Maternal Residential Exposure to Specific Agricultural Pesticide Active Ingredients and Birth Defects in a 2003-2005 North Carolina Birth Cohort

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Abstract

Background: Previously we observed elevated odds ratios (ORs) for total pesticide exposure and ten birth defects: three congenital heart defects and structural defects affecting the gastrointestinal, genitourinary and musculoskeletal systems. This analysis examines association of those defects with exposure to seven commonly applied pesticide active ingredients.

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Methods: Cases were live-born singleton infants from the North Carolina Birth Defects Monitoring Program linked to birth records for 2003–2005; non-cases served as controls (total n=304,906). Pesticide active ingredient exposure was assigned using a previously constructed metric based on crops within 500 meters of residence, dates of pregnancy, and likely chemical application dates for each pesticide-crop combination. ORs (95% CI) were estimated with logistic regression for categories of exposure compared to unexposed. Models were adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status.

Results: Associations varied by birth defect and pesticide combinations. For example, hypospadias was positively associated with exposures to 2,4-D ($OR_{50-<90th\ percentile}$: 1.39 (1.18, 1.64)), mepiquat ($OR_{50-<90th\ percentile}$: 1.10 (0.90, 1.34)), paraquat ($OR_{50-<90th}$: 1.14 (0.93, 1.39)), and pendimethalin ($OR_{50-<90th}$: 1.21 (1.01, 1.44)), but not S-metolachlor ($OR_{50-<90th}$: 1.00 (0.81, 1.22)). Whereas atrial septal defects were positively associated with higher levels of exposure to glyphosate, cyhalothrin, S-metolachlor, mepiquat, and pendimethalin (ORs ranged from 1.22 to 1.35 for 50-<90th exposures, and 1.72 to 2.09 for >90th exposures); associations with paraquat were null or inconsistent (OR 50-<90th: 1.05 (0.87, 1.27).

Conclusion: Our results suggest differing patterns of association for birth defects with residential exposure to seven pesticide active ingredients in North Carolina.

Keywords

agricultural pesticides; birth defects; environmental exposure

Introduction

Birth defects occur in three percent of live births (Mai et al., 2013), are a leading cause of infant mortality, and contribute to continuing health problems throughout development and the life-course (Decoufle, Boyle, Paulozzi, & Lary, 2001; Matthews & MacDorman, 2013). While risk factors for most birth defects are largely unknown and vary by defect, maternal characteristics, medications, genetics, and environmental factors have all been linked to development of birth defects (CDC, 2018). Understanding the factors that contribute to development of birth defects can lead to better interventions and public health protection.

Pesticide exposures before or during pregnancy are one of the environmental factors that have been associated with birth defects (Agopian, Cai, Langlois, Canfield, & Lupo, 2013; Agopian, Langlois, Cai, Canfield, & Lupo, 2013; Agopian, Lupo, Canfield, & Langlois, 2013; Carmichael, Yang, et al., 2014; Carmichael et al., 2013; Garry et al., 2002; Heeren, Tyler, & Mandeya, 2003; Kielb et al., 2014; Kristensen, Irgens, Andersen, Bye, & Sundheim, 1997; Meyer et al., 2006; Ochoa-Acuna & Carbajo, 2009; Rull, Ritz, & Shaw, 2006; Shaw, Wasserman, O'Malley, Nelson, & Jackson, 1999; Shaw et al., 2014). There are several ways in which pesticide exposure is ascertained or estimated, including: questionnaires or surveys on parental occupation (Garry et al., 2002; Kristensen et al., 1997; Shaw et al., 1999); self-reported pesticide use at home or work (Kielb et al., 2014; Shaw et al., 1999); biomarkers of exposure collected during pregnancy (Barr et al., 2010; A. Bradman et al., 2003; M. A. Bradman et al., 1997); pesticide concentrations measured in dust samples from home environments; county-level pesticide use estimates; residential

proximity to agricultural cropland; and/or metrics that employ combinations of agricultural and pesticide use data (Carmichael, Ma, et al., 2014; Carmichael et al., 2013; Rappazzo et al., 2016; Rull & Ritz, 2003; Rull et al., 2006; Shaw et al., 2014; Yang et al., 2014). Several of these metrics may be easier and less costly to calculate (e.g., county-level pesticide use, proximity to residential cropland), but may lack spatial or temporal specificity. Others (e.g., biomarkers, dust samples) to our knowledge have not been used in large scale studies of birth defects due to the difficulty and expense of acquiring these types of samples. In addition, methods that employ occupational assessment and proximity to cropland estimation lack details such as timing of exposure and information on specific pesticides/ active ingredients (Agopian, Cai, et al., 2013; Agopian, Langlois, et al., 2013; Meyer et al., 2006; Ochoa-Acuna & Carbajo, 2009).

