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PATERNAL AND JOINT PARENTAL OCCUPATIONAL PESTICIDE EXPOSURE AND SPINA BIFIDA IN THE NATIONAL BIRTH DEFECTS PREVENTION STUDY, 1997-2002

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Abstract

Objective: Because of persistent concerns over the association between pesticides and spina bifida, we examined the role of paternal occupational pesticide exposure and combined parental occupational pesticide exposures in spina bifida in offspring using data from a large population-based study of birth defects.

Design: Case-control

Methods: Occupational information from fathers of 291 spina bifida cases and 2745 unaffected live born control infants with estimated dates of delivery from 1997–2002 were collected via

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maternal report. Estimated exposure intensity and frequency to insecticides, herbicides, and fungicides were independently assigned by two expert industrial hygienists, blinded to case or control status, with disagreements resolved by consensus. Multivariable logistic regression models were used to estimate adjusted odds ratios (aOR) and 95% confidence intervals (CI) for exposure to any pesticide and to any class of pesticide (yes/no; and by median), and exposure to combinations of pesticides (yes/no) and risk of spina bifida. Adjusted odds ratios were also estimated by parent exposed to pesticides (neither, mother only, father only, both parents).

Results: Joint parental occupational pesticide exposure was positively associated with spina bifida (aOR 1.5, 95% CI 0.9–2.4) when compared to infants with neither maternal nor paternal exposures; a similar association was not observed when only one parent was exposed. There was a suggested positive association between combined paternal insecticide and fungicide exposures and spina bifida (aOR 1.5, 95% CI 0.8–2.8), however nearly all other aORs for spina bifida and paternal pesticide exposures were close to unity.

Conclusions: Overall, there was little evidence that paternal occupational pesticide exposure was associated with spina bifida. Joint parental occupational pesticide exposure was associated with spina bifida, as was paternal occupational exposure to a combination of insecticides and fungicides. However, the small numbers make it difficult to precisely evaluate the role of pesticide classes, individually and in combination.

Background

Neural tube defects (NTDs) affect an estimated 2660 pregnancies in the United States each year (Parker et al., 2010). The most common subtype is spina bifida without anencephaly (1 in 2858 live births) (Parker et al., 2010). Those with spina bifida experience varying degrees of disability, excess mortality, and higher rates of healthcare interventions and expenditures across a lifetime compared to unaffected infants (Mitchell, 2005) (Radcliff et al., 2012) (Ouyang et al., 2007).

In a previous National Birth Defects Prevention Study analysis of maternal occupational pesticide exposure, no association with spina bifida and overall exposure, and a weak association between spina bifida and combined exposure to insecticides and herbicides were observed (Makelarski et al., 2014). Although maternal exposures are most often studied with regard to NTD risk in offspring, paternal exposures may also have teratogenic potential (Friedler, 1996) (Kumar et al., 2013) (Soubry et al., 2014) (Knishkowy and Baker, 1986). Insecticide, fungicide, and herbicide exposures have demonstrated male reproductive toxicity in animal studies (de Liz Oliveira Cavalli et al., 2013) (Eustache et al., 2009) (Stouder and Paoloni-Giacobino, 2011) (Farag et al., 2010). However, results of epidemiologic studies examining associations of paternal occupational pesticide exposure and NTD risk in offspring have been largely equivocal (Schnitzer et al., 1995) (Blatter et al., 2000) (B.M.Blatter et al., 1996) (Shaw et al., 2002) (Fear et al., 2007) (Nordby et al., 2005). Combined maternal and paternal occupational pesticide exposures have also been positively associated with spina bifida (Blatter et al., 2000) (B.M.Blatter et al., 1996) (Kristensen et al., 1997). Paternal exposures might affect birth defect risk 1) by altering the development of sperm during spermatogenesis; 2) by passing into seminal fluid and providing a pathway for

exposure of sexual partners; or 3) by being carried home on clothes, skin, hair, and shoes and contributing to maternal exposure.

A potential limitation of previous analyses is related to exposure assessment. More specifically, many previous studies have relied on self-reported pesticide exposure or employment in the agricultural sector as a proxy for pesticide exposure, which could have resulted in exposure misclassification. Self-report is considered less accurate than expert-assessed exposures (Fritschi et al., 1996). Expert rater review of detailed job descriptions is also considered the gold-standard for retrospective exposure assessment in the absence of biomarkers or direct monitoring data. Additionally, classification of all agricultural workers as exposed to pesticides likely overestimates exposure of these workers, while underestimating pesticide exposure among non-agricultural workers (Daniels et al., 2001) (MacFarlane et al., 2009). Further, lumping pesticides broadly could mask an association with a particular chemical or pesticide class.

To further explore the potential risk of spina bifida in offspring associated with parental pesticide exposure, data from the National Birth Defects Prevention Study (NBDPS), a population-based, case-control study were analyzed. Since industrial hygienists completed a detailed occupational exposure assessment of NBDPS data, the NBDPS presents a unique opportunity to improve upon previous studies and analyze risk for NTDs by pesticide class.

