HHS Public Access

Author manuscript

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2015 November 01.

Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2014 November ; 100(11): 877–886. doi:10.1002/bdra.23293.

Maternal Periconceptional Occupational Pesticide Exposure and Neural Tube Defects

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Abstract

Background—Adverse associations between maternal pesticide exposure and neural tube defects (NTDs) have been suggested but not consistently observed. This study used data from the multisite National Birth Defects Prevention Study to examine associations between maternal periconceptional (1 month preconception through 2 months postconception) occupational pesticide exposure and NTDs.

Methods—Mothers of 502 NTD cases and 2950 unaffected live-born control infants with estimated delivery dates from 1997 through 2002 were included. Duration, categorical intensity scores, and categorical frequency scores for pesticide classes (e.g., insecticides) were assigned using a modified, literature-based job-exposure matrix and maternal-reported occupational histories. Adjusted odds ratios (aORs) and 95% confidence intervals were estimated based on fitted multivariable logistic regression models that described associations between maternal periconceptional occupational pesticide exposure and NTDs. The aORs were estimated for pesticide exposure (any [yes/no] and cumulative exposure [intensity × frequency × duration] to

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The authors declare they have no actual or potential competing financial interests.

any pesticide class, each pesticide class, or combination of pesticide classes) and all NTD cases combined and NTD subtypes.

Results—Positive, but marginally significant or nonsignificant, aORs were observed for exposure to insecticides + herbicides for all NTD cases combined and for spina bifida alone. Similarly, positive aORs were observed for any exposure and cumulative exposure to insecticides + herbicides + fungicides and anencephaly alone and encephalocele alone. All other aORs were near unity.

Conclusion—Pesticide exposure associations varied by NTD subtype and pesticide class. Several aORs were increased, but not significantly. Future work should continue to examine associations between pesticide classes and NTD subtypes using a detailed occupational pesticide exposure assessment and examine pesticide exposures outside the workplace.

Keywords

birth defects; neural tube defects; occupational exposure; pesticides; pregnancy

Introduction

Neural tube defects (NTDs) affect 3000 pregnancies annually in the United States (CDC, 2004). NTDs occur during neurulation, 21 to 28 days postconception, when the neural tube fails to close. Few risk factors have been consistently associated with NTDs, although both environmental (i.e., noninherited) and genetic factors are thought to play a role in their development (Detrait et al., 2005).

In particular, folic acid intake remains the most widely recognized environmental risk factor for NTDs, with randomized controlled trials showing that folic acid supplementation might prevent at least half of all cases (MRC, 1991; Czeizel and Dudas, 1992). Maternal prepregnancy type 1 or type 2 diabetes (Becerra et al., 1990) and maternal use of antiepileptic medications during the days of pregnancy when neurulation occurs (Lammer et al., 1987) have also been associated with NTDs. Additional environmental risk factors for NTDs are less well-studied in humans, and studies have shown mixed results.

One such risk factor is maternal pesticide exposure, which can occur in both the home and the workplace. Residential pesticide exposure can occur through air, water, or food contamination, as well as from home and yard/garden use. Occupational pesticide exposure can occur directly through mixing of chemicals, equipment loading, application, equipment clean-up/repair, or disposal of empty containers, or indirectly through handling of contaminated crops or foods. Occupational exposure can also occur, typically at relatively lower doses, in workplaces that are treated with pesticides. Generally, residential pesticide exposure is expected to be common and to occur at low doses, whereas occupational pesticide exposure is less common, but may occur at higher doses (Garcia, 1998). Positive associations with NTDs have been reported for maternal residential exposure to pesticides (White et al., 1988; Garry et al., 1996; Shaw et al., 1999; Rull et al., 2006; Brender et al., 2010; Yang et al., 2014) and among agricultural populations for conceptions that occurred during the growing season when pesticides are applied (Garry et al., 1996; Kristensen et al.,

1997). The strength of reported associations between maternal occupational pesticide exposure and NTDs has been small to moderate, and associations have not consistently been observed (Zhang et al., 1992; Blatter et al., 1996; Blanco Munoz et al., 2005; Lacasana et al., 2006).

Previous studies of occupational pesticide exposure and NTDs have been limited by sample size (151 cases and 151 controls [Blanco Munoz et al., 2005 and Lacasana et al., 2006], 55 cases and 66 controls [Blatter et al., 1996], 101 cases and 1875 controls [Zhang et al., 1992]) and the exposure assessment. Due to sample size limitations, few analyses were stratified by NTD subtype. Because NTD subtypes may have different etiologies (Mitchell, 2005), combining NTD subtypes may mask underlying associations. Blanco Munoz et al. (2005) reported an odds ratio of 6.5 (95% confidence interval [CI], 1.4–29.6) for occupational pesticide exposure and anencephaly, an estimate considerably higher than those reported from other studies that combined NTD subtypes (Nurminen et al., 1995; Shaw et al., 1999). Different exposure assessment methods may also have contributed to inconsistent findings. Many studies used only selfreports of any exposure (yes/no) to occupational pesticides (Zhang et al., 1992; Blatter et al., 1996; Blanco Munoz et al., 2005; Lacasana et al., 2006).