In previous work (Rappazzo et al., 2016), we used a novel metric of residential proximity to agricultural pesticides that balances computational difficulty and expense with information on temporal and spatial specificity of pesticide application and timing of exposure (Warren et al., 2014). We observed elevated odds ratios (ORs) for total pesticide exposure and ten birth defects, including three congenital heart defects and structural defects affecting the gastrointestinal, genitourinary, and musculoskeletal systems; ORs for highest exposure level compared to lowest ranged from 1.27 to 7.51 for these defects, with most being between 1.27 to 2.22 (Rappazzo et al., 2016). However, the total pesticide metric comprised more than 100 pesticide active ingredients, precluding conclusions on potential contributions of specific pesticide active ingredients.

In this analysis, we expand on the previous work to include estimated exposure to specific pesticide active ingredients that are commonly used in North Carolina. Exposure to these active ingredients was estimated through a combined metric of residential proximity to crops and pesticide use data, and associations with the noted birth defects were calculated.

Methods

Study Population

The study population for our analysis was previously described (Rappazzo et al., 2016; Warren et al., 2014), and is sourced from the NC State Center for Health Statistics. Briefly, the base population was 335,729 geocoded singleton live births in North Carolina from January 1, 2003 to December 31, 2005; births were restricted to those with residence in NC (excluded n = 23,829), gestational age between 20–45 weeks (excluded n = 84), and having data on all demographic characteristics of interest (excluded n = 6,910), leaving an analytic population of 304,906 singleton live births (Rappazzo et al., 2016; Warren et al., 2014).

Outcomes

Data from the NC Birth Defects Monitoring Program (NCBDMP), an active, population-based surveillance system that collects information on medically diagnosed birth defects from delivery hospitals, pediatric and tertiary care hospitals, and other specialty facilities through the first year of life (NCSCHS, 2018) was linked to birth record information to identify cases (Rappazzo et al., 2016; Warren et al., 2014). In our previous analysis, 10 birth

defects (of 42 examined) had a positive association with total pesticide exposure (Rappazzo et al., 2016). These birth defects are used as outcomes here: atrial septal defect (ASD, secundum atrial septal defects, separate from patent foramen ovale), patent ductus arteriosus (PDA) (>2500 gram birth weight), hypoplastic left heart syndrome (HLHS), tracheal esophageal fistula (TEF), hypertrophic pyloric stenosis (HPS), Hirschsprung's disease, hypospadias, upper and lower limb deficiencies, and choanal atresia.

Exposure

Development of the pesticide metric we used as the exposure variable was described previously (Rappazzo et al., 2016; Warren et al., 2014). Briefly, NC crop maps were available for years 2002 and 2008–2010; crop maps for years 2003–2005 were extrapolated from these maps (Warren et al., 2014). A 500 meter (m) buffer was created around each residence at birth and the amount of crop acreage within the buffer was calculated (Warren et al., 2014) Pesticide application data from the National Agricultural Statistics Service were combined with earliest planting and last harvesting dates of each crop and active chemical half-life data to create a probable window of application and to determine if that window overlaps with 1 month before conception through the 3rd month of pregnancy (Warren et al., 2014). Finally, quantities and types of pesticide active ingredients applied during the relevant window in each individual's buffer zone were estimated (Warren et al., 2014). Pesticide exposures were estimated separately based on the individual crop to which they were applied; that is, multiple pesticides may be applied to the same crop during a narrow time window. Similarly, the same pesticide may be applied to multiple crops being cultivated near an individual's residence. Thus, for each study subject, we calculated exposure to a pesticide as the sum of the pounds applied to any crop in the 500 m buffer during the exposure window. From 103 possible pesticide active ingredients, we chose to examine those with over 100,000 application instances, which allows for variability in exposure levels beyond "exposed v. unexposed" for most analyses. Because pesticide exposures were estimated based on crop-type some individuals had multiple exposure estimates for a single pesticide. Seven specific pesticide active ingredients met the application criterion: 2,4-Dichlorophenoxyacetic acid (non dimethyl salt, 2,4-D), mepiquat chloride, pendimethalin, S-metolachlor, Lambda cyhalothrin, glyphosate, and paraquat. With the exception of cyhalothrin, which is an insecticide, the remaining six pesticide active ingredients are herbicidal. Unexposed women either had no crops within their buffer, or the examined pesticides and/or the relevant window of pregnancy occurred during times when no pesticide applications were expected to occur.

