal hooding (an incomplete formation of the prepuce) and chordee (ventral curvature of the penis)[5-7]. Uncorrected hypospadias can cause difficulties in urination, abnormal sexual function, and adverse psychological consequences; for this reason, the majority of hypospadias must be surgically corrected[8,9]. Acute surgical complication rates for hypospadias repair are high, and can lead to failure of the repair[10]. Repeated surgical procedures may not yield normal function and appearance[11,12].

Biology of Hypospadias

Urogenital development between human males and females is virtually identical until approximately the eighth week of gestation, at which time testosterone, an androgen hormone, begins to be released from the fetal testes in response to a surge in luteinizing hormone from the pituitary gland[13]. Dihydrotestosterone induces several urogenital developments, eventually resulting in growth and fusion of the urethral folds to form the

median raphe at the end of the third month[13]. Disruption of androgen activity, whether by reducing production of testosterone, interfering with conversion of testosterone to dihydrotestosterone, or interrupting the normal function of androgen receptors, might arrest this process at any point along the urethral fusion line and result in hypospadias[6]. Increased expression of estrogen and androgen receptors in the penile tissue of boys with hypospadias has been noted previously[14].

Genes involved in estrogen and testosterone protection, or that code for androgen receptors, have been implicated in hypospadias. Other candidate genes for hypsopadias have been identified by evaluating genes that are upregulated during urogenital development and vary in expression between boys with and without hypospadias. These include activating transcription factor 3 (ATF3), connective tissue growth factor (CTGF), and cysteine-rich angiogenic inducer 61 (CYR61) genes; all three genes are estrogen-responsive[15].

Epidemiology of Hypospadias

The epidemiology of hypospadias gives little clue to its etiology, though the disorder is believed to have a multifactorial origin[6,16-19]. Family history, advancing maternal age, body mass index (BMI), alcohol consumption, cigarette smoking, and intrauterine growth retardation are among the suggested risk factors[6,20-23]. *In-utero* exposure to the synthetic estrogen diethylstilbestrol (DES) is a known risk factor for hypospadias[24-26]; intake of other synthetic estrogens and progestins, such as those used in oral contraceptives or assisted reproductive techniques, have also been associated with increased risk of hypospadias in some (but not all) studies[19,27].

Because of their regulatory role in normal fetal genitourinary development, the disruption of normal endocrine hormones has been postulated to play a role in the development of hypospadias[28] along with cryptorchidism, low sperm counts, and testicular cancer through a shared pathway[28-35]. Endocrine disrupting chemicals may have an estrogenic or androgen-antagonist effect. I*n-vitro* or animal studies[13] have shown that pthalates, some solvents, and several common classes of pesticides have demonstrated potential to disrupt endocrine hormones. Among pesticides, chlorphenoxy herbicides, linuron, DDE, glyphosphate, chlozolinate, iprodione, procymidone, and vinclozolin have all been shown to induce hypospadias in rats[36-41].

Epidemiologic studies of pesticide exposure and hypospadias have presented a more conflicted picture. Few studies observed statistically significant changes in risk, and nearly as many demonstrated reduced risk as increased risk. Some of the variability in these outcomes is likely due to small sample sizes or an inability to measure important known confounders.[42] Instability in the point estimates between these studies might also have been caused by variability in methods used to assess pesticide exposure. Of the 13 non-ecologic studies identified, only one evaluated both occupational and residential exposures. Several of these studies evaluated only one parents' exposure, and most were unable to evaluate any other risk factors, due to small sample sizes or limitations in the available data. Almost all previous studies relied on farmwork as a proxy for pesticide exposure; but this is an inadequate marker of actual pesticide exposure[43].

Measuring Pesticide Exposure

Measuring pesticide exposure more accurately is an enormous challenge in epidemiologic studies that rely on recall and retrospective exposure assessment. Self-reported pesticide exposure generally has low validity[44-48], and recall is generally limited to things the subject can see, smell, touch, or hear. Subject recall may also be influenced by the subjects' health status. Job-exposure matrices, in which each unique job title or task is assigned a putative exposure, were developed to reduce recall bias and better account for some types of exposure. These assume, however, that every worker with a given job title or task name shares the same exposure, which may not be highly valid[49,50]. Industrial hygienist-assessed exposure is typically considered a 'gold standard' for defining retrospective occupational exposure, but even this method is subject to exposure misclassification[51-56]. Exposure may also be mediated by personal behaviors, such as the frequency of hand washing and the use of protective equipment[57]. Even when occupational exposure is measured well, pesticide exposures may also occur in the home, from treatments to lawns and gardens, through residues on food, or from aerial drift or water contamination[58-74].

Perhaps the greatest challenge in retrospective pesticide exposure assessment lies in the specificity of assessment. There are over 1,000 pesticide formulations on the market in the U.S., with varying mechanisms of biological action and reactivity. Pesticides can be broadly grouped into categories such as insecticides, fungicides, herbicides, fumigants, and rodenticides based on their targeted organism; but even these categories are highly heterogeneous[75]. Such categories of exposure are, therefore, inexact proxies for exposure to an actual group of chemicals with a shared function and

mechanism; the use of these proxy groups essentially introduces random exposure misclassification that will generally bias results towards the null[76]. The adjuvants used in pesticide formulations may also be biologically active, adding an additional challenge for exposure assessment.

Data Quality and Sample Size Issues in Studies of Birth Defects

Although birth defects as a whole are common, the frequency of an individual type of birth defect (for example, a heart defect or hypospadias) is much lower. Individual defects likely have unique mechanistic pathways; thus, grouping all types of defects together as an outcome group is inappropriate and will likely bias observed results towards the null through outcome misclassification[77]. To examine specific birth defects, one must either assemble an extremely large cohort or use a case-control study design to identify sufficient cases for analysis. Several previous studies have been unable to adjust for important confounders due to their small sample size. Conversely, a number of large registry-based studies have relied on available data, typically from census or birth records. These records are cursory, with information on many important confounders not collected.

Significance

Understanding the risk factors for hypospadias may allow health care providers to give better counsel to patients who are or wish to become pregnant. If an association exists with pesticide exposure, interventions can be targeted towards those at highest risk in order to prevent hypospadias. Each case of hypospadias that is prevented can save a

child from a lifetime of potential medical, social, and psychological difficulties[9]; and save families from enormous treatment costs. Furthermore, environmental risk factors are believed to play a role in the vast majority of birth defects. A critical evaluation of traditional industrial-hygienist reviews provides valuable data that may be helpful in constructing other studies that assess occupational and environmental exposures.

Research Approach and Rationale

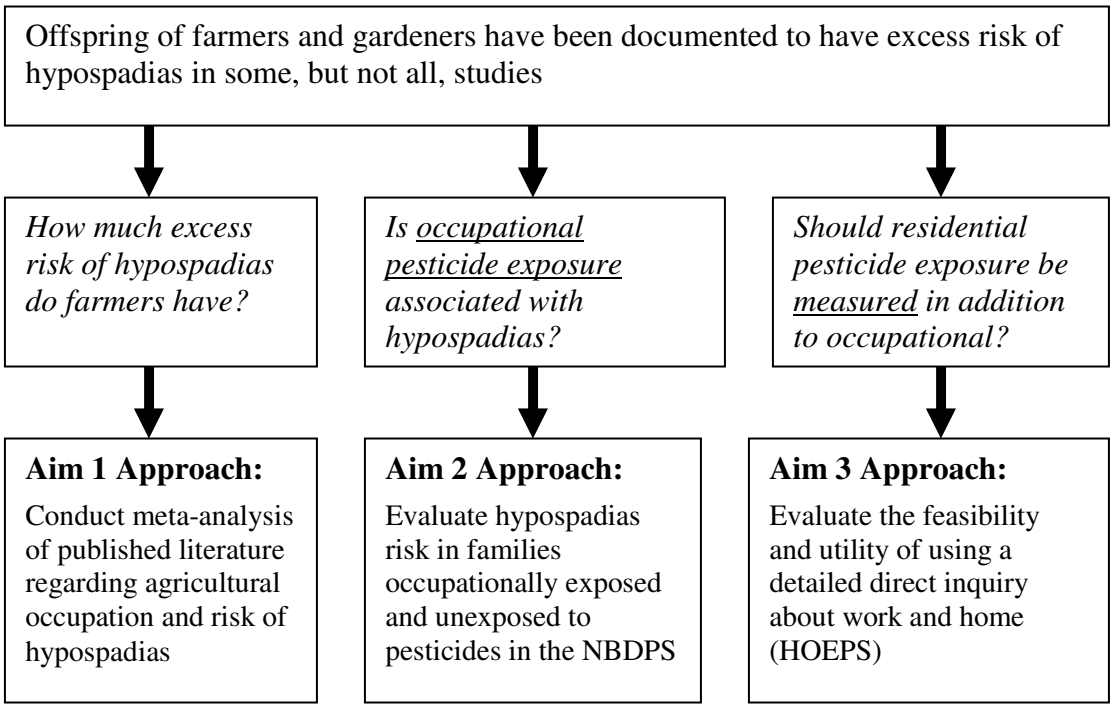
To meet our research goal, we addressed three basic questions, as indicated in the logic model in Figure 1: 1) How much excess risk of hypospadias do farmers have?, 2) Is maternal occupational pesticide exposure associated with hypospadias risk?, and 3) Is it feasible to use additional data—on residential pesticide use, proximity to agriculture or community-wide sprayings, paternal occupation, and the use of personal protective equipment (PPE)—and is reporting frequent enough that these types of data should be assessed in future studies? To answer these questions, we began by conducting a meta-analysis of the current literature, in which summary measures of occupation (such as census occupation code) had been used to assign pesticide exposure. We then used industrial hygienist review of occupational histories, the traditional 'gold-standard' of retrospective pesticide exposure assessment[56], to assess the relation between pesticide exposure and risk of hypospadias. By comparing the risk estimates obtained in the meta-analysis versus the risk estimates obtained with industrial hygienist review, we were able to evaluate the bias that misclassification of exposure might have created. Finally, to evaluate whether our exposure assessment was adequate, we assessed the level of detail

and frequency of reports of residential exposure to pesticides, and whether detailed information on work tasks or PPE could be collected from parents.

Protection of Human Research Subjects

This research draws from the National Birth Defects Prevention Study (NBDPS) and the Home and Occupational Exposure to Pesticides Study (HOEPS). The NBDPS received approval from the Institutional Review Boards of each of the state-based research centers (Arkansas, California, Iowa, Massachusetts, New Jersey, New York, and Texas) along with the Centers for Disease Control and Prevention (CDC). The HOEPS received approval from the Institutional Review Boards of the University of Iowa and the New York State Department of Health.

Figure 1.1. Logic Model



CHAPTER II

PESTICIDES AND HYPOSPADIAS: A META-ANALYSIS

Abstract

Objective: To use meta-analytic techniques to synthesize the findings of the current body of published literature regarding the risk of hypospadias resulting from parental exposure to pesticides.

Materials and Methods: A search of PubMed for original research published in English from January 1966 through March 2008 identified 552 studies, 90 of which were reviewed in detail. Nine studies met all study inclusion criteria. Two reviewers independently abstracted data from each included study. Any disagreements were resolved by consensus. Pooled risk ratios (PRRs) and confidence intervals (CIs) were calculated using both random and fixed-effects models, along with statistical tests of homogeneity.

Results: Elevated but marginally significant risks of hypospadias were associated with maternal occupational exposure (PRR of 1.36, CI = 1.04-1.77), and paternal occupational exposure (PRR of 1.19, CI= 1.00-1.41). Subgroup analyses provided insights into needed designs for future studies. Notably, exposure assessment using a job-exposure matrix resulted in slightly higher estimated risk than agricultural occupation in fathers; but this effect was reversed in mothers, suggesting the importance of indirect and residential pesticide exposures in this group.