Industrial hygienist review of occupational histories is thought to result in less exposure misclassification as compared to self-reports or use of job title alone (Fritschi et al., 1996; Bhatti et al., 2011; Peters et al., 2011). Only two studies of pesticide exposure and NTDs were identified that used maternal occupational histories reviewed by an industrial hygienist (Nurminen et al., 1995; Shaw et al., 1999); however, neither study had sufficient numbers of pesticide-exposed individuals to estimate risks precisely. Also, no studies of maternal occupational pesticide exposure were found that stratified exposure by specific pesticide type. It is likely that not all pesticides disrupt neural tube development; thus, testing for associations of all pesticides combined may bias findings toward the null. Because of the large number of pesticide formulations and overlapping exposure to pesticide types, it is difficult, particularly with a case–control design, to link exposure to a specific pesticide with NTDs; however, exposure assessment may be improved with stratification by pesticide class (e.g., insecticides, fungicides, or herbicides).

To remedy some of the limitations in previous work and extend the knowledge base, data from the National Birth Defects Prevention Study (NBDPS), a multisite population-based case–control study, were used to examine the relationships between maternal periconceptional occupational pesticide exposure and NTD subtypes in offspring.

Methods

STUDY POPULATION, RECRUITMENT, AND INTERVIEWS

The goal of the NBDPS is to investigate genetic and environmental risk factors for more than 30 major structural birth defects. At each site, clinical geneticists reviewed data abstracted from medical records to determine case eligibility based on NBDPS case definitions, confirmatory diagnostic procedures, and exclusion criteria (e.g., known chromosomal or single gene disorders). NBDPS methods are described briefly below;

additional detail is published elsewhere (Yoon et al., 2001; Rasmussen et al., 2003). Each participating site obtained institutional review board approval for the NBDPS.

For this analysis, eligible cases and controls were those with estimated dates of delivery from October 1, 1997 through December 31, 2002 ascertained at eight sites (Arkansas [AR], California [CA], Iowa [IA], Massachusetts [MA], New Jersey [NJ], New York [NY], Texas [TX], and the Centers for Disease Control and Prevention [CDC]/Metropolitan Atlanta). Eligible case deliveries were live births (all sites), fetal deaths (AR, CA, CDC, IA, MA, NY [since year 2000], TX), and elective terminations (AR, CA, CDC, IA, NY [since year 2000], TX) diagnosed with an NTD subtype (modified British Pediatric Association codes): anencephaly and craniorachischisis (740.020 and 740.100), spina bifida (741.001–741.999), or encephalocele (742.000–742.090) (BPA, 1979). NTD cases were classified by subtype and by phenotype, either isolated (no additional major birth defects) or multiple (one or more additional, unrelated defects in a separate organ system) (Rasmussen et al., 2003). Eligible control deliveries were live births without a structural birth defect and randomly selected by each site using birth certificates (AR [since April 2000], CDC [since June 2001], IA, MA, and NJ) or hospital records (AR [through March 2000], CA, CDC [through May 2001], NY, and TX).

Mothers of eligible NTD case and control infants were recruited to complete a telephone interview no earlier than 6 weeks and, in an effort to minimize recall error, no later than 24 months after the estimated date of delivery of the infant. The telephone interview collected information about maternal infectious, chemical, physical, nutritional, and behavioral exposures, as well as information on maternal occupation(s) from 3 months before conception through delivery. Occupational data included company name and description, job title and description, month/ year the job started and ended, average hours worked per day, and average number of days worked per week.

For this analysis, NTD case and control infants were restricted to those whose mothers reported employment during all or part of the relevant periconceptional period (1 month preconception through 2 months postconception); nonemployed mothers were excluded rather than classified as unexposed in an attempt to eliminate confounding by employment status and factors associated with employment status as shown in previous NBDPS analyses (Rocheleau et al., 2013). In addition, case and control mothers who reported use of folate antagonist medications (aminopterin sodium, carbamazepine, cholestyramine resin, methotrexate, oxcarbazepine, pyrimethamine, sulfasalazine, triamterene, trimethoprim, phenytoin, primidone, phenobarbital, valproate sodium) during the periconceptional period and/or were diagnosed with type 1 or type 2 diabetes before or during the index pregnancy were excluded due to suggested associations between these exposures and NTDs in the NBDPS or other studies (Hernández-Díaz et al., 2001; Correa et al., 2008; Matok et al., 2009).