Analysis

Analyses followed those performed in previous work (Rappazzo et al., 2016). Briefly, ORs and 95% confidence intervals (CI) were estimated using logistic regression models. In lieu of testing null hypotheses (OR = 1), we examined patterns and precision of estimates. Cases were those infants with birth defect diagnoses from the NCBDMP and were categorized based on the Centers for Disease Control and Prevention/British Pediatric Association coding system. Cases were examined as "any" case as opposed to "isolated" cases (i.e., only having a single birth defect), as we did not observe substantial differences between these in previous work (Rappazzo et al., 2016). Controls were identified from birth certificates and

included any infant free of diagnosed birth defects. For hypospadias, analyses included only male infants. Exposures were investigated as either *a priori* specified categories of exposed (<10th percentile, 10-<50th percentile, 50-<90th percentile, and >=90th percentile) versus unexposed, or as collapsed versions of this (unexposed, <50th percentile exposed, 50th percentile exposed, or exposed vs. unexposed), as appropriate, to ensure that each exposure category had at least 5 cases for each birth defect. Confounders, identified *a priori* through expert knowledge and literature review, included: maternal race/ethnicity (non-Hispanic white, non-Hispanic Black, Hispanic, Other), maternal education (less than high school (<HS), high school graduate (HS), and greater than high school (>HS)), marital status (married, unmarried), maternal age (<20, 20-<35, >=35 years), and smoking status during pregnancy (ever, never). Individual birth defects were examined as outcomes in separate models with each pesticide active ingredient.

As sensitivity analyses, we examined potential confounding by co-occurring pesticides. This was done using logistic regression models including two pesticide active ingredients for all pesticide-birth defect combinations, estimating the effect of one while adjusting for the other. Given the high correlation between the pesticide active ingredients and the potential for small numbers of exposed cases in any given category of multiple pesticide exposure, these results should be interpreted with caution. In addition, we performed Bayesian logistic regression analogues of all models with a fixed shrinkage prior specification for ASD, PDA, HPS, and hypospadias (other defects not included due to limited case numbers), including all examined pesticide active ingredients. In brief, regression coefficients are assumed to have a weakly informative multivariate normal prior specification, which is similar to the P1 model in MacLehose, Dunson, Herring, and Hoppin (2007). All models are estimated via an MCMC Gibbs sampling algorithm with default prior values specified by the SAS programming software. We run the sampling algorithm in each model for 10,000 iterations and exclude the first 2,000 as a burn-in period. We choose this modeling framework to compare the potential of unstable parameter estimates from the MLE models in our highly correlated predictor setting.

All analyses were performed in SAS 9.4 (SAS Institute, Cary NC) and figures were created in R 3.4.0 and Rstudio 1.0.143 with ggplot2 (R. C. Team, 2017; R. S. Team, 2015; Wickham, 2009). This research was approved by the University of North Carolina at Chapel Hill's Office of Human Research Ethics.

Results

Distributions of demographic factors by case status are presented in Table 1. There were 298,548 controls (152,402 male controls for hypospadias) in our study. Case numbers ranged from over 1,000 for ASD and PDA to 39 for lower limb defects. Distributions across race/ethnicity were similar though there were fewer hypospadias cases with Hispanic mothers, and more cases of ASD and Hirschsprung's were identified as being Black. Male infants predominated in both HPS and Hirschsprung's; cases with Hirschsprung's were also more likely to be born to multiparous individuals than controls. Marital status was also different (higher percent unmarried) for mothers of cases with Hirschsprung's and limb defects. Education levels were similar across cases and controls, while mothers identifying

as smokers were more likely to deliver infants with limb defects. In addition, case infants for ASD, PDA, hypospadias, TEF, choanal atresia, and lower limb defects were more likely than control individuals to have older mothers.