Conclusions: Despite potential exposure misclassification, which would tend to diminish observed associations, the previous literature indicates a modestly increased risk of hypospadias associated with pesticide exposure.

Introduction

Hypospadias is estimated to affect 0.3-1% of live births and is characterized by an abnormal positioning of the meatus, the opening of the urethra, in males[5-7,78]. This malformation is most common among non-Hispanic whites and is least common in Hispanics[78], although recent data suggest that birth prevalence is increasing among nonwhites[7]. There is substantial unexplained variation in hypospadias rates both within and between countries[3,6,79]. Data from the U.S. and several European countries from the 1970s to 1990s showed increases in overall rates which were unlikely to be due to changes in case ascertainment[79], though more recent data seem to indicate that this trend is leveling off or at least not as widespread as once thought [3].

Because sex hormones play a strong role in fetal genitourinary development[7,80], it has been hypothesized that *in utero* exposure to endocrine disrupting chemicals could contribute to hypospadias[33,81]. Such chemicals might have an estrogenic or androgen-antagonist effect[81]. *In-utero* exposure to the synthetic estrogen diethylstilbestrol (DES) is a known risk factor for hypospadias[24-26]. Exposure to other synthetic estrogens and progestins, such as those used in oral contraceptives or assisted reproductive techniques, has also been associated with an increased risk of hypospadias in some[22,27,78,82], but not all, studies[83,84].

Several classes of pesticides have also been shown to have endocrine disrupting potential[85]. Approximately 60% of the herbicides applied in the U.S., by weight, have demonstrated endocrine disrupting or reproductive effects *in vitro* or in animal studies[86], including commercial chlorphenoxy herbicides and glyphosphate. The herbicide linuron, which binds weakly to the androgen receptor, was shown to increase rates of hypospadias

in rats, as were the dicarboximide fungicides chlozolinate, iprodione, procymidone, vinclozolin, and dichlorodiphenyldichloroethylene (DDE)[37,39,87].

Despite laboratory evidence of endocrine-disruption by several pesticide classes, few recommendations have been issued by authoritative expert panels or advisory committees. The National Research Council's comprehensive report on pesticides in the diets of infants and children specifically excluded exposure prior to the third trimester of pregnancy, remarking that "the origins of this broader concern with peri- and postnatal toxicology are inextricably rooted in experimental teratology"[88]. Experimental results in animals, they however note, may not be fully applicable to humans.

Animal models may not accurately reflect the typical human experience of pesticide exposure or metabolism of pesticides[89]. Measurement issues, however, have also plagued many human studies. Pesticide exposure might occur in many settings (occupationally, in the home, or environmentally) and be mediated by personal behaviors and the use of protective equipment[71,90,91]. Even when pesticide exposure has been well measured, the low frequency of hypospadias has resulted in several studies that were underpowered to adequately detect a clinically significant increase in risk[92-96]. Further, the potential adverse effects of adjuvants used in commercial products—which typically make up 50-60% of the total product weight—have rarely been considered[85].

This meta-analysis was conducted to systematically review the available evidence of an association between pesticide exposure and hypospadias, to provide a quantitative summary of the estimated risk, and to identify areas where further study might be needed. Although a meta-analysis cannot overcome variations in measurement, it might be able to

overcome a lack of precision and present a composite estimate of the association between pesticide exposure and hypospadias [97].

Methods

Relevant published studies from January 1966 through March 2008 were identified using Pub Med searches and reviewing references from selected citations. Search terms were included as both key words and medical subject headings (MeSH). Exposure terms used were pesticides, fungicides, fumigants, insecticides, herbicides, agriculture, agricultural chemicals, occupation, maternal occupation, paternal occupation, parental occupation, and hypospadias risk factors. Outcome terms used were hypospadias, genitourinary defects, birth defect, birth malformation, congenital malformation, congenital anomaly, and birth anomaly. The latter broad terms were selected to identify articles that examined multiple birth defects and might have reported results for hypospadias. Publications were restricted to those on humans.

Identified articles were reviewed for suitability for inclusion in the meta-analysis first by title, followed by abstract review, and then by full text review (Figure 1). Among the excluded studies were two that solely examined the effects of serum DDT/DDE [98,99]; with widespread bans on use of DDT beginning in the 1970s, these studies have decreasing relevance and potential for intervention. Two other studies that used other congenital defects as a control group were also excluded[100,101]. Associations have been suggested between pesticides and several types of birth defects[18]; inclusion of these defects in the control group might significantly bias results towards the null. Because meta-analytic methods require that all risk estimates included in calculation of the PRR

be independent from one another, two additional studies were excluded due to overlap between their study populations and other study populations[18,102]. In each instance, the study with the larger sample size or more extensive adjustment of covariates was used in calculating the final PRRs. To assess bias introduced by excluding these two studies, the PRR was recalculated using the excluded studies while removing the alternative studies from the overlapping population.

Reported risk ratios (either odds ratios or relative risks), confidence intervals (CIs), raw percentages, and raw data were abstracted, where available, by two independent reviewers (CMR and PAR). Discrepancies in abstracted data were resolved by consensus. Inclusion criteria were presentation of either a reported risk ratio estimate and CI, or sufficient raw data to calculate a risk ratio between some measure of individual pesticide exposure and risk of hypospadias. Abstracted data from studies identified as suitable for inclusion were used to conduct a meta-analysis for reported type (maternal or paternal) of occupational exposure. Where data permitted, pooled risk ratios (PRRs) were calculated for study design and exposure period.

The natural log of the risk estimate and its variance was required for each study, and was either calculated from the reported risk ratios and CIs (n=7) where available or from the raw (crude) data (n= 2). The most highly analytically-adjusted risk ratios available were used for calculation of the PRRs in the final model. Most exposure data were presented as a dichotomous variable (exposed/unexposed); multi-level exposure categories were similarly dichotomized to reduce the potential overuse of the highest exposure category.

PRRs were calculated using both fixed effects and random effects models[103,104]. Data were stratified into subgroups according to study design, exposure period, and exposure assessment. The consistency of associations across studies was assessed using statistical tests of homogeneity[103]. Where multiple risk ratios were available from a single publication, the PRRs and CIs were recalculated using each available risk ratio estimate, regardless of inclusion in the final model, in order to detect a large change in the calculated pooled PRRs and CIs associated with which risk ratio was selected from the multiple ratios presented in a particular study.

Results

Among the nine studies pooled in this meta-analysis, six of the studies evaluated both maternal and paternal exposure, two evaluated paternal exposure only, and one examined maternal exposure only (Table 1). Overall, cases and controls were most often identified from hospital or health care records, and the numbers of subjects enrolled was reported for most studies. Five studies provided risk ratios adjusted for potential confounders. Only one study attempted to stratify by severity or location of hypospadias, by evaluating all hypospadias and then restricting to those requiring surgical repair. The authors' assumption was that only more severe hypospadias (e.g. distal or proximal) would receive repair; this assumption may not be appropriate in other settings. Outcome ascertainment might have been variable between the remaining studies, depending on how cases were identified. First-degree (glanular) hypospadias may not be immediately apparent at birth or prior to circumcision; therefore these cases may not be identified based on birth records.

Pesticide exposure was most commonly assessed as self-reported agricultural occupation, though occupation recorded on the birth record or in the medical chart was also used. Two studies used a job-exposure matrix (JEM) to assess the probability of occupational exposure to pesticides, and one of these compared self-reported agricultural work to JEM-assigned exposure. The relevant period of exposure varied across all studies, with exposure at any time during the pregnancy ± pre-pregnancy period most often reported.

PRRs stratified by maternal and paternal exposure, study design, and exposure period are presented in Table 2. The studies reported homogenous PRR estimates ($p>0.40$), thus fixed and random effects were similar or identical. Only results using random effects models are shown to provide the most conservative estimates. Total maternal occupation in agriculture or other pesticide-exposed occupations was associated with an excess risk of hypospadias (PRR of 1.36; CI = 1.04 to 1.77), and total paternal occupation in agriculture or other pesticide-exposed occupations was also associated with a small excess risk (PRR 1.19; CI= 1.00 to 1.41) using all studies. Restriction of analyses to those studies with covariate adjustment produced small elevations in the PRR estimates. Self-reported maternal exposure produced similar results, but when maternal exposure was assigned by JEM the PRR dropped below unity. Results of a cohort analysis could not be calculated for paternal exposure; but the PRR for the two cohort studies of maternal exposure was elevated (PRR 1.51, CI=1.06 to 2.16). Whether using a JEM or self-reported exposure, paternal pesticide exposure produced PRRs greater than unity but non-significant. Data for evaluating the exposure period were only available for

paternal studies, but assessment of exposure in the periconceptional and spermatogenesis ± periconceptional periods produced PRRs that were non-significant and less than unity.

Discussion

This meta-analysis showed that maternal occupational exposure to pesticides or agricultural work was associated with a 36% increased risk of hypospadias overall, and paternal occupational exposure to pesticides or agricultural work was associated with a 19% increased risk of hypospadias. Though modest, these elevated risks may be clinically relevant given the enormous psychological and economic impact of hypospadias on families. The elevated risk observed in this meta-analysis may be an underestimate. Challenges in exposure assessment created the potential for misclassification in the pooled studies; this could have biased the risk ratio estimates towards the null. Given the spectrum of severity of hypospadias, there was potential for incomplete case ascertainment in some previous studies; this also may have diluted the observed overall effect of pesticide exposure.

The relationship between pesticide exposure and birth defects might be difficult to examine due to the potential critical period, which is typically defined as between gestational weeks 8 and 14 but which may also involve latent effects[7,24,26,78]. Assessment of exposure only during weeks 8-14 might be misleading when subjects are continuously employed in a given occupation, creating the illusion of a 'critical period' when the actual critical exposure might have occurred outside this time frame.

Although maternal exposure during early gestation could alter the normal fetal environment and disrupt embryogenesis[105], high levels of pesticides have also been

measured in seminal fluids[80]. Consequently, both parents' exposures might be relevant. Of the nine studies included in this analysis, three did not assess both parents' occupations[94,106,107], three assessed maternal and paternal exposures separately[108-110], two assigned exposure to both parents based on exposure by either parent, citing the familial nature of farm work[111,112], and only one considered each parent's exposure separately while adjusting for the other parent's exposure[113].

Residential pesticide exposure could not be evaluated in this meta-analysis, because only one included study evaluated probable exposure to pesticides at either work or at home[113]. Non-occupationally exposed individuals have been demonstrated to carry substantial levels of non-persistent pesticide metabolites in biological samples[114]. Of the studies identified for this meta-analysis, two used a JEM to assign probable exposure, one asked for self-reports of pesticide exposure, and the remainder relied on either documented or self-reported work in agriculture. Expert assessment of a detailed occupational history by industrial hygienists has been demonstrated to be superior to self-assessed exposure[115], and JEMs are based on many assumptions that might lead to misclassification[116]. Any exposures might be modified by practices such as the use of personal protective equipment and the frequency of handwashing[90,117-119], though these were not assessed in any of the included studies. None of the studies were able to assess risks associated with specific pesticide brands or classes. The exposure misclassification in these studies would likely be random and therefore most likely bias results towards the null.

Rather than subjectively assigning a 'quality' score to the exposure assessment used by the included studies, we examined methodologic subcategories to elucidate

potential sources of misclassification that may have caused underestimation of the true risk. Limiting analysis to studies with covariate adjustment yielded slight increases in the PRRs for all analyses, suggesting that cruder assessment might tend to bias the observed associations towards the null. The PRR obtained from cohort studies was also increased over that calculated from case-control studies; this might be due to better exposure assessment available in the cohort studies.