EXPOSURE ASSESSMENT

Based on an extensive literature review and reported dermal measurements of pesticide exposure, the National Cancer Institute previously developed over 25 job- and task-exposure matrices (TEMs) to assign exposure to the classes of herbicides, insecticides, and fungicides

by job title or task, decade, type of application, and protective equipment (Samanic et al., 2008). An industrial hygienist from the Battelle Center for Public Health Research and Evaluation (Seattle, WA), in collaboration with the National Institute for Occupational Safety and Health, modified these TEMs using expert judgment to reflect subject-specific job descriptions provided in the NBDPS maternal interview reports to assign estimated pesticide exposure variables. She assigned a probability score (0, >0–33%, 34–66%, 67–89%, and ≥90%) for maternal occupational exposure to each of three classes of pesticides (insecticides, herbicides, and fungicides) for each maternal job reported. She also estimated the average number of hours (<2, 2–10, 11–19, or >19) exposed to each pesticide class based on a 40-hr work week. Lastly, she estimated a dermal intensity, or dose, representing exposure to each pesticide class by categories of quantitative levels (<1, 1–9, 10–99, and ≥100 mg/hr).

For each maternal job reported, the hours worked per week were calculated based on reported typical hours worked per day multiplied by the typical number of days per week worked. For reported jobs with missing hours per day and/or days per week (<1% of all jobs), an 8-hr day and/or a 5-day work week were assumed. The exposure assessment team verified individual maternal reports that exceeded 12 hours per day and 7 days per week for accuracy and imposed a 16-hr limit per day to 28 jobs.

For this analysis, mothers who had a probability of 0 for occupational pesticide exposure for all reported jobs during the periconceptional period were classified as unexposed and were used as the referent exposure group. Mothers who had a probability >0 for at least one reported job during the periconceptional period were classified as exposed. For each job with a probability >0, the assigned exposure intensity, estimated hours exposed per week, and maternal reports of typical hours worked per week were used to estimate cumulative occupational exposure to each pesticide class during the 1 month before conception through the first 2 months of pregnancy as follows:

$$\text{estimated exposure intensity, mg/hr} \times \frac{\text{estimated hours exposed per week}}{\text{40hours per week}} \times \frac{\text{reported typical hours worked per week}}{\text{7days per week}} \times \text{n}$$

The cumulative exposure to each pesticide class was then classified as 0, less than the median cumulative exposure in control mothers (>0 to <50%), or at or above the median cumulative exposure in control mothers (≥50%).

COVARIABLES

Relevant covariables evaluated included maternal age at delivery (<21, 21–25, 26–30, 31–35, >35 years), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), education (<12, 12, 13–15, >15 years), gravidity (0, 1, 2, ≥3), prepregnancy body mass index (BMI) (<18.5, 18.5–24.9, 25–29.9, ≥30 kg/m^2), periconceptional smoking (yes/no), and NBDPS site. Additionally, dietary food folate intake (<600 or ≥600 μg, daily) and periconceptional use of vitamins and/or supplements containing folic acid (yes/ no) were examined. Dietary food folate intake was assigned using the responses to the food frequency items in the NBDPS about usual intake during the year prior to conception, (Willett et al.,

1985, 1987), as well as reports of breakfast cereals consumed during the 2 months following conception. Dietary food folate intake was estimated using the reported food frequencies, the standardized serving size on which a question item was based, and the United States Department of Agriculture National Standard Reference 16-1 (USDA, 2004). Also, each vitamin and dietary supplement reportedly used during the periconceptional period was assessed to determine whether it contained folic acid. Mothers were classified into those who took vitamins and/or supplements containing folic acid during the periconceptional period and those who did not.

STATISTICAL ANALYSIS

Analyses were conducted using SAS software, version 9.2 (SAS Institute, 2007). Using the chi-square test, descriptive analyses of selected infant and maternal characteristics were conducted comparing each NTD subtype to control infants. Crude odds ratios (cORs) and 95% CIs were estimated to examine associations between maternal periconceptional occupational exposure (yes/no) to any pesticide class and all NTD cases combined and each NTD subtype. Similarly, cORs and 95% CIs were estimated to examine the associations between cumulative exposure (0, >0 to <50%, ≥50%) to any pesticide class and all NTD cases combined and each NTD subtype. When the number of exposed case mothers was at least five, analyses were also conducted by individual (insecticides only, herbicides only, and fungicides only) and combined (insecticides + herbicides, insecticides + fungicides, herbicides + fungicides, and insecticides + herbicides + fungicides) pesticide classes.