Methods

Study Population, Recruitment, and Interviews

The NBDPS included cases diagnosed with one or more of 45 non-chromosomal major birth defects identified from population-based birth defects surveillance programs in ten NBDPS sites (Arkansas [AR], California [CA], Georgia [GA], Iowa [IA], Massachusetts [MA], North Carolina [NC], New Jersey [NJ], New York [NY], Texas [TX], and Utah [UT]) and unaffected controls to examine associations with various risk factors (Reefhuis et al., 2015). This analysis included cases and controls with an estimated date of delivery from October 1, 1997 through December 31, 2002. Eligible case deliveries were diagnosed with spina bifida (modified British Pediatric Association code [BPA] 741.001–741.999). Clinical geneticists reviewed data abstracted from hospital reports and medical records to confirm case eligibility based upon the following criteria: defect definition; confirmatory diagnostic procedures; prenatal diagnosis; and exclusions. Cases were classified as either isolated (no other unrelated major defects or conditions of known etiology present) or multiple (occurring in conjunction with another unrelated major defect of unknown etiology) (Rasmussen et al., 2003).(table 3)

Cases consisted of live births from all sites, fetal deaths from AR, CA, GA, IA, MA, and TX and elective terminations from AR, CA, GA (beginning in 1999), IA and TX. New York began including fetal deaths and elective terminations in 2000. Control infants were liveborn infants without major birth defects randomly selected from birth hospital records (AR [through March 2000], CA, GA [through May 2001], NY, and TX) or birth certificates (AR [since April 2000], GA [since June 2001], IA, MA, and NJ) (Makelarski et al., 2014) (Reefhuis et al., 2015).

Case and control mothers were interviewed in English or Spanish language using a computer-assisted telephone interview 6 weeks to 24 months after the estimated date of delivery. The questionnaire covered information on maternal health and medications, the index pregnancy and previous pregnancies, diet and substance use, residence, family demographics, lifestyle, and occupation. Requested parental occupational data included company name and description, job title and description, month and year start and stop dates, average hours worked per day, average days per week for each job, and chemicals and substances handled on the job.

Exclusions

Mothers of 780 NTD case (71% of eligible) and 4141 control infants (68% of eligible) were interviewed. Given that the exposure classification for paternal occupation was limited to those cases and controls with employed mothers, analyses were restricted to the 489 case and 2799 control families whose mother and father both worked for at least one month at any time during the period three months before conception through the end of pregnancy, and who provided sufficient job descriptions that industrial hygienists could assess potential pesticide exposure. Work was defined to include paid work, self-employment, or volunteer work performed outside the home or at home. Due to previous reports of associations with NTDs, cases and controls were also excluded if they had a family history of NTDs (n=3 cases/5 controls) or if their mother reported having a previous diagnosis of type 1 or type 2 diabetes mellitus (n=5 cases/15 controls) or used a medication known to be associated with birth defects (aminopterin sodium, carbamazepine, cholestyramine resin, methotrexate, pyrimethamine, sulfasalazine, triamterene, trimethoprim, trimethoprim HCL, trimethoprim sulfate, phenytoin, phenytoin sodium, primidone, phenobarbital, phenobarbital sodium, valproic acid) (n=11 cases/30 controls) (Werler et al., 2003) (Hernández-Díaz et al., 2001) (Hernández-Díaz et al., 2000). One case and four controls were excluded due to missing maternal education data. Data were complete for all other covariates. The final sample size consisted of 291 spina bifida cases, 123 anencephaly cases, 55 encephalocele cases, and 2745 controls. Due to the small numbers of anencephaly and encephalocele cases, the analyses reported herein are limited to spina bifida. Ninety percent of the spina bifida cases were of isolated phenotype and 10% had multiple defects. All spina bifida cases were analyzed regardless of phenotype due to the small number of cases with multiple defects.

Exposure Assessment

Each parental job reported by the mother was classified using the 1997 North American Industry Classification System (NAICS) codes and the 2000 Standard Occupational Classification (SOC- 2000) codes. The pesticide exposure assessment of mothers in the NBDPS has been described previously (Makelarski et al., 2014). Similar to maternal assessment, the paternal exposure assessment was conducted in three phases by two industrial hygienists, blinded to case or control status, with expertise in occupational pesticide exposure and extensive experience performing retrospective exposure assessments. First, an industrial hygienist conducted an initial review of all jobs, selecting any jobs that might possibly have any type of pesticide exposure for a detailed review. A second expert industrial hygienist also reviewed a 10% sample of the jobs as a quality control check; agreement between raters was nearly 100%. In the second phase of review, jobs flagged for