Publication bias, in which positive studies are more likely to be published than null studies, might have artificially inflated the calculated PRRs[97]. It is worth noting that only one of the included studies contributed a statistically significant result[111], suggesting that publication bias is unlikely for this disease-exposure relationship. Bias might also be introduced in a meta-analysis when authors make exclusion decisions. To assess potential bias introduced by authors' inclusion decisions, analyses were conducted in which the PRR was recalculated after adding back each excluded study, both singly and in combination while removing any overlapping study. These analyses found very little (<10%) change in either the magnitude or significance of the PRRs.

The results of this meta-analysis are further strengthened by their consistency with animal studies demonstrating the teratogenic potential of pesticides. The anti-androgenic effects of selected pesticides are well-defined from *in vivo* studies[120], and are important in light of the androgen-dependent normal urethral development in the fetus[6,13,33,92]. It is also possible that estrogenic or other endocrine disrupting effects of pesticides might play a role [81,121-123].

In conclusion, this meta-analysis of nine published studies demonstrated an elevated risk of hypospadias associated with maternal occupational exposure to pesticides

or agricultural work, with a suggested increased risk for paternal exposure that was stronger when analysis was restricted to those studies with covariate adjustment. Potential misclassification of exposure, which would likely attenuate the relative risk estimates, posed a large threat in all publications used in this meta-analysis. This misclassification could mask a stronger relationship between specific types of pesticides and hypospadias, since pesticides and their adjuvants comprise a chemically diverse class. Despite this potential, the 19-51% increase in risk of hypospadias defects observed here may be clinically relevant given the economic, social, and psychological burden of this malformation. Results of this meta-analysis are consistent with evidence from animal and developmental models. Future studies of pesticide exposure and hypospadias should attempt to describe pesticide exposure in terms of both quantity and type of pesticide.

Figure 2.1: Flow scheme of process used to select studies for inclusion in the meta-analysis

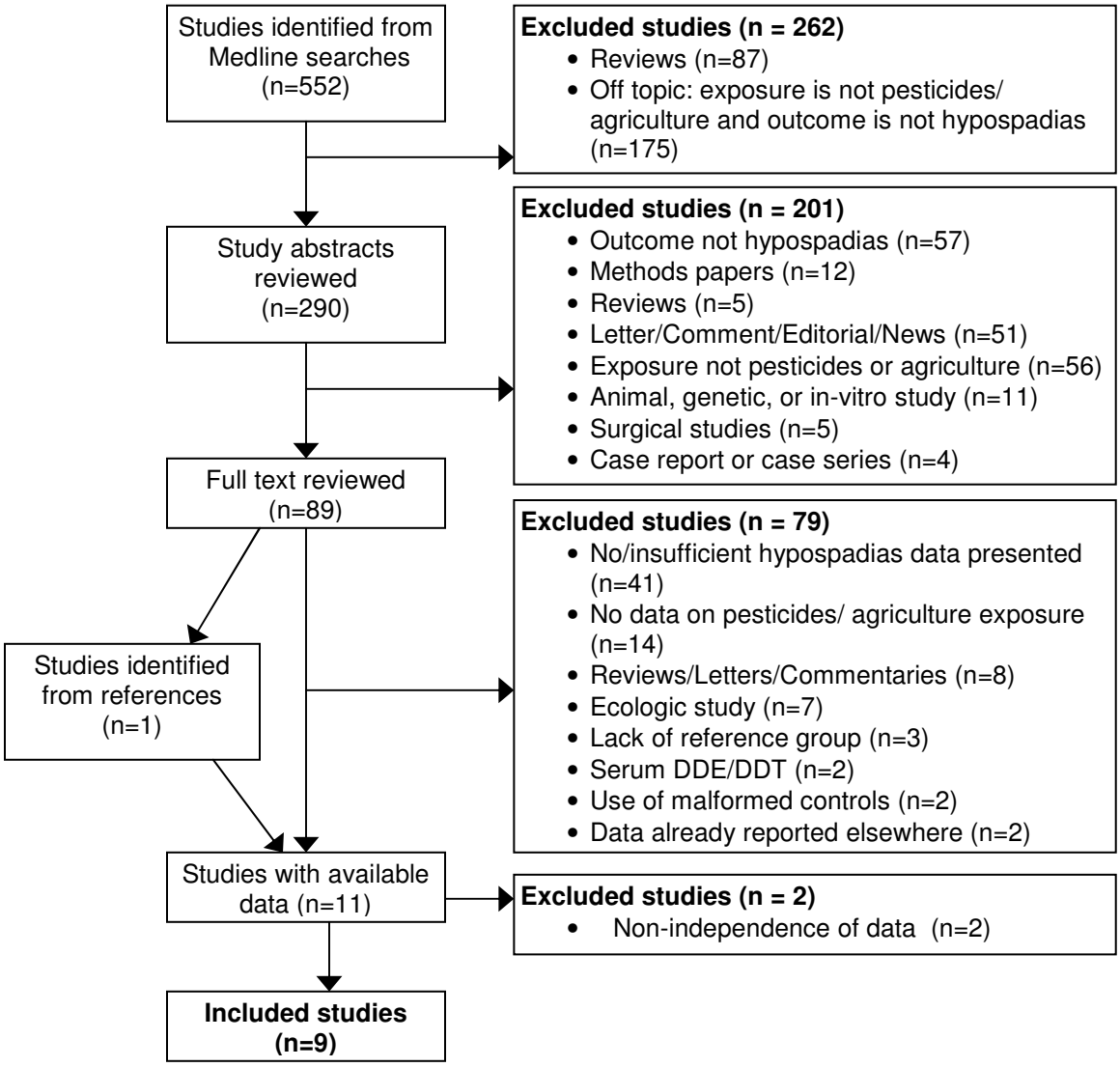


Table 2.1: Characteristics of study methods for the association between parental pesticide exposure and hypospadias

					MATERNAL EXPOSURE		PATERNAL EXPOSURE	
Lead author and year	**Study Population and Period**	**Subject Selection (Case: Control)**	**Exposure Assessment**	**Covariate Adjustment***	**Exposure Levels**	**Exposure Period**	**Exposure Levels**	**Exposure Period**
Schwartz 1986	California, USA 1975-1978	Hospital (12: 2,451)	Medical chart review	NO	Agricultural occupation vs. nonagricultural occupation	Prenatal	Agricultural Occupation vs. Nonagricultural Occupation	Prenatal
Schnitzer 1995	Georgia, USA 1968-1990	Birth register (463: 2,388)	Parental interview	NO			Farm manager or worker (yes/no)	During 2 years before birth
Olshan 1991	British Columbia 1952-1973	Birth defects registry (838 :28,830)	Occupation recorded on birth record	YES			Occupation "farm managers and workers" (yes/no)	Birth
Garcia 1999	Spain 1993-1994	Hospitals (18: 18)	Parental interview	YES	Agricultural work ever (yes/no) Agricultural work during acute risk periods (yes/no)	Ever / 1 month prior through first trimester	Pesticide application ever (yes/no) Pesticide application in critical period (yes/no)	Ever / 3 months prior through first trimester
Carbone 2006	Ragusa, Italy 1998-2002	Pediatric services (43: 203)	Parental interview	YES	Work in agriculture (yes/no) Exposure to pesticides[a]	Before/ during pregnancy	Work in agriculture[b] Exposure to pesticides[c]	Before/ during pregnancy
Pierik 2004	The Netherlands 1991-2001	Child health care centers (56: 313)	Parental interviews and JEM	NO	Probable pesticide exposure (yes/no)	First trimester	Probable pesticide exposure (yes/no)	Periconception
Weidner 1998	Denmark 1983-1992	Birth Cohort (1,345: 23,273)	Maternal interview	YES	Farmer (yes/no) Gardener (yes/no)	Occupation during year of conception	Farmer (yes/no) Gardener (yes/no)	Occupation during year of conception
Zhu 2006	Denmark 1997-2003	Birth Cohort (172: 62,432)	Maternal interview	NO	Farmer (yes/no) Gardener (yes/no)	Occupation in pregnancy and 3 months prior		
Kristensen 1997	Norway 1967-1991	Birth register (270: 253,498)	Birth record linked to agricultural census	YES	Parents farmers (yes/no) Use of tractor spraying equipment Tractor spraying + grain cultivation.	Occupation at time of pregnancy and birth	Parents farmers (yes/no) Use of tractor spraying equipment, tractor spraying + grain cultivation.	Occupation at time of pregnancy and birth

[a] Exposure to pesticides was classified as: impropable or probable.

[b] Agricultural work was classified as: no, in related occupations, work in the field, or work in a greenhouse.

[c] Exposure to pesticides was classified as : improbable, probable, or highly probable.

* Carbone et al. adjusted for birth weight, parity, mother's age, mother's education, TTP, condom use, mother's gynecological diseases, father's urogenital diseases, use of anti-abortion drugs, mother's alcohol use during pregnancy, other parent's exposure to pesticides. Garcia et al. matched cases and controls according to hospital and approximate date of birth. Kristensen et al. adjusted for year of birth, maternal age, geographical region, and parental consanguinity. Weidner et al. adjusted for year of birth and birth weight. Vrijheid et al. adjusted for year of birth, region, maternal age, social class of mother, social class of father. Olshan adjusted for parental ages, race, and outcome of previous pregnancies.

Table 2.2: Results from exposure-specific meta-analyses using a random effects model

Category	All Studies				Studies with Covariate Adjustment [a]			
	N	OR	95% CI	Homogeneity p-value	N	OR	95% CI	Homogeneity p-value
Maternal Occupation [c]	7	1.36	1.04-1.77	0.77	4	1.40	1.06-1.84	0.63
Study Design								
Case-Control	5	1.19	0.80-1.76	0.68	3	1.25	0.82-1.91	0.52
Cohort	2	1.51	1.06-2.16	0.94	1	NC [b]	NC [b]	NC [b]
Exposure Period								
Pregnancy ± prepregnancy	7	1.36	1.04-1.77	0.77	4	1.40	1.06-1.84	0.63
Exposure Assessment [d, e]								
Agricultural occupation	5	1.36	1.01-1.82	0.53	4	1.42	1.05-1.92	0.60
JEM-assessed pesticide exposure [f]	2	0.93	0.24-3.65	0.34	1	NC [b]	NC [b]	NC [b]
Paternal Occupation [g]	8	1.19	1.00-1.41	0.69	5	1.23	1.03-1.47	0.74
Study Design								
Case-Control	7	1.11	0.92-1.34	0.86	4	1.16	0.95-1.41	0.95
Cohort [b]	1	NC [b]	NC [b]	NC [b]	1	NC [b]	NC [b]	NC [b]
Exposure Period								
Pregnancy ± prepregnancy	8	1.19	1.00-1.41	0.69	5	1.23	1.03-1.47	0.74
Periconceptive [h]	2	0.85	0.30-2.44	0.85	1	NC [b]	NC [b]	NC [b]
Spermatogenesis ± periconception [i]	2	0.84	0.41-1.73	0.44	1	NC [b]	NC [b]	NC [b]
Exposure Assessment [e]								
Agricultural occupation	7	1.20	0.99-1.46	0.52	5	1.25	1.02-1.53	0.60
JEM-assessed pesticide exposure	2	1.28	0.50-3.27	0.29	1	NC [b]	NC [b]	NC [b]

OR=odds ratio; CI=confidence interval; NC=not calculated

[a] Studies 1, 2, 6, and 8 excluded from Studies with Covariate Adjustment due to lack of sufficient data.

[b] Insufficient number of studies to calculate a pooled risk estimate.

[c] Studies 2 and 3 excluded from maternal occupation analyses due to a lack of data.

[d] Study 8 assessed exposure as occupation in "gardening" and was excluded.