Results of the descriptive analyses were used to construct the most parsimonious multivariable logistic regression model for all NTDs combined and any (yes/no) maternal periconceptional occupational pesticide exposure. Covariables included in the preliminary model were those that were associated ($p < 0.10$) with any pesticide exposure and/or outcome; age, BMI, and food folate were entered as continuous variables. Beginning with the least significant covariable (highest *p*-value), backward variable selection was used to exclude covariables from the preliminary model based on the Wald chi-square statistic. Covariables for which exclusion from the model resulted in a change of greater than 20% in the parameter estimate(s) of pesticide exposure were returned to the model. If the pesticide exposure parameter estimate(s) changed by less than 20%, the fit of the full model and reduced model were compared using the log-likelihood ratio test. Covariables for which the log-likelihood ratio test was significant ($p < 0.05$) were returned to the model and those that were not significant remained excluded. Based on the final multivariable logistic regression model, adjusted odds ratios (aORs) and 95% CIs were estimated between any maternal periconceptional occupational exposure to pesticide or pesticide class and also between any maternal cumulative exposure to pesticide or pesticide class and NTDs (all NTD cases combined, NTD subtypes, and NTD phenotypes). Multivariable regression models were built for each exposure-outcome combination; however, the resultant models were not materially different. As such, the model used for exposure to any pesticide and all NTD cases combined was applied in all analyses; aORs for the main effect did not materially change. Lastly, subanalyses estimated aORs by: phenotype (isolated/multiple); family history of NTDs (yes/no); and restriction to sites that included live births, fetal deaths, and elective terminations. Because few jobs were considered to have a low probability of

exposure (>80% of jobs had probability scores ≥67%), a subanalysis stratified by probability score was not conducted.

Results

Interview data were collected from mothers of 958 (68% of eligible) NTD case and 5008 (66% of eligible) control infants; 521 case and 2997 control mothers met the criterion of employment during all or part of the relevant periconceptional period. Of these, 18 case and 47 control interviews were excluded as follows: incomplete maternal interviews (cases n = 1; controls n = 4), maternal diagnosis of type 1 or type 2 diabetes before or during the index pregnancy (cases n = 7; controls n = 15), or maternal periconceptional exposure to folic acid antagonists (cases n = 10; controls n = 28). To improve homogeneity of NTD subtypes, the maternal interview for one NTD case diagnosed with multiple NTD subtypes was also excluded, leaving 502 NTD cases (126 with anencephaly, 5 with craniorachischisis, 310 with spina bifida, and 61 with encephalocele) and 2950 controls eligible for analysis. Where pesticide exposure information was missing, inconsistent, or could not be evaluated based on the information provided (cases = 6; controls = 20), mothers were excluded from calculations of the odds ratio estimates.

Compared with control mothers, case mothers were more likely ($p < 0.05$) to be younger, Hispanic, less educated, have 3 or more pregnancies, or to be obese; the proportion of NTD cases also differed among sites (Table 1). When stratified by subtype, differences between control mothers and spina bifida mothers tended to parallel those for all NTD cases combined; mothers of cases with anencephaly or encephalocele only differed from control mothers by the proportion of infants per site.

As shown in Table 2, 30% of control mothers and 33% of case mothers were evaluated as being occupationally exposed to any pesticide. Of these, 81% of control mothers and 80% of case mothers held jobs with a probability of exposure that was ≥67% (data not shown); most of this exposure was to insecticides only. Compared with control mothers, the estimated median cumulative exposure to insecticides was higher for mothers of anencephaly and spina bifida cases. Compared with control mothers, the estimated median exposure to fungicides was higher for mothers of spina bifida cases, whereas the estimated median cumulative exposure for each of the three pesticide classes was near equal or lower for mothers of encephalocele cases.

In crude analyses, statistically significant, positive associations were observed for insecticides+herbicides with all NTD cases combined and with spina bifida, as well as for insecticides+herbicides+fungicides with all NTD cases combined and with anencephaly (data not shown). The final adjusted regression model included maternal education, prepregnancy BMI, and site. The aORs for insecticides + herbicides tended to parallel those for crude analyses, although aORs for insecticides+herbicides+fungicides were positive for anencephaly or encephalocele but not for all NTDs combined (Table 3). A significantly increased aOR was only seen for spina bifida (insecticides+herbicides). Sample sizes precluded analyses of exposure to herbicides only; however, numbers allowed estimation of odds of exposure to each pesticide class if exposure to the other remaining classes was

considered irrelevant (e.g., exposure to herbicides regardless of exposure to insecticides and/or fungicides). Using this approach, the aORs were near unity for mothers exposed to insecticides, fungicides, or herbicides (data not shown).

Examination of maternal cumulative exposure (0, >0 to <50%, ≥50%) to any pesticide and also to insecticides only produced aORs near unity for all NTD cases combined and for the NTD subtype groups examined. The aORs for maternal cumulative exposure to insecticides + herbicides were positive, but not significant, for all NTD cases combined and for spina bifida cases, as were the aORs for maternal cumulative exposure to insecticides + herbicides + fungicides for anencephaly and encephalocele. Dose-dependent effects were only observed for anencephaly. The majority of jobs assigned as exposed to insecticides + herbicides provided services to buildings (janitorial, landscaping, or pest control; 40.7%) or traveller accommodation (26.4%). Jobs considered exposed to insecticides + herbicides + fungicides were most commonly in food/drink service places (26.7%), grocery or specialty food stores (18.7%), and agriculture (crop or animal production, or support activities for these; 16.7%) (data not shown).