detailed review were assessed independently by each rater and assigned categorical scores for exposure intensity and frequency to herbicides, insecticides, and fungicides if the job was considered exposed. In the third phase, any discrepancies between the raters were resolved by a consensus conference between both raters. Fathers with no probable exposure to any pesticide were considered unexposed. Cumulative pesticide exposure dose was estimated by quantitatively mapping each categorical frequency score (as hours per week in a standard 40-hour work week: 0, 5, or 15) and categorical intensity scores (as mg/hour: 0, 1, 5, 50, 100). An approximate estimated dose for each paternal job was calculated by combining these quantitatively mapped scores, reported typical hours per day and days per week worked, and start and stop dates as: (estimated exposure intensity, mg/hr) * [(exposure frequency, hours/wk) / (40 hours/week)] * [(typical hours worked per week)/(7 days per week)] * (number of days worked in the exposure window) for each class of pesticide (Makelarski et al., 2014). This approach has been described previously (Samanic et al., 2008). Paternal pesticide exposures were calculated for the period three months before conception through the third month of pregnancy, and were limited to the three months before conception and one month after for this analysis. Because this calculation uses mapped categorical scores, rather than absolute measurements of intensity and frequency, the calculated approximate dose likely has greater relative versus absolute validity; consequently the approximate dose was used to classify fathers into categories of lower and higher estimated doses. Exposure to each class of pesticide was summed across jobs to create a total estimated exposure dose per father. Cumulative exposure to any pesticide was the sum of total herbicide, insecticide, and fungicide exposures. For each class, cumulative exposures were divided into median categories (>0 to <=50, >50) based on distribution among exposed controls. Mothers with any pesticide exposure during the month before pregnancy through the first month of pregnancy were classified as exposed.

Covariables

Education was categorized as <12, 12, 13–15, and >15 years. Race/ethnicity was classified as non-Hispanic white, non-Hispanic black, Hispanic, and other. Maternal body mass index (BMI) was calculated in kg/m^2 and categorized as underweight (BMI <18.5), normal weight (BMI=18.5–24.9), overweight (BMI =25–29.9), and obese (BMI >=30). Food folate intake from both naturally occurring and fortified sources was divided between <600ug daily and >=600 ug daily. Folic acid supplementation was considered to be any use of a single or multi-vitamin supplement containing folic acid during the month before conception through the second month of pregnancy.

Potentially relevant confounders were identified from previous literature examining maternal and paternal exposures and NTDs. Using a directed acyclic graph (DAG), education, race/ethnicity, and NBDPS site were identified as potential confounders. The utility and methodology of DAGs has been discussed elsewhere (Greenland et al., 1999) (Hernán et al., 2002). Thirty-two spina bifida cases and 194 controls were missing data on paternal race/ethnicity, and 4 cases and 33 controls were missing data on paternal education. Because maternal and paternal education levels and maternal and paternal race/ethnicity were highly correlated, maternal rather than paternal race/ethnicity and education level were used in the

models providing a larger sample size and increased statistical power (Desrosiers et al., 2012).

Statistical Analysis

Analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary NC, 2010). Bivariate analyses examined the distribution of infant, maternal, and paternal characteristics, including pesticide exposure, among controls and spina bifida cases.

Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were estimated for spina bifida for the following paternal occupational exposure patterns: any pesticide exposure; insecticides only; herbicides only; fungicides only; insecticides and herbicides combined; insecticides and fungicides combined; and insecticides, herbicides, and fungicides combined. No cases were exposed to herbicides and fungicides combined. Any exposures to insecticide, herbicide, and fungicide were entered into a single model. Cumulative and individual class exposures were also categorized by the median cut-point of estimated cumulative dose. Finally, using no maternal and no paternal occupational pesticide exposures as a referent group, the associations between spina bifida and only maternal occupational exposure to any pesticide, only paternal occupational exposure to any pesticide, and joint parental occupational exposure to any pesticide were estimated. Odds ratios were not calculated for strata with four or fewer cases. A sub-analysis was conducted restricting the sample to sites/years that collected data on live birth, fetal deaths, and elective terminations.

Results

Overall, case mothers were more likely to be multiparous, of Hispanic ethnicity, and have a BMI >=30 compared to control mothers (Table 1); case fathers were also more likely to be of Hispanic ethnicity than control fathers. Parents of cases completed fewer years of schooling than parents of controls. The proportion of cases varied across sites.

Eleven percent of control fathers were considered probably exposed to any pesticide (Table 2). Of these 308 exposed control fathers, the most common exposure pattern was insecticides, herbicides, and fungicides combined (n=100), followed by insecticides and fungicides combined (n=69). Of the pesticide classes, the largest number of control fathers were exposed to any fungicide (n=234), with 65 control fathers exposed to fungicides only. Herbicide exposure was the least common, with 138 control fathers exposed to any herbicide, 19 of which were exposed to herbicides only. Both parents of 124 control infants were exposed to any pesticide.