[e] Study 5 assessed exposure based on self-reported occupation in agriculture, and also used a JEM to assess pesticide exposure. Data from each evaluation were available, and therefore used in each of the exposure assessment categories.

[f] JEM = Job-exposure Matrix

[g] Study 8 excluded from paternal occupation analyses due to lack of data on paternal exposures.

[h] Study includes any part of the 3 months prior to conception plus early pregnancy.

[i] Exposure period assessed included the approximate time period of spermatogenesis, roughly 4 months prior to conception.

CHAPTER III

MATERNAL OCCUPATIONAL PESTICIDE EXPOSURE AND RISK OF HYPOSPADIAS IN THE NATIONAL BIRTH DEFECTS PREVENTION STUDY

Abstract

Hypospadias is a common congenital malformation among males in which the urethral opening is dorsally malpositioned. Exposure to potential endocrine disrupting chemicals, such as pesticides, has been suggested as a possible etiologic factor but previous epidemiologic studies have been inconsistent. Using data from a multicenter population-based case-control study, we examined maternal occupational exposure to fungicides, insecticides, and herbicides during the month prior to conception through the first trimester of pregnancy for 647 hypospadias case infants and 1496 unaffected male control infants with estimated dates of delivery from October 1997 through December 2002. Pesticide exposures were assigned by multiple expert raters based on a job history completed by mothers during a computer-assisted telephone interview. Multivariable logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for relevant covariates. Maternal occupational exposure to any pesticides (yes/no) was not associated with an increased risk of hypospadias (OR = 0.83, 95% CI = 0.6-1.1). Likewise, there was little or no increased risk associated with cumulative insecticide (OR = 1.09; 95% CI = 0.9- 1.3), herbicide (OR = 1.05; 95% CI = 0.9- 1.2), or fungicide (OR = 0.91; 95% CI = 0.7-1.2) exposure. These results suggest that maternal occupational pesticide exposure may not be a risk factor for hypospadias; however, further research is needed to measure other potential sources of pesticide exposure.

Introduction

Hypospadias is a congenital malformation in which the meatus, the urethral opening, is dorsally malpositioned. The severity of hypospadias varies and is classified as 1st, 2nd, or 3rd degree depending on the position of the meatus. A 3rd degree hypospadias is the most severe and difficult to correct. Overall, hypospadias occurs in 0.3-1% of live births[5-7]. Uncorrected hypospadias can cause difficulties in urination, abnormal sexual function, and adverse psychological consequences; for this reason, the majority of hypospadias must be surgically corrected[8,9]. Severe hypospadias may require several surgeries and still have an improper appearance and function[12].

Published reports of the epidemiology of hypospadias give little clue to its etiology, though the disorder is believed to have a multifactorial origin[5,16]. Family history, advancing maternal age, and intrauterine growth retardation (a low birthweight for gestational age, either idiopathic or due to multiple gestation) have all been suggested as risk factors[3,7,17,124,125]. *In-utero* exposure to the synthetic estrogen diethylstilbestrol (DES) is a recognized risk factor for hypospadias[24-26]; while DES has been recognized as a teratogen since 1971, it has raised interest in the role of estrogen in the etiology of hypospadias. Intake of other synthetic estrogens and progestins, such as those used in oral contraceptives or assisted reproductive techniques, have also been associated with increased risk of hypospadias in some (but not all) studies[27,78].

Because of their regulatory role in fetal genitourinary development, the disruption of endocrine hormones has been postulated to play a role in the development of hypospadias. Endocrine disrupting chemicals may have an estrogenic or androgen-

antagonist effect[28,33,126]. Several common classes of pesticides have demonstrated potential to disrupt endocrine hormones in *in-vitro* or in animal studies[13,127]. These include the chlorphenoxy herbicides, linuron, p,p'-dichlorodiphenyldichloroethylene (DDE), and glyphosphate; as well as the fungicides chlozolinate, iprodione, procymidone, and vinclozolin. Each of these has been shown to induce hypospadias in rats[37,39,40].

Epidemiologic studies of pesticide exposure and hypospadias, however, have presented a more conflicted picture. Few studies observed statistically significant changes in risk, with nearly equal numbers of studies having reported point estimates for reduced risks as increased risks. Part of the variability in these outcomes is likely due to small sample sizes or the inability to measure important known confounders. Instability in the point estimates between these studies may also be a caused by variability in the methods of pesticide exposure assessment. A meta-analysis of 7 non-ecologic studies on this topic noted a weak but marginally statistically significant association between probable maternal pesticide exposure and hypospadias (pooled risk ratio = 1.36, 95% CI 1.04-1.77)[42]. Five of those studies relied on farmwork as a proxy for pesticide exposure; but this type of work is associated with many other exposures, including solvents, fertilizers, and animal wastes. The remaining two studies utilized a job-exposure matrix, which is more cost-effective but less sensitive and specific than expert industrial hygienist review of detailed work histories[50,128]. Small sample sizes or limited availability of exposure data in most of these studies also restricted the evaluation of confounders and effect modifiers.

The paucity of data and conflicting results regarding pesticide exposures and hypospadias indicates the need for improved, rather than simply additional, study on this topic. The large and detailed data set available from the National Birth Defects Prevention Study (NBDPS), which includes industrial-hygienist assessments of work exposures from a detailed self-reported job history, provides an opportunity to overcome important limitations that plagued many past studies of risk factors for hypospadias.

Methods

Study Design

The NBDPS is a population-based case-control study of more than 30 different birth defects initially conducted by 8 state-based surveillance systems (Arkansas, California, Iowa, Massachusetts, New Jersey, New York, and Texas) and the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia). The details of the study design and methods have been described elsewhere[129]. Briefly, the NBDPS began in 1997 and is the largest case-control study of birth defects in the United States. Affected children and fetuses are identified through active case ascertainment by surveillance programs. Controls are a random sample of all unaffected live births in the areas covered by the state-based birth defects surveillance systems (i.e. the NBDPS catchment areas). Controls were identified from hospital delivery logs (Arkansas, California, CDC [1997-2000], New York, Texas) or birth certificate files (CDC [2001-2002], Iowa, Massachusetts, and New Jersey).

Eligible cases were defined as having a diagnosis of a second- or third-degree hypospadias, with or without chordee, (modified British Paediatric Association codes

752.606, 752.607, 752.626, or 752.627). Abstracted medical records, including operative reports when available, were reviewed at each study center by a clinical geneticist to identify potential cases. Records indicative of first-degree hypospadias (including coronal or glandular) were not collected, due to variable documentation in the medical records. Hypospadias without location information, epispadias, ambiguous genitalia with female karyotypes or without further description, and chordee alone were also not collected. All potential cases were reviewed by a second clinical geneticist to confirm that each case met eligibility and diagnosis criteria. Cases were further classified as isolated (no other major anomaly) or multiple (at least one other major unrelated anomaly was concurrent). As hypospadias is specific to male infants, controls were also restricted to male infants.

Mothers of cases and controls were required to speak either English or Spanish and have access to a telephone. Computer-assisted telephone interviews (CATIs) took place no sooner than 6 weeks and no later than 24 months after the estimated delivery date of the infant. The interview collected data on demographics, pregnancy characteristics, family history, medical and prenatal care, diet and lifestyle, and occupational history.

Study Variables

Subjects included in this analysis had an estimated due date (identified from abstracted medical records) from October 1, 1997 to December 31, 2002. During the interview, mothers were asked to provide information on any jobs worked for one month or more during the three months prior to pregnancy (B3) through the end of the pregnancy (P9 or date of infant's birth, DOB), including both full- and part-time jobs and

those worked at home, on a farm, or outside the home. For each job, the mother provided the name of the employer, a job title, descriptions of what the company produced or the service it provided, main job activities or duties, and chemicals or substances handled or machines used on the job. Each mother also provided the month and year she started and, if applicable, ended each job. Mothers also reported the usual number of days worked per week and hours worked per day[129]. Where subjects reported ending a job in the same month and year as beginning a different job, the 15th day of the month was considered the end date for the preceding job, with the latter job assumed to begin on the 16th day of the month. Otherwise, jobs were assigned as beginning on the first day of a reported month and ending on the last day of a reported month.

Though mothers reported jobs held in the 3 months prior to pregnancy and throughout the entire pregnancy, the critical period of exposure is believed to be the periconceptional period and the first trimester of pregnancy. Jobs were considered relevant to this critical period if they occurred 1 month prior to conception through the end of the first trimester (or termination of the pregnancy, if the pregnancy ended in less than 89 days from the date of conception).

The occupational history was classified by an industrial hygienist contracted by the National Institute for Occupational Safety and Health. Each job was assigned a North American Industry Classification System (NAICS)[130] and 2000 Standard Occupational Classification (SOC-2000)[131] code. These codes, combined with the job-specific information provided by the mother, were used to estimate the probability of exposure (0, <1%, 1-33%, 34-66%, 67-89%, 90% or greater) to insecticides, herbicides, and fungicides. Exposed jobs (probability >0) were also given a score for probable exposure

intensity (<1, 1-9, 10-99, or100+ mg/hr) and frequency in a typical work week (<2, 2-9, 10-19, and 20+ hours per week). Hours per week was calculated based on self-reported usual hours worked per day times the usual days per week worked. Observations exceeding 12 hrs/day and 7 days/week were individually verified for accuracy. Where hours per day or days per week worked were unknown, an 8-hour day and/or a 5-day work week were assumed (<0.9% of jobs). Cumulative work exposure incorporated probability of exposure, exposure intensity, hours worked, and number of days worked during the critical period (B1-P3), and was calculated as: (probability of exposure) * (exposure intensity) * [(typical frequency)/(40 hours per week)] * [(hours worked per week)/(7 days per week)] * (number of days worked in the exposure window).

Potential confounders and effect modifiers evaluated were maternal age, parity, previous history of miscarriages, singleton or multiple pregnancy, the gestational age and birth weight of the index infant, maternal alcohol consumption or cigarette smoking during the month prior to conception or any time during the first trimester of pregnancy, use of a folic acid-containing supplement, and maternal pre-pregnancy BMI. Maternal and paternal race, income category, maternal and paternal highest education level attained, maternal and paternal birthplace outside the U.S., language of the interview, and study center were also assessed. Since study center reflects geography, and could relate to the types of agricultural products and chemicals used, interaction terms for study center and pesticide class were assessed. A reported family history of hypospadias in a first-degree relative (father or full brother) was also examined.

Use of progesterone or progestin-containing drugs has been suggested as a risk factor for hypospadias in a previous analysis of these data[27]. It is unclear whether

progesterone itself or the reason for taking progesterone might be the true risk factor. Progesterone and its synthetic analogues are primarily taken to prevent conception, to improve fertility, or to prevent pregnancy complications; the latter indications[27] could be a consequence of a shared endocrine disrupting event that also led to development of hypospadias. As such, adjusting for the indication is inappropriate and would cause an attenuation of the observed effect. A sub-analysis was conducted to examine the effects of separately adjusting for use of progestins and indication variables. Indication variables were the use of any fertility medication or procedure by either parent (yes/no), use of any medication to prevent pregnancy complications (yes/no), and use of any contraceptive (yes/no). All drugs taken in the month prior to pregnancy through the first trimester of pregnancy, for any indication, were then classified as containing progesterone/progestins (yes/no). Because progestins used in contraceptives are typically of lower dose than those used for other indications, progestins were further categorized as being taken for contraception versus any other indication.

Subjects with missing exposure or outcome data were excluded. Since item non-response was most likely non-random, a 'missing' category was created for all categorical covariates with substantial (n >10) missing responses; this technique has been described elsewhere[132]. No continuous variables considered for inclusion in the models had substantial missing responses, thus imputation of missing continuous values was not performed.