For each pesticide class examined, aORs for isolated NTDs were similar to those for all NTDs combined (isolated + multiple) (data not shown). Among mothers of NTD cases with multiple defects (n = 61), cumulative exposure at or above the median (≥84.375 mg) to any pesticide was positively associated (aOR: 2.1, 95% CI, 1.2–3.9) with an NTD compared with those with no exposure. In addition, sub-analyses by family history of an NTD (yes/no) produced no appreciable difference in the aORs. Furthermore, restriction of analyses to sites which included live births, stillbirths, and elective terminations also produced little change in the aORs (data not shown).

Discussion

Maternal periconceptional occupational exposure to any pesticide (yes/no) or insecticides only was not associated with all isolated NTD cases combined or individual NTD subtypes. Generally, small increases or decreases in risk were observed in adjusted analyses. For mothers occupationally exposed to insecticides + herbicides, the aORs for all NTDs combined were increased, but not statistically significant; the aOR was significantly increased for spina bifida alone. The aORs were increased, but nonsignificant, for anencephaly alone and encephalocele alone in mothers exposed to insecticides + herbicides + fungicides; however, other than spina bifida, none of the associations exhibited an exposure-response relationship. Results for maternal cumulative exposure to a pesticide class were generally similar to those for any exposure to that class, and with the exception of anencephaly only, dose-dependent effects were not observed. Lack of dose-dependent effects for other subtypes may reflect the relatively small difference in doses between the lower and higher exposed groups; most jobs considered exposed in this population-based sample of women had very low intensity and/or frequency exposure to pesticides.

The general lack of significant associations and dose-response relationships may indicate that periconceptional maternal occupational exposure to pesticides at the level observed in this population does not increase the risk of NTDs. The positive associations observed were

supported by some (Blatter et al., 1996; Blanco Munoz et al., 2005; Lacasana et al., 2006) but not all (Zhang et al., 1992; Nurminen et al., 1995; Shaw et al., 1999) previous studies in humans. Two of the three previous studies that reported significant associations (Blanco Munoz et al., 2005; Lacasana et al., 2006) were limited to cases diagnosed with anencephaly and analysis of job titles (comparing those employed in agriculture to those not employed in agriculture), adjusted for selected maternal covariables. The odds ratios reported in each study (aOR: 6.5, 95% CI, 1.4–29.6; aOR: 4.57, 95% CI, 1.1–20.0) were markedly higher than those reported in the current study. The third study (Blatter et al., 1996) was also restricted to mothers employed in agriculture and reported an association for spina bifida adjusted for selected maternal covariables (aOR: 3.4, 95% CI, 3.0–9.0). These increased odds may be due to higher or more frequent exposures, greater prevalence, or different pesticides than experienced by the study subjects in our population.

Mechanisms by which pesticides have been linked with NTDs are poorly understood, although animal studies provide some insights. Methyl carbamate, chlorpyrifos, and other organophosphate insecticides are cholinesterase inhibitors, and in animal studies, cholinesterase inhibition has been shown to alter cell proliferation and differentiation during neurulation (Slotkin, 2004). In particular, chlorpyrifos has also been shown to lead to excessive neuroepithelial cell death during neurulation in rat embryos (Roy et al., 1998), possibly resulting in too few cells for neural tube closure.

The current case–control study of maternal occupational exposure to pesticides and NTDs used data from one of the largest U.S. population-based studies of birth defects; comparison of selected characteristics of control mothers who participated in the NBDPS and all live births in the same geographic areas showed that NBDPS control participants tended to be similar to all live births (Cogswell et al., 2009). Also, assignment of maternal occupational pesticide exposure was based on a substantial literature review and industrial hygienist review of maternal reports of occupational histories. This approach is considered the “gold standard” for exposure assessment where direct monitoring data are unavailable, unlike previous studies which used maternal self-reports only or job title only. Exposure assessment through industrial hygienist review is expected to decrease the risk of exposure misclassification compared with use of self-report or job title alone (Fritschi et al., 1996). Two prior studies of NTDs and pesticide exposure that used industrial hygienist review to assess exposure (Nurminen et al., 1995; Shaw et al., 1999) were limited by sample size. In addition, no prior studies reported results from stratifying exposure by pesticide class. Lastly, NBDPS data permitted attempts to estimate aORs by phenotype, restricting to families without a family history of NTDs, and restricting to sites that included live births, fetal deaths, and elective terminations.