There was a suggested positive association between combined paternal occupational exposure to insecticides and fungicides and spina bifida in offspring (aOR 1.5, 95% CI 0.8 – 2.8); however, nearly all other adjusted ORs for paternal pesticide exposure were close to unity.

While the exposure of one parent was not associated with an increased risk of spina bifida, when both parents were exposed the association changed direction from an adjusted OR of

0.8 for maternal exposure only (95% CI 0.5–1.3) and an adjusted OR of 0.8 for paternal exposure only (95% CI 0.6–1.8) to an adjusted OR of 1.5 (95% CI 0.9 – 2.4) for joint parental occupational exposure to any pesticide. There were too few cases with both parents exposed (n=25) to characterize exposure by pesticide class or pattern. [Data not shown.]

Analyses restricted to sites that collected data on live births, fetal deaths, and elective terminations decreased the sample size to 1914 and increased the percentages of exposed spina bifida cases (16.7%) and controls (14.3%). However, this restriction did not produce materially different estimates, although statistical precision was reduced.

Discussion

This investigation of paternal and maternal occupational pesticide exposures and risk of spina bifida produced largely null associations, though imprecise modest positive associations were observed between paternal occupational exposure to insecticides and fungicides combined. Additionally, joint parental occupational exposure to pesticides was positively associated with spina bifida. We did not observe a similar association between maternal-only or paternal-only exposure to pesticides. Stratification by pesticide class and exposure pattern produced small numbers of exposed cases and imprecise odds ratio estimates. Previous studies of paternal pesticide exposure and spina bifida produced suggestive, but inconclusive results. Differences in outcomes, sample selection, and exposure assessment limited comparisons.

The association between joint parental occupational exposure to any pesticide and spina bifida raises the question whether maternal exposure is driving the association. Using NBDPS data and an exposure classification as described above, Makelarski et al. (2014) observed an increased association between spina bifida and maternal exposure to insecticides and herbicides combined, however an association was not observed for any pesticide exposure (yes/no). Other studies have observed positive associations between spina bifida and maternal occupational exposures to pesticides (B.M.Blatter et al., 1996).However, in the current analysis there was no association between any maternal-only pesticide exposure and spina bifida.

Exposure contributions from both parents may result in a higher total pesticide dose that reaches a threshold effect. There was not sufficient sample size to analyze the dose in this analysis. Several other analyses have examined joint parental pesticide exposure and the risk of spina bifida. A study in the Netherlands observed an OR of 5.6 (95% CI 1.8–17.8) for maternal agricultural employment and spina bifida (B.M.Blatter et al., 1996). However, there was no increased risk based on paternal employment alone. The OR for spina bifida increased to 19.7 if both parents were employed in the agricultural sector, although no confidence interval was reported. Using the family unit as an exposure category, children of Norwegian farmers who worked in greenhouses or orchards had a greater risk of spina bifida (OR 2.76, 95% CI 1.07–7.13) (Kristensen et al., 1997). Other European analyses of maternal agricultural employment and spina bifida produced mixed results when the sample was restricted to only those women whose husbands were also agricultural workers. In Spain, the

OR decreased from 2.2 to 1.7, but in Hungary it increased from 1.1 to 2.0 (Blatter et al., 2000).

The failure to include early terminations and early losses in the sample could result in selection bias if those women who electively terminated a NTD-affected pregnancy were more or less likely to have a partner occupationally exposed to pesticides (Cragan et al., 1995) (Howards et al., 2015). In this sample, although we do not know how complete the ascertainment of terminations and losses is at sites collecting it, the restriction to only sites and years that included terminations reduced the sample size and widened confidence intervals with no substantive change in the results.

The majority of the studies on paternal pesticide exposure and NTDs consider membership in a broad occupational category, such as farmworkers, as the marker of exposure compared to all other occupations. This methodology has resulted in misclassification of exposure, as some agricultural workers are not exposed to pesticides, and many non-agricultural workers may be exposed (Daniels et al., 2001) (MacFarlane et al., 2009).

The few epidemiological studies that have assessed exposure by individual pesticide, or groups of related pesticides have observed mixed results, with some chemicals showing associations with NTDs, and others not. The fungicide mancozeb was moderately associated with an increase in all NTDs among the children of Norwegian farmers in the one analysis that examined both maternal and paternal exposure (Nordby et al., 2005). Other analyses looked at only maternal exposures. Maternal occupational and hobby exposure to insecticides suggested an association with all NTDs (OR 1.3, 95% CI.81–2.1) (Shaw et al., 1999). In addition, Rull et al. and Yang et al. observed associations between all NTDs as a group and spina bifida in particular, and maternal residential exposures to a handful of individual pesticides and chemical groups (Yang et al., 2014) (Rull et al., 2006).