Statistical Analysis

All analyses were conducted using SAS 9.1.3 (SAS, Cary, NC). Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine the

association between maternal occupational pesticide exposure and hypospadias. Pesticide exposure was first evaluated as a bivariate variable (any pesticide exposure vs. none), then by pesticide exposure type (none, insecticides only, herbicides + insecticides, fungicides + insecticides, fungicides+herbicides+insecticides); due to extensive overlap with insecticide exposure, it was not possible to evaluate fungicide and herbicide exposure separately. Finally, the cumulative exposure estimate (in total milligrams) to fungicides, insecticides, and herbicides was evaluated.

Maternal and paternal characteristics, pregnancy history, and pregnancy characteristics were compared between case and control pregnancies using the Pearson chi-square or, where appropriate, the Fisher exact test. Results of this analysis were used to guide logistic regression model selection. A hybrid stepwise selection scheme was used to select the most parsimonious model that adequately fit the data. We also performed a subanalysis restricting to subjects with complete data for all covariates included in the final model.

Results

Birth mothers of 646 cases and 1493 controls that completed the NBDPS interview were evaluated in this study. As shown in Table 1, case mothers were more likely than control mothers to be older, have a higher household income, be primiparous, use birth control, or use medications to prevent pregnancy complications. Both fathers and mothers of case infants were more likely to have completed college, and less likely to be of Hispanic ethnicity. Preterm birth, low birth weight, and multiple gestation were

also more common among case infants than control infants. The proportion of cases also varied by study center.

A family history of hypospadias among first-degree relatives was strongly associated with hypospadias, but this observation was based on 2 controls with a freported amily history. Consequently, the main analysis excluded all subjects with a first-degree family history of hypospadias; a sub-analysis including these subjects did not substantially alter the ORs for the main effects (data not shown).

Adjustment for use fertility treatments or procedures by either parent (yes/no), use of any contraceptive, and use of medications to prevent pregnancy complications did not substantially alter the ORs for pesticide exposure. Likewise, adjustment for use of any progesterone/ progestin caused little (<10%) change in the ORs.

Substantial item non-response was noted for paternal race/ethnicity (10.2% missing), use of fertility treatments or procedures by either parent (5.9% missing), and income (9.8% missing). Though all medication use was solicited in the interview, subjects were not asked whether the medications were taken to prevent pregnancy complications until a later version of the CATI; consequently, indication is missing for a number of women (23.0% missing). Restricting the analysis to subjects with complete response for all covariates in the model altered the ORs slightly for a 1-gram increase in cumulative insecticide, herbicide, and fungicide; but all ORs remained non-significant.

Any maternal occupational pesticide exposure (yes/no) during the month prior to conception and during the first trimester of pregnancy appeared to confer a reduced risk in the crude analysis (OR= 0.69; 95% CI= 0.6 - 0.9), but this appeared to be attributable to other differences between the exposed and unexposed populations such as maternal

age, infant birthweight, and infant gestational age. No association was observed between hypospadias and any maternal occupational pesticide exposure after adjustment for center, maternal and paternal race, maternal age, infant gestational age, birthweight, parity, and maternally-reported occupational work by anyone else in the household (adjusted OR= 0.83; 95% CI= 0.6-1.1). Most subjects who were exposed to pesticides were either exposed only to insecticides or to all three classes of pesticides (Table 2).

Cumulative maternal occupational exposure to fungicides, insecticides, and herbicides during the periconceptional period was also not associated with hypospadias, both before and after adjustment for center, maternal and paternal race, maternal age, gestational age of the infant, infant birthweight, parity, and self-reported occupational work by anyone else in the mother's household (Table 3).

Discussion

Overall, this study showed no evidence that maternal occupational exposure to pesticides during the month prior to conception through the first trimester of pregnancy was associated with increased risk of second- or third-degree hypospadias. This is consistent with two previous studies using a JEM to assign maternal pesticide exposure[109,133]; to our knowledge this is the first study to evaluate maternal occupational pesticide exposure based on expert industrial hygienists' ratings in a population-based setting. Exposure assessments performed by industrial hygienists are generally more accurate than self-reported exposure or assigning exposure based on job title alone[134]. We used multiple raters with broad industry experience and expertise in coding retrospective exposure histories, provided raters with training to calibrate their exposure

estimates, and used consensus conferences to resolve discrepant ratings; these techniques are effective in reducing misclassification errors[134,135].

Previous studies of the potential association between pesticide exposure and hypospadias have faced several methodological limitations, including small sample sizes or very limited data due to the use of existing records. In either case, their ability to assess potential confounders or effect modifiers was severely limited. With the large sample size and detailed information available in our data set, we were able to identify and adjust for relevant factors such as low birth weight, parity, and maternal age. In our bivariate analysis, we observed several associations with hypospadias that have been identified elsewhere: advancing maternal age[3], low parity[23,136,137], low birth weight[23,136,137], multiple gestation[23], family history of hypospadias[23,136,137], and parental subfertility[138-140]. Hispanic race was also associated with reduced risk of hypospadias in our analysis, as previously described[141] in the literature. Our consistency with other research in describing these associations supports our study's overall findings.

Our study also benefitted from both a large sample size and the availability of high-quality interview and outcome data. The NBDPS uses a computer-assisted telephone interview with pre-programmed probes to elicit detailed responses from subjects in a systemized manner. These steps may reduce variations in data quality[142,143]. Since creating a timeline may improve subjects' recall[144], all NBDPS subjects complete a pregnancy calendar at the beginning of their interview.

Despite the strengths of this study, these data must be interpreted cautiously. This is one of the first population-based studies to use industrial hygienist classifications of pesticide exposure. Most pesticide-exposed women in the population were only exposed

to very low pesticide levels. The lack of an association between pesticides and hypospadias at the low doses observed in our study does not indicate the lack of a relationship between higher maternal doses of pesticides and hypospadias.

Due to its inconsistency with animal data, we must consider that our negative findings may be a reflection of exposure misclassification (particularly if occupational exposure is only a small component of total pesticide exposure), rather than indicating a lack of association between maternal pesticide exposure and hypospadias. Animal studies indicate that maternal exposure to certain pesticides at critical periods in fetal development can induce hypospadias, possibly through an endocrine disruption mechanism[36,37,39,120]. Hypospadias occurs when fusion of the urogenital urethral folds is interrupted; this process is largely controlled by endocrine hormones produced by the fetal testes[7,28]. Exposure to other endocrine disrupting chemicals, such as progestins[19,27] and DES[24,25], are also associated with hypospadias.

Although industrial hygienist assessments have been shown to be more accurate than self-reports or JEMs, they are still subject to exposure misclassification[48,145]; such misclassification of exposure will generally bias results towards the null[135,146,147]. A case-control study design, though efficient for rare outcomes like hypospadias, generally precludes the collection of more accurate direct monitoring data. We were also unable to account for exposure modifiers such as the use of personal protective equipment, hygiene, or ventilation during exposed tasks. Because data were based on recall, we were also not able to examine specific pesticides or functional classes of pesticides. The broad categories of fungicides, insecticides, and herbicides each contain a diverse array of

chemicals that are unlikely to all exhibit similar properties. Less specific measures of exposure generally dilute any observed associations[76], as they tend to mix effects.

Even if maternal occupational exposure was perfectly measured, other sources of pesticide exposure may not be adequately accounted for. Mothers can be exposed to pesticides through pesticide drift or community-wide sprayings, through residential applications of pesticides in and around the home, and through carry-home exposures from other household members who may be exposed to pesticides. Self-reported work of any household member with pesticides was a relevant covariate in our analysis, suggesting that this may be an important exposure source. Paternal occupational exposure may also be linked to hypospadias risk by exerting effects during spermatogenesis. We were unable to account for paternal pesticide exposure in our study; this prevents us from examining paternally-mediated risk through sperm damage or carry-home exposures. Future work can attempt to reduce exposure misclassification by measuring all sources of potential pesticide exposure – occupational, residential, and of both parents.

Finally, though previous work has identified an increased risk of hypospadias in the sons of women who work in agriculture[42], agricultural workers experience a number of unique exposures that are not limited to pesticides, including fertilizers, diesel fuel, animal wastes, and hormones. Hormones are often mixed with livestock feed to promote growth, enhance milk or egg production, or influence the development of lean muscle mass. Given that endocrine disruption is believed to play a causal role in the development of hypospadias, potential hormone exposures among agricultural workers deserves further study. The reported associations between maternal agricultural work

and hypospadias, and our observed lack of an association between maternal pesticide exposure and hypospadias, is consistent with the hypothesis that an agricultural exposure other than pesticides may be associated with hypospadias.

In conclusion, this study found no association between low-dose maternal occupational pesticide exposure and hypospadias. This study was not able to address the potential contribution of paternal pesticide exposure to hypospadias risk, to assess the effects of non-occupational pesticide exposures, or to examine whether other agricultural exposures (such as hormones) are associated with hypospadias. We also could not assess the likelihood or magnitude of potential exposure misclassification. Future studies should focus on improving study designs to address these shortcomings.

Table 3.1: Demographic Characteristics of Hypospadias Case and Control Families, NBDPS 1997-2002.

		Cases [a] N (%)[b] N = 646	***Controls*** [a] N (%)[b] N = 1493
Maternal Characteristics	Age at conception[c]		
	Less than 20 years old	28 (4.3)	129 (8.6)
	20-24 years old	109 (16.9)	323 (21.6)
	25-29 years old	148 (22.9)	403 (27.0)
	30-34 years old	228 (35.3)	413 (27.7)
	More than 35 years old	133 (20.6)	225 (15.1)
	Education level[c]		
	Did not complete high school	39 (6.0)	144 (9.6)
	Completed high school	309 (47.8)	837 (56.1)
	Completed college or higher	298 (46.1)	509 (34.1)
	Race and ethnicity[c]		
	White, non-Hispanic	461 (71.4)	985 (66.0)
	Black, non-Hispanic	93 (14.4)	178 (11.9)
	Hispanic	54 (8.4)	245 (16.4)
	Other	34 (5.3)	82 (5.5)
	Household Income[c]		
	Less than $10,000 annually	45 (7.0)	178 (11.9)
	10,000-29,999 annually	122 (18.9)	357 (23.9)
	30,000-49,999 annually	108 (16.7)	255 (17.1)
	50,000 or more annually	332 (51.4)	532 (35.6)
	Pre-pregnancy BMI		
	<18.5 (under weight)	29 (4.5)	82 (5.5)
	18.5-24.9 (normal weight)	350 (54.2)	839 (56.2)
	25.0-29.9 (over weight)	149 (23.1)	341 (22.8)
	≥30 (obese)	109 (16.9)	202 (13.5)
	Smoked cigarettes (B1- P3)	122 (18.9)	324 (21.7)
	Drank alcohol (B1- P3)	304 (47.1)	667 (44.7)
	Used folic acid supplements, B1-P1[c]	396 (61.3)	776 (60.0)
	Parous[c]	264 (40.9)	838 (56.1)
	Used any birth control[d]	154 (23.8)	468 (31.3)
	Used progesterone/ progestin B1-P3[e]	100 (15.5)	181 (12.1)

Table 3.1 Continued

		Cases [a] N (%)[b]	***Controls*** [a] N (%)[b]
Paternal Characteristics	Education level[d]		
	Did not complete high school	234 (36.2)	649 (43.5)
	Completed high school	134 (20.7)	321 (21.5)
	Completed college or higher	259 (40.1)	493 (33.0)
	Race and ethnicity[c]		
	White, non-Hispanic	372 (57.6)	899 (60.2)
	Black, non-Hispanic	91 (14.1)	174 (11.7)
	Hispanic	45 (7.0)	205 (13.7)
	Other	41 (6.3)	94 (6.3)
Infant Characteristics	Gestational age[c]		
	Very preterm (<32 weeks)	55 (8.5)	23 (1.5)
	Preterm (32-36 weeks)	125 (19.3)	106 (7.1)
	Term (37-45 weeks)	466 (72.1)	1364 (91.4)
	Birthweight[c]		
	<2500g	175 (27.1)	89 (6.0)
	≥ 2500g	465 (72.0)	1395 (93.4)
	Plurality[c]		
	Singleton	588 (91.0)	1444 (96.7)
	Two or more	56 (8.7)	47 (3.1)
	Conceived during/after fertility treatments or procedures [c]	81 (12.5)	66 (4.4)
	First-degree relative with hypospadias[c]	30 (4.6)	2 (0.1)
	Study Center[c]		
	Arkansas	74 (11.5)	183 (12.3)
	California	23 (3.6)	172 (11.5)
	Iowa	18 (2.8)	220 (14.7)
	Massachusetts	108 (16.7)	220 (14.7)
	New Jersey	237 (36.7)	185 (12.4)
	New York	54 (8.4)	181 (12.1)
	Texas	17 (2.6)	144 (9.7)
	CDC/Atlanta	115 (17.8)	188 (12.6)

B1-P3 = during the one month prior to conception through the third month of pregnancy. B1-P1 = during the one month prior to conception through the first month of pregnancy

[a] Subcategory totals may vary due to missing or incomplete data.