Despite the efforts made to improve exposure and outcome classification compared with previous studies, limitations remained. Our sample of exposed NTD case and control mothers was larger than previous studies, yet small sample sizes limited some analyses for NTD subtypes, phenotypes, pesticide classes, and exposure probability categories, resulting in either imprecise odds ratios or the inability to calculate odds ratios for specific exposure strata. Post-hoc power calculations, given the sample size and a 30% rate of maternal occupational pesticide exposure in controls, showed the minimum detectable odds ratio for

an association between any maternal occupational exposure (yes/no) and all NTDs combined was 1.39 (90% power, alpha 0.05 two-sided test). Also, although NBDPS interview data permitted adjustment for several possible covariables, data on pesticide exposure outside the workplace—such as using pesticides at home, residing on a farm, or residing near land in crop production— were not collected and could not be considered analytically. Residential exposure to pesticides is generally expected to be at lower levels (Garcia, 1998) than occupational exposure; however, it is possible that residential exposure exceeded some of the occupational exposures reported here. For example, residential exposure to pesticides could have impacted the association being tested if exposure to the particular type of residential pesticides was associated with NTDs and with the occupational exposure (as might be expected, for example, of agricultural workers who also live near the fields where they work). Additionally, cumulative occupational exposure in this population was generally low; therefore, results may not be generalizable to those in occupations with relatively high exposure intensities, such as agricultural populations. Furthermore, we could not identify specific pesticides, and we did not evaluate paternal exposure to which the mothers may have been exposed. Lastly, overlap among exposure to pesticide classes was high, limiting analysis to individuals exposed to insecticides only, insecticides + herbicides, and insecticides + herbicides + fungicides.

In summary, the association between specific NTDs and maternal periconceptional occupational exposure to pesticides was investigated within a large, case–control study. Results showed slightly increased, but not statistically precise, associations between exposure to some pesticide classes and some NTD subtypes. Although some previous studies corroborate these results, and efforts were made to improve on the design of these studies, findings should be interpreted cautiously due to limitations in sample size and exposure classification. Also, a large number of associations were tested, and significant findings may be due to chance. Future studies should aim to increase the sample size, particularly in less prevalent subtypes, of mothers exposed to herbicides or fungicides. Investigators should consider characterizing associations in highly exposed populations, such as agricultural populations, rather than examining the general population as was done here. In addition, future studies should aim to use similar detailed exposure assessment methods, while also collecting data on exposure to pesticides outside of the workplace.

Acknowledgments

We thank the Principal Investigators and study staff at each NBDPS site for their contributions to study design and data collection. We also thank the California Department of Public Health Maternal Child and Adolescent Health Division for providing data. We are grateful for the expert administrative assistance provided by Ms. Julee Bormet in the preparation of this manuscript. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the California Department of Public Health.

This work was funded by grants (5U01DD000492 and 1U01DD001035) from the Centers for Disease Control and Prevention, which participated in study design and data collection. Coding of drug information in the National Birth Defects Prevention Study used the Slone Drug Dictionary under license from the Slone Epidemiology Center of Boston University. Nutritional analysis was supported by grant no. DK56350 from the Nutrition Epidemiology Core of the University of North Carolina Clinical Nutrition Research Center. Exposure assessment was supported by contract 200-2000-08018 from the National Institute for Occupational Safety and Health.

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TABLE 1

Selected Characteristics of Infants and Birth Mothers for Controls and Neural Tube Defect Cases, National Birth Defects Prevention Study, 1997–2002

	Controls (*n* = 2,950)	All NTDs (*n* = 502)	Anencephaly (*n* = 126)	Spina Bifida (*n* =310)	Encephalocele (*n* = 61)
Characteristic	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**
Infant					
Sex					
Male	1,468 (49.8)	230 (48.4)	43 (40.6)	158 (52.0)	26 (42.6)
Female	1,480 (50.2)	245 (51.6)	63 (59.4)	146 (48.0)	35 (57.4)
Phenotype					
Isolated	NA	441 (87.9)	114 (90.5)	280 (90.3)	44 (72.1)
Multiple	NA	61 (12.2)	12 (9.5)	30 (9.7)	17 (27.9)
Mother					
Age at delivery (years)[c]					
<21	361 (12.2)	70 (13.9)	19 (15.1)	43 (13.9)	8 (13.1)
21–25	664 (22.5)	112 (22.3)	27 (21.4)	70 (22.6)	14 (23.0)
26–30	845 (28.6)	160 (31.9)	39 (31.0)	103 (33.2)	16 (26.2)
31–35	746 (25.3)	96 (19.1)	31 (24.6)	50 (16.1)	15 (24.6)
>35	334 (11.3)	64 (12.7)	10 (7.9)	44 (14.2)	8 (13.1)
Race/ethnicity[c]					
Non-Hispanic White	1,910 (64.9)	295 (58.8)	72 (57.1)	188 (60.6)	32 (52.5)
Non-Hispanic Black	373 (12.7)	57 (11.4)	16 (12.7)	31 (10.0)	10 (16.4)
Hispanic	521 (17.7)	124 (24.7)	31 (24.6)	74 (23.9)	17 (27.9)
Other	139 (4.7)	26 (5.2)	7 (5.6)	17 (5.5)	2 (3.3)
Education (years)[c]					
<12	295 (10.0)	72 (14.3)	17 (13.5)	44 (14.2)	8 (13.1)
12	729 (24.7)	152 (30.3)	38 (30.2)	94 (30.3)	19 (31.1)
13–15	885 (30.0)	145 (28.9)	32 (25.4)	97 (31.3)	16 (26.2)
>15	1,039 (35.2)	133 (26.5)	39 (31.0)	75 (24.2)	18 (29.5)