Strengths and Limitations

One strength of the NBDPS is a definition of work that includes paid work, self-employment, and volunteer work, which captures cottage industries and unreported employment that may have occupational exposures to pesticides that would not otherwise have been included. Another strength of the NBDPS is the exposure assessment by industrial hygienists, blinded to information on case or control status, that captured the pesticide exposures of many occupations other than agricultural work, including maintenance workers and property managers. This assessment was also able to categorize exposure by pesticide class: insecticide, fungicide, and herbicide. However, we were not able to analyze exposure to particular chemicals, leaving the possibility that the effect of an individual chemical was masked. The limited sample size also precluded analyses of narrower exposure designations, such as herbicide only or insecticide combined with herbicide.

The major limitation of this study is that paternal occupational data were collected via maternal self-report. In one study of birth defects, only 59% of maternal reports of paternal occupation matched the paternal report (Schnitzer et al., 1995). Few studies have collected occupational data directly from the father. Blatter et al. (1997) observed a suggested risk of

spina bifida with a moderate/high level of paternal occupational exposure (OR 1.7, 95% CI . 7–4.0). Although exposure data were collected directly from the fathers, they were interviewed 2–15 years after the birth, which could have impacted recall and observed associations. In addition, the sample was conditioned upon maternal employment, which excluded households where only the father was employed. This restriction was chosen a priori to prevent confounding by characteristics associated with parental employment status, however as a consequence these results may not reflect those that would be observed in the households in which only one parent works. Finally, unmeasured residential pesticide exposures may have produced exposure misclassification that biased the results toward the null.

Conclusion

Paternal occupational exposure to a combination of insecticides and fungicides, as well as joint parental occupational exposure to any pesticide were modestly associated with spina bifida.

Although additional analyses revealed largely null associations, the small numbers make it difficult to evaluate the role of pesticide classes, individually and in combination, for spina bifida. Future research that accurately measures exposure to individual pesticides is warranted.

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References

Blatter BM, Hermens R, Bakker M, Roeleveld N, Verbeek AL, Zielhuis GA, 1997 Paternal occupational exposure around conception and spina bifida in offspring. Am. J. Ind. Med. 32, 283–291. [PubMed: 9219659]

Blatter BM, Roeleveld N, Bermejo E, Martínez-Frías ML, Siffel C, Czeizel AE, 2000 Spina bifida and parental occupation: results from three malformation monitoring programs in Europe. Eur. J. Epidemiol. 16, 343–351. [PubMed: 10959942]

Blatter BM, Roeleveld N, Zielhuis GA, Gabreëls FJ, Verbeek AL, 1996 Maternal occupational exposure during pregnancy and the risk of spina bifida. Occup. Environ. Med. 53, 80–86. [PubMed: 8777455]

Blatter BM, Roeleveld N, Zielhuis GA, Mullaart RA, Gabreëls FJ, 1996 Spina bifida and parental occupation. Epidemiol. Camb. Mass 7, 188–193.

Cragan JD, Roberts HE, Edmonds LD, Khoury MJ, Kirby RS, Shaw GM, Velie EM, Merz RD, Forrester MB, Williamson RA, Krishnamurti DS, Stevenson RE, Dean JH, 1995 Surveillance for anencephaly and spina bifida and the impact of prenatal diagnosis--United States, 1985–1994. MMWR CDC Surveill. Summ. Morb. Mortal. Wkly. Rep. CDC Surveill. Summ. Cent. Dis. Control 44, 1–13.

Daniels JL, Olshan AF, Teschke K, Hertz-Picciotto I, Savitz DA, Blatt J, 2001 Comparison of assessment methods for pesticide exposure in a case-control interview study. Am. J. Epidemiol. 153, 1227–1232. [PubMed: 11415959]

de Liz Oliveira Cavalli VL, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, Wilhelm Filho D, Mena Barreto Silva FR, Pessoa-Pureur R, Zamoner A, 2013 Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. Free Radic. Biol. Med. 65, 335–346. doi:10.1016/j.freeradbiomed.2013.06.043 [PubMed: 23820267]

Desrosiers TA, Herring AH, Shapira SK, Hooiveld M, Luben TJ, Herdt-Losavio ML, Lin S, Olshan AF, National Birth Defects Prevention Study, 2012 Paternal occupation and birth defects: findings from the National Birth Defects Prevention Study. Occup. Environ. Med. 69, 534–542. doi:10.1136/oemed-2011-100372 [PubMed: 22782864]

Eustache F, Mondon F, Canivenc-Lavier MC, Lesaffre C, Fulla Y, Berges R, Cravedi JP, Vaiman D, Auger J, 2009 Chronic dietary exposure to a low-dose mixture of genistein and vinclozolin modifies the reproductive axis, testis transcriptome, and fertility. Environ. Health Perspect. 117, 1272–1279. doi:10.1289/ehp.0800158 [PubMed: 19672408]