[b] Percentages may not total 100 because counts of rounding.

[c] p-value <0.0001

[d] p-value <0.001

[e] p-value <0.05

Table 3.2: Reported Patterns of Exposure for Pesticides among Case and Control Mothers, NBDPS 1997-2002

Exposure, 1 month prior to conception through 1st trimester	**Hypospadias** N[a] (%[b])	**Controls** N[a] (%[b])
Total pesticide exposure[a]		
Any	157 (24.3)	473 (31.7)
None	489 (75.7)	1020 (68.3)
Pesticide exposure, by class		
None	489 (75.7)	1020 (68.3)
Herbicides only	0 (0)	2 (0.1)
Insecticides only	115 (17.8)	326 (21.8)
Fungicides only	0 (0)	0 (0)
Herbicides + insecticides	9 (1.4)	28 (1.9)
Herbicides + fungicides	0 (0)	0 (0)
Insecticide + fungicides	2 (0.3)	5 (0.3)
Herbicides + insecticides + fungicides	31 (4.8)	112 (7.5)
Mean pesticide exposure, in grams, among exposed	Mean (SD)	Mean (SD)
Herbicides	2.00g (5.5g)	1.15g (4.4g)
Insecticides	0.56g (2.7g)	0.44g (2.4g)
Fungicides	0.96g (2.9g)	0.98g (4.6g)

[a] p-value <0.001

[b] Percentages may not total due to rounding.

Table 3.3: Crude and Adjusted Odds Ratios per 1- Gram Increases in Pesticide Exposure, by Class, NBDPS 1997-2002

	per 1.0 gram increase in cumulative exposure	
Cumulative Exposure, 1 month prior to conception through 1st trimester	**Crude OR (95% CI)**	**Adjusted OR (95% CI) [a]**
Insecticides	1.02 (0.9-1.2)	1.09 (0.9- 1.3)
Fungicides	0.89 (0.7-1.1)	0.91 (0.7-1.2)
Herbicides	1.07 (0.9-1.2)	1.05 (0.9- 1.2)

[a] adjusted for exposure to all other pesticide classes, birthweight, use of medications to prevent pregnancy complications, parity, maternal and paternal race, maternal age, infant birthweight and gestational age, center, use of any birth control, self-reported work of any other household member with pesticides, and an interaction term for maternal race and center.

CHAPTER IV

THE FEASIBILITY OF COLLECTING RETROSPECTIVE HOME AND OCCUPATIONAL PESTICIDE EXPOSURE DATA FROM BOTH PARENTS IN A STUDY OF BIRTH DEFECTS

Abstract

A number of studies have examined occupational pesticide exposure and risk of birth defects; many cited poor or missing information on paternal occupation exposure, missing information on personal protective equipment and behaviors that may modify occupational exposure, and a lack of information on residential pesticide exposures as important sources of unmeasured confounding. We conducted a pilot case-control study among participating families in the National Birth Defects Prevention Study to solicit detailed residential, maternal occupational, and paternal occupational pesticide exposure information. Subjects were randomly assigned to receive either a written questionnaire or a computer-assisted telephone interview. The impact of the survey instrument on participation, recruitment, and response patterns was also evaluated. Survey responses were examined to describe pesticide use during the six months prior to pregnancy and in each trimester of pregnancy. Mothers and fathers assigned to the questionnaire were significantly more likely to complete participation than those assigned to the interview, though for each instrument, participation rates for mothers were higher than those for fathers. Residential pesticide use during the six months prior to pregnancy and during pregnancy was fairly common among control mothers: 45% reported that their home had been treated for insect or rodent pests; 47% reported that their lawn or garden had been treated for weeds or insect pests; 16% used a lawn service; 26% reported that a pet had

been treated for fleas, ticks, or mites (including flea and tick preventives); 17% reported community-wide sprayings for pests; and 16% reported that their workplaces were treated for pests. Case mothers were more likely to report that their home had been treated of insect or rodent pests (50%) or that a pet had been treated for fleas, ticks, or mites (36.5%). Most mothers were able to recall which pests their homes, pets, or lawns were treated for; the time period the treatments occurred; the treatmenr type; and who performed the treatment. Our results suggest that collection of information on residential pesticide use is feasible, and the impact of residential pesticide use on birth defects risk should be assessed in future studies.

Introduction

Birth defects occur in approximately 1 of 33 births in the United States, and are a major cause of both infant mortality and morbidity[148]. Genetic or other known causes account for only about 30% of birth defects. In recent years, a great deal of research has attempted to examine whether environmental or occupational exposures, such as pesticides, could play an etiologic role in the development of birth defects.

Most studies of pesticide exposure have evaluated only occupational exposure, often based on job titles that are taken from birth records or self-report[80,111,149,150]. Occupational title, as reported on birth records, is often not consistent with self-reported occupation during parental interviews; this produces exposure misclassification[151]. People with a farm-related job title are also most frequently considered pesticide-exposed; however, a recent study shows that farm-related job title is a poor measure of actual pesticide exposure[43].

Even if job title were a suitable proxy for pesticide exposure, it lacks information on the quantity or types of pesticides to which an individual might be exposed. Exposure may be affected by personal behaviors, such as the frequency of hand washing and the use of protective equipment[64,72,152]. Pesticide exposure also does not occur strictly through work. Residential pesticide applications, such as treating for insects in the home or weeds in the lawn, as well as drift from aerial pesticide sprayings, water contamination, or soil contamination can contribute to pesticide exposure[59,60,70,153-155]. Few studies have examined combined pesticide exposure from multiple sources, possibly due to a belief that these exposures are insignificant or that collecting such information would be infeasible.

In order to better assess exposure to pesticides, the Home and Occupational Exposure to Pesticides Study (HOEPS) was designed as a pilot follow-up project to the National Birth Defects Prevention Study (NBDPS). The purpose of this pilot was to: 1) determine whether suitable participation from both parents could be obtained in a detailed follow-up study of residential and occupational pesticide exposure, and whether the administration of either a questionnaire or interview influenced this participation; 2) determine whether parents would be able to recall details of residential and occupational pesticide exposure and modifiers (e.g. use of protective equipment) of such exposure; and 3) determine whether residential pesticide use during pregnancy and the months prior to conception was frequent enough to warrant more substantial consideration in future studies of pesticide exposure.

Methods

National Birth Defects Prevention Study

Parents of case or control infants who had completed participation in the NBDPS were recruited. The NBDPS is an ongoing population-based case-control study of birth defects conducted in nine states in the US. Details of the NBDPS have been described elsewhere[129]; in brief, cases of over 30 major structural birth defects were identified through ten population-based active surveillance systems. Unaffected controls were identified from birth records or medical records in the same catchment areas of the surveillance registries. Mothers of cases and controls completed telephone interviews of their medical and reproductive histories, lifestyle factors, and pregnancy characteristics. Buccal cell samples were collected from the index child and birth parents.

Home and Occupational Exposure to Pesticides Study

Data collection for the HOEPS included administration of a survey instrument either by self-administered questionnaire or by computer-assisted telephone interview to mothers and fathers (if available) of index children. The survey (both questionnaire and interview) covered four time periods: the six months prior to conception (B6) and the first (T1), second (T2), and third (T3) trimesters of pregnancy. For both parents, a personalized pregnancy calendar was provided to assist them in relating calendar dates to these time periods, along with a pesticide card that listed common pesticides by both chemical and trade name. The maternal survey included a module to record residential pesticide treatments during B6-T3 to determine if the pesticide was used to: treat areas in or around the home for insect or rodent pests; treat the lawn or garden for weeds or pests; treat pets for fleas, ticks, or mites; treat houseplants for insect or fungal pests; or use a

lawn service. If mothers responded 'yes' to any of these questions, they were asked to report the target pest(s); how many times the treatments had been performed during B6-T3; who performed the treatments (herself, her baby's biological father, or someone else); what type of treatment was used (e.g. a spray, bomb, bait or trap); the name of the specific product that was used, if known; and whether the product was stored inside the home. The maternal survey also included a module on environmental pesticide exposure, with questions on community sprayings for pests and which type of pest was targeted and proximity of maternal residence to a field where commercial crops were grown, orchard, or commercial livestock confinement area. In addition, the maternal survey asked when, if any months, the father resided in the same residence with the mother during B6-T3.

The maternal survey also included a module on occupational pesticide exposure, with questions asking if the mother had performed agricultural work or completed other work, such as providing pest control or handling cut flowers, which might bring her into contact with pesticides. If such tasks had been performed, the survey then asked for the frequency and duration of exposure, the time periods in which exposure occurred, and whether any personal protective equipment or special hygiene was used. A hygiene section inquired when and where a mother washed herself and her clothing after working in areas with potential pesticide exposure. She was also asked whether her workplaces had been treated for any pests during B6-T3, and whether she experienced any symptoms or medical treatments she believed might be related to pesticide exposure. The paternal survey included the occupational module only.

Eligible mothers were randomized to receive either a questionnaire or interview and were recruited between June 2007 and June 2008. Though the NBDPS began in

1997, recruitment for the HOEPS was restricted to deliveries that occurred between January 1, 2004 and December 31, 2005 to reduce the potential for recall bias. Cases selected were limited to those from the Iowa and New York centers diagnosed with either hypospadias or a heart defect, as previous studies have suggested an association between pesticides and these defects[42,156], and mothers of these cases were not enrolled in other ongoing follow-up studies. We attempted to recruit one control per case. Because only mothers participate in the NBDPS interview, recruitment for the HOEPS was initiated with mothers; biological fathers were not contacted for the HOEPS unless permission was given by mothers.

Each mother was initially approached by mail to inform her of the HOEPS and to invite them to participate. Mothers were asked to return an address and telephone update sheet in a pre-addressed, stamped envelope. Regardless of whether the address update form was received, two weeks after the initial letter mothers were sent an introductory packet that included financial compensation; for those randomized to the questionnaire arm, a questionnaire and pre-addressed stamped envelope were included. Up to three reminder call cycles (the subject was called in the morning, afternoon, and evening and a message was left only at the last call attempt; the call cycle ended if the subject was contacted during any of the call attempts) and two reminder letters were sent two weeks apart. If no response was received by the mother following these contacts, a letter was then sent to all non-respondents, informing them that it would be their last contact. For mothers assigned to the questionnaire, a copy of the questionnaire and pre-addressed stamped envelope were enclosed with the final decision letter. If study staff never had direct contact with a mother, the decision letter was sent via certified mail. Following the

return of the questionnaire or completion of the telephone interview, mothers were asked for permission to contact the biological father of the index child. If permission was given, contact information for the father was requested. Fathers were recruited with the same protocol used for mothers.