Characteristic	Controls (*n* = 2,950)	All NTDs (*n* = 502)	Anencephaly (*n* = 126)	Spina Bifida (*n* =310)	Encephalocele (*n* = 61)
	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**
Gravidity[c]					
0	899 (30.5)	137 (27.3)	36 (28.6)	82 (26.5)	17 (27.9)
1	922 (31.3)	148 (29.5)	41 (32.5)	90 (29.0)	17 (27.9)
2	606 (20.5)	101 (20.1)	23 (18.3)	66 (21.3)	12 (19.7)
≥3	522 (17.7)	116 (23.1)	26 (20.6)	72 (23.2)	15 (24.6)
Pre-pregnancy BMI (kg/m^2)[c]					
Underweight (<18.5)	150 (5.2)	17 (3.5)	6 (4.9)	9 (3.0)	2 (3.5)
Normal weight (18.5–24.9)	1,662 (57.5)	249 (51.4)	73 (59.4)	140 (46.7)	34 (59.6)
Overweight (25–29.9)	652 (22.6)	104 (21.5)	28 (22.8)	67 (22.3)	9 (15.8)
Obese (≥30)	425 (14.7)	114 (23.6)	16 (13.0)	84 (28.0)	12 (21.1)
Periconceptional smoking					
Yes	600 (20.3)	91 (18.1)	18 (14.3)	62 (20.0)	10 (16.4)
No	2,350 (79.7)	411 (81.9)	108 (85.7)	248 (80.0)	51 (83.6)
Periconceptional folic acid supplementation					
Yes	2,295 (79.0)	382 (77.2)	101 (80.8)	230 (75.7)	47 (77.0)
No	611 (21.0)	113 (22.8)	24 (19.2)	74 (24.3)	14 (23.0)
Pre-pregnancy food folate intake (μg daily)					
<600	1,892 (64.1)	324 (64.5)	85 (67.5)	195 (62.9)	40 (65.6)
≥600	1,058 (35.9)	178 (35.5)	41 (32.5)	115 (37.1)	21 (34.4)
NBDPS site[c]					
Arkansas	369 (12.5)	83 (16.5)	22 (17.5)	49 (15.8)	10 (16.4)
California	343 (11.6)	83 (16.5)	28 (22.2)	51 (16.5)	3 (4.9)
Iowa	409 (13.9)	80 (15.9)	16 (12.7)	58 (18.7)	6 (9.8)
Massachusetts	424 (14.4)	35 (7.0)	7 (5.6)	20 (6.5)	8 (13.1)
New Jersey	414 (14.0)	47 (9.4)	9 (7.1)	34 (11.0)	4 (6.6)
New York	334 (11.3)	43 (8.6)	7 (5.6)	27 (8.7)	9 (14.8)
Texas	311 (10.5)	71 (14.1)	20 (15.9)	40 (12.9)	10 (16.4)

	Controls (*n* = 2,950)	All NTDs (*n* = 502)	Anencephaly (*n* = 126)	Spina Bifida (*n* =310)	Encephalocele (*n* = 61)
Characteristic	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**	***n*[a] (%)[b]**
CDC/Atlanta, Georgia	346 (11.7)	60 (12.0)	17 (13.5)	31 (10.0)	11 (18.0)

Periconceptional period corresponded to 1 month preconception through 2 months postconception

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

[b] Due to missing data and rounding, percentages may not total 100.

[c] The *p*-values for all NTDs compared with controls: maternal age at delivery ($p = 0.0407$), race/ethnicity ($p = 0.0023$), education ($p<.0001$), gravidity ($p = 0.0333$), pre-pregnancy BMI ($p <.0001$), and NBDPS site ($p<.0001$).

NTDs, neural tube defects; NA, not applicable; BMI, body mass index; NBDPS, National Birth Defects Prevention Study; CDC, Centers for Disease Control and Prevention.

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2015 November 01.

TABLE 2

Maternal Periconceptional Occupational Pesticide Exposure by Controls and Neural Tube Defect Cases, National Birth Defects Prevention Study, 1997–2002

Periconceptional Exposure	**Controls *n* = 2,930**		**All NTDs *n* = 496**		**Anencephaly *n* = 123**		**Spina Bifida *n* = 307**		**Encephalocele *n* = 61**	
Pesticide exposure[a]	*n* (%)[b]		*n* (%)[b]		*n* (%)[b]		*n* (%)[b]		*n* (%)[b]	
Any	888 (30.3)		162 (32.7)		43 (34.2)		97 (31.6)		20 (32.8)	
None	2,042 (69.7)		334 (67.3)		81 (65.9)		210 (68.4)		41 (67.2)	
Pesticide exposure by class[c]										
Insecticides only	616 (21.0)		91 (18.3)		21 (20.6)		58 (18.9)		10 (16.4)	
Insecticides+herbicides	52 (1.8)		19 (3.8)		4 (4.7)		14 (4.6)		1 (1.6)	
Insecticides+herbicides+fungicides	211 (7.2)		52 (10.5)		17 (17.4)		25 (8.1)		9 (14.8)	
Cumulative pesticide exposure among exposed (mg)	*n*	Median	*n*	Median	*n*	Median	*n*	Median	*n*	Median
Insecticides	885	62.6	162	96.4	42	96.4	97	84.4	20	59.5
Herbicides	266	49.8	71	40.2	21	48.2	39	35.5	10	33.4
Fungicides	217	64.3	52	63.9	17	67.5	25	96.4	9	34.7