Farag AT, Radwan AH, Sorour F, El Okazy A, El-Agamy E-S, El-Sebae AE-K, 2010 Chlorpyrifos induced reproductive toxicity in male mice. Reprod. Toxicol. Elmsford N 29, 80–85. doi:10.1016/j.reprotox.2009.10.003

Fear NT, Hey K, Vincent T, Murphy M, 2007 Paternal occupation and neural tube defects: a case-control study based on the Oxford Record Linkage Study register. Paediatr. Perinat. Epidemiol. 21, 163–168. doi:10.1111/j.1365-3016.2007.00793.x [PubMed: 17302646]

Friedler G, 1996 Paternal exposures: impact on reproductive and developmental outcome. An overview. Pharmacol. Biochem. Behav. 55, 691–700. [PubMed: 8981601]

Fritschi L, Siemiatycki J, Richardson L, 1996 Self-assessed versus expert-assessed occupational exposures. Am. J. Epidemiol. 144, 521–527. [PubMed: 8781468]

Greenland S, Pearl J, Robins JM, 1999 Causal diagrams for epidemiologic research. Epidemiol. Camb. Mass 10, 37–48.

Hernández-Díaz S, Werler MM, Walker AM, Mitchell AA, 2001 Neural tube defects in relation to use of folic acid antagonists during pregnancy. Am. J. Epidemiol. 153, 961–968. [PubMed: 11384952]

Hernández-Díaz S, Werler MM, Walker AM, Mitchell AA, 2000 Folic acid antagonists during pregnancy and the risk of birth defects. N. Engl. J. Med. 343, 1608–1614. doi:10.1056/NEJM200011303432204 [PubMed: 11096168]

Hernán MA, Hernández-Díaz S, Werler MM, Mitchell AA, 2002 Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am. J. Epidemiol. 155, 176–184. [PubMed: 11790682]

Howards PP, Johnson CY, Honein MA, Flanders WD, National Birth Defects Prevention Study, 2015 Adjusting for bias due to incomplete case ascertainment in case-control studies of birth defects. Am. J. Epidemiol. 181, 595–607. doi:10.1093/aje/kwu323 [PubMed: 25792608]

Knishkowy B, Baker EL, 1986 Transmission of occupational disease to family contacts. Am. J. Ind. Med. 9, 543–550. [PubMed: 2426943]

Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L, 1997 Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiol. Camb. Mass 8, 537–544.

Kumar M, Kumar K, Jain S, Hassan T, Dada R, 2013 Novel insights into the genetic and epigenetic paternal contribution to the human embryo. Clin. São Paulo Braz. 68 Suppl 1, 5–14.

MacFarlane E, Glass D, Fritschi L, 2009 Is farm-related job title an adequate surrogate for pesticide exposure in occupational cancer epidemiology? Occup. Environ. Med. 66, 497–501. doi:10.1136/oem.2008.041566 [PubMed: 19221114]

Makelarski JA, Romitti PA, Rocheleau CM, Burns TL, Stewart PA, Waters MA, Lawson CC, Bell EM, Lin S, Shaw GM, Olney RS, the National Birth Defects Prevention Study, 2014 Maternal periconceptional occupational pesticide exposure and neural tube defects. Birt. Defects Res. A. Clin. Mol. Teratol. doi:10.1002/bdra.23293

Mitchell LE, 2005 Epidemiology of neural tube defects. Am. J. Med. Genet. C Semin. Med. Genet. 135C, 88–94. doi:10.1002/ajmg.c.30057 [PubMed: 15800877]

Nordby K-C, Andersen A, Irgens LM, Kristensen P, 2005 Indicators of mancozeb exposure in relation to thyroid cancer and neural tube defects in farmers' families. Scand. J. Work. Environ. Health 31, 89–96. [PubMed: 15864902]

Ouyang L, Grosse SD, Armour BS, Waitzman NJ, 2007 Health care expenditures of children and adults with spina bifida in a privately insured U.S. population. Birt. Defects Res. A. Clin. Mol. Teratol. 79, 552–558. doi:10.1002/bdra.20360

Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, Anderson P, Mason CA, Collins JS, Kirby RS, Correa A, National Birth Defects Prevention Network, 2010 Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004–2006. Birt. Defects Res. A. Clin. Mol. Teratol. 88, 1008–1016. doi:10.1002/bdra.20735

Radcliff E, Cassell CH, Tanner JP, Kirby RS, Watkins S, Correia J, Peterson C, Grosse SD, 2012 Hospital use, associated costs, and payer status for infants born with spina bifida. Birt. Defects Res. A. Clin. Mol. Teratol. 94, 1044–1053. doi:10.1002/bdra.23084

Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA, National Birth Defects Prevention Study, 2003 Guidelines for case classification for the National Birth Defects Prevention Study. Birt. Defects Res. A. Clin. Mol. Teratol. 67, 193–201. doi:10.1002/bdra.10012