Statistical Analysis

All analyses were conducted with SAS 9.1.3 (SAS Cary, NC). Descriptive analyses examined participation rates by case or control status and type of survey instrument administered; statistical significance ($p<0.05$) of the difference in participation rates was calculated using the Fisher's Exact test. The mean number of days between mailingof the introductory packet and return of a completed questionnaire or completion of an interview was calculated for both mothers and fathers. The mean number of contacts conducted after mailing the introductory packet was also calculated, stratified by instrument; a contact was defined as either a letter being sent or a call cycle being completed. Unpaired T-Tests were used to test whether the difference in mean number of days or number of contacts was statistically significant between instruments ($p<0.05$).

The frequency of self-reported residential pesticide use to treat the home, pets, houseplants, and lawns or gardens; community sprayings; workplace treatments for pests; and work with pesticides was examined for cases and controls. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated to assess the association between measures of pesticide exposure and risk of hypospadias or any heart defect. For treatment of pets, the home, and lawn or gardens, the OR was further evaluated by comparison of those exposed during the T1 to those unexposed at any time during B6-T3. ORs and CIs were calculated for cell counts >5.

Results

Overall, 268 of 394 (68%) eligible mothers participated, including 138 case mothers (participation rate of 72.6%) and 130 control mothers (participation rate of 63.7%). A current address or telephone number was not identified for almost 13% of eligible subjects (Table 4.1). Mothers assigned to the questionnaire were significantly more likely to participate than those assigned to the interview (75.1% vs. 60.9%, respectively), but were less likely to give permission to contact the father (75.0% vs. 85.8%,respectively, Table 4.3). Fathers assigned to the questionnaire, however, had significantly higher participation rates than those assigned to the interview (81.4% vs. 53.4%, table 4.1). Mothers who reportedly lived with the child's biological father during B6-T3 were more likely to give permission to contact the father, compared to mothers who did not live with the father at all during that time period (84.9% vs. 62.5%, data not shown).

For cases, participation rates were similar between those assigned to the questionnaire (76.8%) and those assigned to the interview (68.4%); participation rates for control mothers varied considerably between the questionnaire (73.5%) and interview (53.9%). Despite these gains in participation, the distribution of other demographic characteristics (maternal age, race, education, and family income) was similar between mothers who participated in HOEPS and all eligible mothers (Table 4.2). Cohabitating with the biological father was during the entire period of B6-T3 was reported by 83.3% of mothers assigned to the interview and 88.5% of mothers assigned to the questionnaire.

Mothers who completed the questionnaire finished participation, on average, almost 11 days earlier than those who completed the interview; maternal participants assigned to the questionnaire also required 3 fewer reminder contacts (Table 4.3). These differences between questionnaire and interview participants were statistically significant ($p < 0.05$). The time between the birth of the index child and completion of HOEPS was similar between mothers and fathers, averaging of 2.7 years for mothers (range: 1.5-4.6 years) and 2.9 years for father (range: 1.7-4.6 years) (Table 4.3).

In both the interview and questionnaire, mothers were able to report 'do not know' for questions on residential pesticide use. Maternal participants who completed the questionnaire were slightly more likely than those who complete to interview to report 'do not know' for residential pesticide applications (data not shown), but overall reporting of 'do not know' was infrequent (range <1 – 5%). Despite having the option to report 'unknown,' the majority of respondents (> 95%) reporting residential pesticide uses were able to recall the type of pest that was treated for, who performed the treatment (mother, father, or someone else) during which time period the treatment was performed (B6, T1, T2, and T3), the pesticide application method, and whether the pesticide used for that treatment was stored in their homes. Recall of the precise chemical product used was much lower.

Maternal occupational exposure to pesticides was reported infrequently in the HOEPS. Less than 5% of mothers reported working on a farm, orchard, or with livestock There was no significant difference in rates of cases between farmers and non-farmers (table 4.4). Residential pesticide uses during B6-T3 were fairly common. Among control mothers, during B6-T3 44.6% reported that their home had been treated for insect

or rodent pests, almost 47% reported that their lawn or garden had been treated for weeds or insect pests, 16.2% reported using lawn service, and 26.2% reported that a pet had been treated for fleas, ticks, or mites (including flea and tick preventives). In addition, 16.9% of control mothers reported community-wide sprayings for pests and 16.2% reported treatment of their workplace for pests (Table 4.3).

Treating the home for pests or treating a pet for fleas, ticks, or mites during B6-T3 was more common among mothers of children with a heart defect or hypospadias, though these totals were based on small numbers. Use of a pesticide treatment on a pet anytime during B6-T3, compared to not using a treatment during B6-T3, was associated with an increased and marginally significant risk of having a child with a heart defect (OR = 1.75; 95% CI = 1.0-3.0). Use of a pesticide treatment in or around the home during the first trimester, compared to not using a treatment during B6-T3, was also associated with a slightly increased but non-significant risk of having a child with a heart defect (OR = 1.29; 95% CI = 0.8-2.1), though this number was not statistically significant. Use of a lawn company, treating one's lawn or garden for weeds or pests, living in a community that conducted pest sprayings, having a workplace that treated for pests, or working on a farm or orchard were not associated with an increased risk of having a child with a heart defect (table 4.4).

Discussion

Overall, participation of mothers was fair in the HOEPS. Fathers who were contacted had reasonable participation rates, however they were also lost to participation when the mother could not be located or refused participation in HOEPS or when the

mother did not give permission to contact the father; overall the participation rate of potentially eligible fathers was 37.3%. Information provided from fathers directly, however, may be more accurate than those reported by proxy. These results show that obtaining information from fathers, in addition to mothers, is feasible though participation rates may be lower. Mothers who resided with the index child's biological father six months before and during the index pregnancy were more likely to give permission for researchers to contact the father; this could be an important source of selection bias in evaluating paternal exposures, and also has implications for determining how much carry-home exposure fathers contribute. Future studies should collect information on whether or not parents resided together during the critical exposure period for stratification purposes.

Compared to those assigned to the interview, participation rates were higher for those assigned to the questionnaire and fewer reminder calls or letters were needed before subjects completed participation. The largest difference in participation rates occurred among control subjects. Response rates for the questionnaire were higher in most demographic groups (by maternal age, income, education, and race) compared to the interview, but the distribution of participating subjects across those demographic groups were similar to the distribution of eligible subjects. This suggests that recruited subjects are likely representative of eligible subjects, and that the increased response to the questionnaire did not occur within a specific demographic subgroup. Future studies that evaluate the use of a mailed questionnaire should consider whether the increased cost of postage and printing for a questionnaire will be offset by savings in recruitment costs and increased participation rates.

Fewer mothers completing the questionnaire gave permission to contact fathers, compared to mothers completing the interview; this was not explained by underlying differences in the percentages of mothers who reported living with their child's biological father during the entire time period of B6-T3. Narrative comments by some mothers indicated that the father had assisted in completing the mother's questionnaire, and the mothers felt that the father had nothing further to add (particularly if he were unexposed to pesticides, in her opinion). Sending both questionnaires to the mother at the same time, and allowing her to pass the questionnaire to the father immediately if they share a residence, might increase paternal participation.

This study provides a valuable comparison of survey instruments. Since subjects were randomized to the interview or questionnaire, self-selection effects or confounding is unlikely to affect the comparison of the two instruments. This is supported by non-significant differences on several maternal demographic characteristics (age, race, education, and income) between mother assigned to each instrument, and between those eligible and those who completed participation in the HOEPS.

In assessing pesticide exposure, this study displays several strengths. Participation rates were fair, and mothers participating in the HOEPS were representative of the eligible mothers according to age at conception, income, race, and education level. Our use of a pregnancy calendar may have improved recall. Case status was based on abstracted medical records, and was reviewed by a clinical geneticist. Data obtained via the survey instruments accounted for both residential and occupational pesticide exposures, and included self-reported information on pesticide exposure from biological fathers.

There are several important limitations to consider in this study, however. Overall participation rates may not be fully applicable to all study populations, as participants in the HOEPS had already completed participation in the NBDPS and may have been more likely to participate in further studies; however all HOEPS subjects were subject to the same enrollment condition (participation in NBDPS) and recruited in the same manner, regardless of which survey instrument they were assigned to. Recall bias cannot be ruled out as an explanation for the elevated odds ratios; but if recall bias resulted in cases over-reporting pesticide exposure, we would have expected to see elevated odds ratios for all pesticide treatments. Similar rates of pesticide treatments on lawns and gardens or at work, between cases and controls, suggests that recall bias is an unlikely explanation for the elevated ORs we observed. Due to low occupational exposure reported in this pilot by mothers, and low participation rates by fathers, we were unable to examine the effects of hygiene practices or personal protective equipment at work for either parent. We were also unable to examine the joint effects of residential and occupational pesticide exposure from both parents; such an analysis could shed better light on the relationship between pesticide exposure and certain types of birth defects. The HOEPS also had only a small number of hypospadias cases, which prevented calculation of odds ratios for pesticide exposure and hypospadias. Due to small sample sizes, we were not able to adjust for relevant confounders, such as age and parity, in our comparison of heart defect rates among women who used reported using pesticides in or around their homes, lawns and gardens, or on their pets, and heart defect rates among those women who did not.

Overall, however, the frequency of self-reported residential pesticide use by women during their index pregnancy and the six months prior to conception was substantial, and could be an important source of exposure misclassification in studies that only examine occupational exposure. Although this study is based on very small numbers, the elevated odds ratios observed for some types of residential exposure (treating pets or the home) merit further examination. Expanding the HOEPS to other centers, for additional birth years, could provide an enhanced sample size and greater statistical power.

Table 4.1: Participation of Families Contacted for the HOEPS Pilot

	Control (n = 204)	Hypospadias Cases (n = 25)	Heart Cases (n = 165)
	N (%)	N (%)	N (%)
Participation Status of Mother			
Hard refusal	18 (8.8)	5 (20.0)	10 (6.1)
Soft refusal with contact	20 (9.8)	5 (20.0)	12 (7.3)
Soft refusal no contact	2 (1.0)	0 (0)	1 (0.6)
Unlocatable	33 (16.2)	1 (4.0)	17 (10.3)
Complete	130 (63.7)	14 (56.0)	124 (75.2)
Excluded	1 (0.5)	0 (0)	1 (0.6)
	Control (n = 94) [a]	Hypospadias Cases (n=12) [a]	Heart Cases (n=109) [a]
Participation Status of Father [a]			
Hard refusal	8 (8.5)	0 (0)	4 (3.7)
Soft refusal with contact	16 (17.0)	3 (25.0)	31 (28.4)
Unlocatable	3 (3.2)	0 (0)	2 (1.8)
Complete	66 (70.2)	9 (75.0)	72 (66.1)
Excluded	1 (1.1)	0 (0)	0 (0)

[a] Fathers are only eligible if the index child's mother participated and gave permission to contact the father

[b] The denominator for father's participation status is the number of eligible fathers

Table 4.2: Demographics of Eligible and Participating Mothers, HOEPS Pilot, by Survey Instrument.