Periconceptional period corresponded to 1 month preconception through 2 months postconception

[a] Missing, incomplete, or questionable data on pesticides exposure were distributed as follows: controls (*n* = 20), all cases (*n* = 6), anencephaly (*n* = 3), spina bifida (*n* = 3), encephalocele (*n* = 0).

[b] Due to rounding, percentages may not total 100.

[c] No case and three control mothers were exposed to herbicides only, no case or control mothers were exposed to fungicides only or jointly exposed to herbicides and fungicides, and no case and six control mothers were jointly exposed to insecticides and fungicides.

NTDs, neural tube defects.

TABLE 3

Adjusted Odds Ratio Estimates for Neural Tube Defects Associated with Periconceptional (1 Month Preconception through 2 Months Postconception) Maternal Occupational Exposure to Pesticides, National Birth Defects Prevention Study, 1997–2002

		All NTDs		Anencephaly		Spina Bifida		Encephalocele	
	Controls *n*	*n*	Odds ratio (95% CI)	*n*	Odds ratio (95% CI)	*n*	Odds ratio (95% CI)	*n*	Odds ratio (95% CI)
Dichotomous pesticide exposure[a,b]									
None	2042	334	Ref	81	Ref	210	Ref	41	Ref
Any pesticide	888	162	0.9 (0.7, 1.1)	43	1.0 (0.6, 1.4)	97	0.9 (0.7, 1.2)	20	0.9 (0.5, 1.7)
Insecticides only	616	91	0.8 (0.6, 1.0)	21	0.7 (0.4, 1.2)	58	0.8 (0.6, 1.1)	10	0.8 (0.4, 1.6)
Insecticides+herbicides	52	19	1.7 (0.9, 3.1)	4	NC	14	**2.1 (1.0, 4.1)**	1	NC
Insecticides+herbicides+fungicides	211	52	1.1 (0.8, 1.6)	17	1.6 (0.9, 2.8)	25	0.9 (0.5, 1.4)	9	1.6 (0.7, 3.7)
Estimated cumulative exposure (mg)[a,c]									
None	2042	334	Ref	81	Ref	210	Ref	41	Ref
Any pesticide									
>0 and <84.375	441	63	0.8 (0.6, 1.1)	13	0.7 (0.4, 1.3)	42	0.9 (0.6, 1.3)	7	0.8 (0.4, 1.9)
≥84.375	447	99	1.0 (0.7, 1.3)	29	1.2 (0.7, 1.9)	55	0.9 (0.6, 1.2)	13	1.0 (0.5, 2.1)
Insecticides Only									
>0 and <52.607	308	29	**0.6 (0.4, 0.9)**	3	NC	19	0.7 (0.4, 1.1)	7	1.4 (0.6, 3.2)
≥52.607	308	62	0.9 (0.7, 1.3)	18	1.1 (0.6, 1.9)	39	1.0 (0.7, 1.4)	3	NC
Insecticides+herbicides									
>0 and <9.482	26	10	1.7 (0.7, 3.9)	2	NC	8	2.1 (0.8, 5.3)	0	NC
≥9.482	26	9	1.7 (0.7, 4.0)	2	NC	6	2.0 (0.7, 5.5)	1	NC

		All NTDs		Anencephaly		Spina Bifida		Encephalocele	
	Controls *n*	*n*	Odds ratio (95% CI)	*n*	Odds ratio (95% CI)	*n*	Odds ratio (95% CI)	*n*	Odds ratio (95% CI)
Insecticides+herbicides+fungicides									
>0 and <245.089	105	22	1.1 (0.6, 1.7)	7	1.4 (0.6, 3.2)	9	0.7 (0.4, 1.5)	6	2.1 (0.8, 5.6)
≥245.089	106	30	1.2 (0.8, 2.0)	10	1.8 (0.8, 3.9)	16	1.0 (0.5, 1.9)	3	NC

[a] Missing or incomplete data for pesticides exposure were distributed as follows: controls (*n* = 71), all cases (*n* = 22), anencephaly (*n* = 58), spina bifida (*n* = 13), and encephalocele (*n* = 1).

[b] Analyses adjusted for maternal body mass index (continuous), maternal education, and site.

[c] Cut-points were based on exposure in control mothers and calculated as exposure less than the median (50%) and greater than or equal to the median.

NTD, neural tube defect; CI, confidence interval; Ref, Reference; NC, not calculated.