Reefhuis J, Gilboa SM, Anderka M, Browne ML, Feldkamp ML, Hobbs CA, Jenkins MM, Langlois PH, Newsome KB, Olshan AF, Romitti PA, Shapira SK, Shaw GM, Tinker SC, Honein MA, National Birth Defects Prevention Study, 2015 The National Birth Defects Prevention Study: A review of the methods. Birt. Defects Res. A. Clin. Mol. Teratol. 103, 656–669. doi:10.1002/bdra.23384

Rull RP, Ritz B, Shaw GM, 2006 Neural tube defects and maternal residential proximity to agricultural pesticide applications. Am. J. Epidemiol. 163, 743–753. doi:10.1093/aje/kwj101 [PubMed: 16495467]

Samanic CM, De Roos AJ, Stewart PA, Rajaraman P, Waters MA, Inskip PD, 2008 Occupational exposure to pesticides and risk of adult brain tumors. Am. J. Epidemiol. 167, 976–985. doi:10.1093/aje/kwm401 [PubMed: 18299277]

Schnitzer PG, Olshan AF, Erickson JD, 1995 Paternal occupation and risk of birth defects in offspring. Epidemiol. Camb. Mass 6, 577–583.

Shaw GM, Nelson V, Olshan AF, 2002 Paternal occupational group and risk of offspring with neural tube defects. Paediatr. Perinat. Epidemiol. 16, 328–333. [PubMed: 12445149]

Shaw GM, Wasserman CR, O'Malley CD, Nelson V, Jackson RJ, 1999 Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiol. Camb. Mass 10, 60–66.

Soubry A, Hoyo C, Jirtle RL, Murphy SK, 2014 A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. BioEssays News Rev. Mol. Cell. Dev. Biol. 36, 359–371. doi:10.1002/bies.201300113

Stouder C, Paoloni-Giacobino A, 2011 Specific transgenerational imprinting effects of the endocrine disruptor methoxychlor on male gametes. Reprod. Camb. Engl. 141, 207–216. doi:10.1530/REP-10-0400

Werler MM, Bower C, Payne J, Serna P, 2003 Findings on potential teratogens from a case-control study in Western Australia. Aust. N. Z. J. Obstet. Gynaecol. 43, 443–447. [PubMed: 14712948]

Yang W, Carmichael SL, Roberts EM, Kegley SE, Padula AM, English PB, Shaw GM, 2014 Residential Agricultural Pesticide Exposures and Risk of Neural Tube Defects and Orofacial Clefts Among Offspring in the San Joaquin Valley of California. Am. J. Epidemiol. 179, 740–748. doi:10.1093/aje/kwt324 [PubMed: 24553680]

Table 1.

Selected characteristics of control infants, spina bifida cases, and their birth parents (n= 3036), National Birth Defects Prevention Study, 1997–2002

	Control Infants (n= 2745)		Spina Bifida Cases (n=291)	
Characteristic	**n**	**(%)**	**n**	**(%)**
Infant				
Sex				
Male	1374	50.1	152	52.2
Female	1369	49.9	133	45.7
Gestational Age				
< 37 Weeks	229	8.3	87	29.9
≥37 Weeks	2516	91.7	204	70.1
Phenotype				
Isolated			261	89.7
Multiple			30	10.3
Mother				
Age at Delivery (years)				
< 20	198	7.2	20	6.9
20–34	2127	77.5	224	77.0
>34	420	15.3	47	16.2
Race/Ethnicity				
Non-Hispanic White	1818	66.2	182	62.5
Non-Hispanic Black	325	11.8	27	9.3
Hispanic	476	17.3	68	23.4
Other	126	4.6	14	4.8
Education (years)				
< 12	251	9.1	36	12.4
12	661	24.1	89	30.6
13–15	820	29.9	95	32.6
>15	1013	36.9	71	24.4
Parity				
0	1218	44.4	107	36.8
1	964	35.1	107	36.8
2+	563	20.5	77	26.5
Prepregnancy BMI (kg/m^2)				
Underweight (<18.5)	135	5.0	8	2.8
Normal (18.5 –24.9)	1560	57.9	130	46.3
Overweight (25–29.9)	607	22.5	63	22.4
Obese (≥30)	392	14.6	80	28.5
Periconceptional smoking				
Yes	561	20.4	59	20.3