	Recruited to HOEPS		Participated in HOEPS	
	Questionnaire (n=197)	Interview (n=197)	Questionnaire (n=148)	Interview (n=120)
Case status	N (%)	N (%)	N (%)	N (%)
Cases	95 (48.2)	95 (48.2)	73 (49.3)	65 (54.2)
Controls	102 (51.8)	102 (51.8)	75 (50.7)	55 (45.8)
Center				
Iowa	124 (62.9)	123 (62.4)	92 (62.2)	78 (65.0)
New York	73 (37.1)	74 (37.6)	56 (37.8)	42 (35.0)
Mother's age at conception				
<20 years	18 (9.1)	15 (7.6)	10 (6.8)	7 (5.8)
20-24 years	49 (24.9)	45 (22.8)	36 (24.3)	24 (20.0)
25-29 years	57 (28.9)	60 (30.5)	42 (28.4)	40 (33.3)
30-34 years	49 (24.9)	57 (28.9)	39 (36.4)	34 (28.3)
35 years or older	24 (12.2)	20 (10.2)	21 (14.2)	15 (12.5)
Mother's race/ethnicity [a]				
White, non-Hispanic	171 (88.1)	176 (89.3)	134 (90.5)	108 (90.0)
Other race	23 (11.9)	21 (10.7)	13 (8.8)	12 (10.0)
Mother's education				
Less than 12 years, or did not complete high school	13 (6.6)	11 (5.6)	6 (4.1)	5 (4.2)
12 years, completed HS, or equivalent	38 (19.3)	43 (21.8)	27 (18.2)	25 (20.8)
1-3 years college, or completed technical college	70 (35.5)	68 (34.5)	53 (35.8)	37 (30.8)
4 years or more of college; bachelor's degree or higher	76 (38.6)	75 (38.1)	62 (41.9)	53 (44.2)
Family's annual income [a]				
<$20,000	37 (19.4)	42 (21.9)	24 (16.2)	22 (18.3)
$20,000 - $39,999	53 (27.8)	52 (27.1)	39 (26.4)	32 (26.7)
>$40,000	101 (52.9)	98 (51.0)	80 (54.1)	63 (52.5)

[a] Missing data not shown; percentages do not equal 100 due to missing data

Table 4.3: Participation and Recruitment of Subjects to HOEPS pilot, by Survey Instrument

	Interview (n =197) *N (%)*	Questionnaire (n =197) *N (%)*	Total (n =394) *N (%)*
Mother's participation rate [a]	120 (60.9)	148 (75.1)	268 (68.0)
Mother gave permission to contact father [a,b]	103 (85.8)	111 (75.0)	214 (79.9)
Father's participation rate [a,c]	55 (53.4)	92 (81.4)	147 (68.1)
	Mean	*Mean*	*Mean*
Time to completion (participants only)			
Mother [a]	36.5 days	25.9 days	30.6 days
Father [a]	35.3 days	36.7 days	36.2 days
Number of recruitment contacts needed			
Mother [a]	6.4 contacts	3.4 contacts	4.7 contacts
Father [a]	5.9 contacts	2.8 contacts	4.2 contacts
Time Elapsed from child's birth to completion of HOEPS			
Mother	999 days	999 days	999 days
Father	1082 days	1045 days	1059 days

[a] p-value <0.05

[b] the denominator for permission rate is the number of mothers who participated

[c] among fathers that project staff were given permission to contact

Table 4.4: Maternal exposure to pesticides, B6-T3, in the HOEPS Pilot

	Control (n =130) N (%)	Case (n =138) N (%)	OR (95% CI) Exposure during B6-T3 [a]
Treated home for pests	58 (44.6)	69 (50.0)	1.29 (0.8-2.1)
Treated pets for fleas, ticks, or mites	34 (26.2)	50(36.5)	1.75 (1.0-3.0) [b]
Treated houseplants for pests	1 (0.8)	1 (0.8)	NA[c]
Used a lawn company to treat the lawn	21 (16.2)	18 (13.0)	0.78 (0.4-1.7)[d]
Treated the lawn or garden	61(46.9)	64 (46.4)	1.00 (0.6-1.7)[e]
Community sprayed for pests	22 (16.9)	25 (18.1)	1.15 (0.6-2.2)
Mother's workplace treated for pests	21 (16.2)	20 (14.5)	0.82 (0.4-1.7)
Mother worked on a farm or orchard	6 (4.6)	6 (4.3)	0.87 (0.3-3.0)

[a] calculated only among heart defect cases and controls; rates could not be calculated separately for hypospadias cases due to the small number of hypospadias in the pilot group. Reference group is no exposure during B6-T3.

[b] p-value <0.05

[c] not calculated due to low cell counts

[d] adjusted for home use of lawn treatments

[e] adjusted for use of a lawn company

CHAPTER V

LESSONS LEARNED AND FUTURE DIRECTIONS IN THE STUDY OF PESTICIDE EXPOSURE AND BIRTH DEFECTS

Often the people most highly exposed to a given substance are those who work with it, and the most common group of workers exposed to pesticides are farmers. Previous work examining pesticide exposure and risk of hypospadias has therefore often used farm work or agricultural job title as a proxy for actual pesticide exposure[42]. Using this proxy measure, the net effect of these studies has shown a small increase in risk of hypospadias associated with both paternal and maternal work in agriculture. Because agricultural job title has been shown to be a poor proxy for pesticide exposure[43], we would expect considerable exposure misclassification in previous studies. Generally, this misclassification would cause the observed OR to be biased towards the null, or show no effect. The persistence of a small effect, despite this heavy bias towards the null, suggested that a much larger effect could exist. We hypothesized that with better pesticide measurement, we might observe a stronger effect.

To better measure maternal occupational pesticide exposure, we used expert rater-assigned exposure to pesticides, based on detailed job history reports. This approach is widely accepted as more accurate than self-report or job title[56,142]. Additionally, this approach allowed us to add an estimated quantity of exposure. We also achieved greater specificity, by being able to distinguish between broad classes of pesticides – herbicides, fungicides, and insecticides. Though still imperfect measures, we hoped that these methods would reduce exposure misclassification and allow us to more accurately

estimate the OR. We found, however, no association between maternal occupational pesticide exposure and hypospadias.

Our negative findings could be explained in multiple ways. The first is that there is no association between maternal pesticide exposure and hypospadias. The excess risk of hypospadias observed in agricultural workers could be due to other unique exposures among those workers—fertilizers, diesel exhaust, zoonotic infections, and hormones added to feed to improve production or promote growth[157,158]. The latter may be the most plausible risk factor, given the current data suggesting a strong role for endocrine disruption in the etiology of hypospadias. Another possible explanation for our negative finding is that no association exists between maternal pesticide exposure and hypospadias *at the levels of pesticide exposure observed in our study*. The NBDPS is a population-based study, and most workers considered exposed to pesticides in that population had very low levels of cumulative occupational pesticide exposure. A true, dose-dependent relationship could exist between pesticides and hypospadias, but the pesticide 'dose' in the population sample was too near the baseline population dose to determine any relationship. Examining pesticide exposure and hypospadias risk in a highly exposed occupational group could yield different results. The would be consistent with animal data, which have shown that hypospadias can be induced by exposing dams to pesticides[36,37,39,159]. Finally, we can interpret our negative finding as a reflection of inadequate measurement of total maternal pesticide exposure, since our study only collected occupational data. Misclassification of exposure could occur if we did not collect adequate data to allow our expert raters to correctly classify exposure, or due to a lack of information on non-occupational pesticide exposure. Paternal exposure to

pesticides could also play a role in the etiology of hypospadias; the increased risk of hypospadias among women who work in agriculture could be explained by carry-home paternal exposures.

These explanations are not mutually exclusive, and could all be partially valid. To determine whether the final explanation (exposure misclassification) was viable, or even feasible to address, we conducted a pilot study to evaluate the feasibility of collecting detailed occupational information (including information on PPE) from mothers and fathers, as well as residential pesticide exposure. Most studies of birth defects associated with paternal exposure have relied on either job title from birth certificates or from paternal job reports provided by maternal respondents, although each approach is of questionable validity[151,160,161]. Residential pesticide exposure during pregnancy and preconception periods has not been well measured; it was unknown whether exposures were frequent enough to potentially account for substantial exposure misclassification, or whether it was feasible to collect details of residential pesticide exposure in a retrospective study.

In our pilot study, we found that it was feasible to obtain occupational information directly from fathers, though lower response rates might limit the generalizability of our results. We also found that residential pesticide use was very frequent during pregnancies and the six months preceding pregnancy. Though mothers were generally not able to recall the precise chemicals used in residential applications, they were able to report the target organism, application method (e.g. spray, bait, or granules), who applied the treatment, how many times the treatment was applied, and the approximate time periods during which these applications occurred. From these data, pesticide experts

could narrow down the potential pesticide types to which a subject was exposed and perhaps identify targets for further research. Future work that quantifies the amount of pesticide exposure involved in these types of non-occupational activities may allow us to better calculate total pesticide exposure, or at least to understand what range of background pesticide levels we could expect in non-occupationally exposed workers.

As we move forward in exploring the relationship between maternal pesticides and hypospadias—or other birth defects—we recommend that future studies account for residential exposure; such an assessment is feasible and appropriate. Those who work with pesticides may still comprise the most heavily exposed to pesticides, but our results suggest that they are not the most frequently exposed to pesticides. Failure to account for both occupational and residential exposure—particularly among those with low occupational exposure—could cause substantial exposure misclassification. With this knowledge, we can attempt to measure all sources of pesticide exposure more accurately in future studies.

In future studies, biological monitoring for pesticide metabolites may offer a more efficient and accurate method to measure pesticide exposure[162-168]. Biological measures account for the actual absorbed dose from all exposure routes, and are generally very specific. It does not require subjects to remember the precise pesticide product used; indeed, it does not rely on personal recall at all. Biomonitoring studies, however, are not without challenges. Researchers must identify a suitable metabolite or marker of exposure, identify the optimal biological sample materials (e.g. blood, hair, urine, or some other body fluid/tissue) for the metabolite or marker, and be able to collect such samples during the appropriate time period[164,165,169]. Obtaining the optimal biological

sample may be too invasive and unacceptable to research subjects, or too expensive to sample. The best biological sample will vary depending on the pesticide being targeted, since each has unique chemical properties[162,163,170-172]. The time window for collecting a biomarker will also vary; some biomarkers are persistent and last in bodily tissues long after exposure occurs, while others may only be detected for a few days after exposure occurs.

Unless biomarkers of pesticide exposure are extremely persistent (with a half-life measured in years) a biomarker study of pesticide exposure and birth defects would require the assembly of a cohort of women who are pregnant or who may become pregnant, so that biological samples may be collected at relevant time intervals. Because birth defects are somewhat rare, it would be necessary to assemble an extremely large cohort study. This is an expensive and time-consuming endeavor, but would allow an objective measurement of the exposure.

In the absence of such data, the literature can move forward by working towards better exposure assessment. Occupational exposure assessment has made considerable strides, largely through direct monitoring studies. Studies of direct monitoring of non-occupational pesticide exposures are scarce, but provide valuable tools for predicting an absorbed dose associated with tasks, such as petting a dog that is wearing a flea collar.

Through the HOEPS, we identified areas for future study. We observed an elevated risk for having a child with either a hypospadias or heart defect among mothers whose homes were treated for pests before or during pregnancy, or whose pets were treated for pests during these time periods. Although the sample size was too small for us

to fully explore these potential associations, future (larger) studies may be able to better elucidate these relationships.

The HOEPS gave us valuable insight into effective tools to recruit subjects. We were able to critically evaluate whether a questionnaire or computer-assisted telephone interview performed better in terms of response rate, recruitment time, and cost. We obtained a higher response rate with less recruitment effort using the questionnaire; such information may guide other epidemiological studies in deciding which mode of survey administration to implement.

In conclusion, though we have not definitively answered the question of whether pesticide exposure is associated with hypospadias risk, we have identified important areas for future research and, through our explorations, identified important issues regarding pesticide exposure assessment. We hope that these findings will help researchers improve measurements in future studies, so that we can better understand and prevent birth defects.

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