Characteristic	Control Infants (n= 2745) n	(%)	Spina Bifida Cases (n=291) n	(%)
No	2184	79.6	232	79.7
Periconceptional Folic Acid Supplement Use				
Yes	2171	80.3	221	77.3
No	533	19.7	65	22.7
Pre-pregnancy Food Folate Intake				
<600 μg daily	1874	68.3	191	65.6
≥ 600 μg daily	870	31.7	100	34.4
Father				
Age at Delivery (years)				
< 20	57	2.1	5	1.7
20–34	1833	68.1	195	67.9
>34	802	29.8	87	30.3
Race/Ethnicity				
Non-Hispanic White	1779	65.0	167	57.6
Non-Hispanic Black	349	12.8	30	10.3
Hispanic	474	17.3	79	27.2
Other	134	4.9	14	4.8
Education (years)				
< 12	292	10.8	52	18.1
12	841	31.0	95	33.1
13–15	645	23.8	71	24.7
>15	934	34.4	69	24.0
Site				
Arkansas	341	12.4	47	16.2
California	307	11.2	44	15.1
Georgia	330	12.0	28	9.6
Iowa	388	14.1	57	19.6
Massachusetts	402	14.6	20	6.9
New Jersey	386	14.1	31	10.7
New York	313	11.4	24	8.2
Texas	278	10.1	40	13.7

Exclusion included infants with a family history of neural tube defects, maternal use of folic acid antagonist medication, and pre-pregnancy diagnosis of type I or type II diabetes mellitus.

Abbreviations: BMI, Body Mass Index.

Table 2.

Paternal and joint parental pesticide exposure patterns during the three months before conception through the first month of pregnancy in 3036 control infants and spina bifida cases, National Birth Defects Prevention Study, 1997–2002

	Control Infants (n=2745)		Spina Bifida Cases (n=291)	
Exposure categories	**n**	**%**	**n**	**%**
Paternal Exposure				
None	2437	88.8	247	84.9
Any Pesticide	308	11.2	44	15.1
Any Insecticide	224	8.2	34	11.7
Any Herbicide	138	5.0	15	5.2
Any Fungicide	234	8.5	33	11.3
Insecticide Only	36	1.3	6	2.1
Fungicide Only	65	2.4	9	3.1
Herbicide Only	19	0.7	1	0.3
Insecticide + Fungicide	69	2.5	14	4.8
Insecticide + Herbicide	19	0.7	4	1.4
Fungicide + Herbicide			0	
Insecticide + Herbicide + Fungicide	100	3.6	10	3.4
Joint Parental Exposure *				
No Maternal and No Paternal Exposure	1745	64.0	183	64.2
Maternal Exposure Only	673	24.7	59	20.7
Paternal Exposure Only	183	6.7	18	6.3
Any Maternal and Any Paternal Exposure	124	4.6	25	8.8

* Excluded 20 control and 6 case infants due to missing maternal exposure data.

Table 3.

Adjusted odds ratio estimates for 3036 control infants and spina bifida cases associated with paternal and joint parental occupational exposure to pesticides during the period from 3 months before conception through the first month of pregnancy, National Birth Defects Prevention Study, 1997–2002

	Control Infants (n=2745)	Spina Bifida Cases (n=291)			
		n	aOR (95% CI)		
Paternal Exposure					
No Pesticide Exposure	2437	247	ref		
Any Pesticide	308	44	1.1	0.8	1.6
No Pesticide Exposure	2437	247	ref		
Insecticide Only	36	6	1.3	0.6	3.3
Fungicide Only	65	9	1.3	0.6	2.7
Herbicide Only	19	1	nc		
Insecticide + Fungicide	69	14	1.5	0.8	2.8
Insecticide + Herbicide	19	4	nc		
Fungicide + Herbicide		0	nc		
Insecticide + Herbicide + Fungicide	100	10	0.8	0.4	1.5
Exposure by Median (mg)					
All Exposure Combined					
None	2437	247	ref		
Below Median	154	23	1.3	0.8	2.1
Above Median	154	21	1.0	0.6	1.6
All Insecticide					
None	2521	257	ref		
Below Median	103	18	1.4	0.8	2.4
Above Median	121	16	0.9	0.5	1.6
All Herbicide					
None	2607	276	ref		
Below Median	66	9	1.1	0.5	2.2
Above Median	72	6	0.6	0.3	1.5

	Control Infants (n=2745)	Spina Bifida Cases (n=291)			
		n	aOR (95% CI)		
All Fungicide					
None	2511	258	ref		
Below Median	117	15	1.1	0.6	2.0
Above Median	117	18	1.2	0.7	2.0
Any exposure to a pesticide class controlled for other classes					
Any insecticide	224	34	1.4	0.8	2.7
Any herbicide	138	15	0.6	0.3	125
Any fungicide	234	33	1.1	0.6	1.9
Joint Parental Exposure *					
No maternal and no paternal exposure	1726	184	ref		
Any maternal exposure only	673	59	0.8	0.5	1.3
Any paternal exposure only	183	18	0.8	0.6	1.8
Any maternal and any paternal exposure	127	25	1.5	0.9	2.4

Models excluded infants with a family history of neural tube defects, previous maternal diagnosis of type I or II diabetes mellitus, and maternal use of folic acid agonist medications. Adjusted for maternal race/ethnicity, maternal education level, and site.

* Excluded 20 control and 6 case infants missing maternal exposure data.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; ref, referent group; nc, not calculated.