Exposures to pesticide active ingredients were not appreciably different by demographic factors (Supplemental Table S.1). Generally, over 50% of births fell into the unexposed categories, except for paraquat where the proportion of unexposed individuals was generally less than 20%, due to ubiquitous use as a commercial weed killer (EPA, 2018) (Tables 2a and 2b). For two birth defects, the numbers of unexposed cases for paraquat were below 5 (choanal atresia and lower limb defects), therefore results for those models should be viewed cautiously. Pesticide active ingredient correlations varied from low to high, with Spearman's correlation coefficients ranging from 0.17 for 2,4-D and cyhalothrin to 0.78 for cyhalothrin and pendimethalin, though the majority were in the moderate (0.4 – 0.7) to high (>0.7) range (Table 3); correlations were similar using either five- or three-category exposure levels.

Associations with pesticide active ingredients varied across those birth defects examined with five categories of exposure, though with many positive associations observed (Supplemental Table S.2). ORs for ASD were elevated at the highest level of exposure (>= 90th percentile) for all examined pesticide active ingredients, though patterns at lower levels of exposure were not consistent across different pesticides (Figure 1). Some associations exhibited monotonic increasing associations with increasing exposure level (cyhalothrin, pendimethalin), while others appeared to have threshold effects or were non-linear with generally elevated ORs (2,4-D, glyphosate, mepiquat, metolachlor). Paraquat exhibited a U-shaped response, with elevated ORs only for the <10th and >=90th exposure levels. When adjusting for other pesticide active ingredients, associations with ASD are attenuated towards the null at the lowest levels of exposure across several pesticide active ingredients (Supplemental Table S.3, Supplemental Figure S.1). Associations with 2,4-D and pendimethalin remain robust to co-exposure adjustment, while ORs for metolachlor and paraquat move toward the null at all levels. Associations for cyhalothrin and glyphosate are robust to adjustment, except with pesticide active ingredients with which they have a high correlation (e.g., cyhalothrin and mepiquat have a correlation of ~0.70).

Associations for PDA were somewhat different (Figure 2): no evidence of an association for metolachlor was observed; ORs with paraquat were elevated only at the highest exposure level; ORs for cyhalothrin, glyphosate, and pendimethalin showed either slight elevations from the null or apparent threshold effects; and monotonic increases in the ORs were observed for mepiquat and 2,4-D. With adjustment for an additional pesticide active ingredient, associations for mepiquat were attenuated except at the highest exposure level, while 2,4-D and pendimethalin remained largely unchanged (Supplemental Table S.4, Supplemental Figure S.2). With HPS, ORs were all either elevated or showing threshold effects, though patterns with increasing exposure levels differed across pesticide active ingredients (Figure 3). HPS ORs were largely robust to co-pesticide adjustment, with the potential exception of cyhalothrin and those active ingredients with which it had a high correlation (Supplemental Table S.5, Supplemental Figure S.3). Associations for hypospadias were similar to those for PDA, with mepiquat and 2, 4-D having increasing ORs with increasing exposure levels (Figure 4). Associations for hypospadias with

pendimethalin and mepiquat were attenuated toward the null with adjustment for an additional pesticide active ingredient, while associations for metolachlor at the lowest and highest exposure levels moved away from the null, and associations for 2,4-D cyhalothrin, and paraquat remained largely unchanged (Supplemental Table S.6, Supplemental Figure S. 4). Results for Bayesian sensitivity analyses, for models with all pesticide active ingredients, were frequently attenuated towards the null, and less precise (Supplemental Table S.7). Many associations remained similar to those observed in the primary analyses, though several were fully attenuated or crossed the null.

Associations for those birth defects examined with the 3-category exposure levels were also varied, though again we observed a number of positive associations. While some birth defects had positive associations with paraquat exposure, confidence intervals were generally unstable (Figures 5–10, Supplemental Table S.2). HLHS was not associated with 2,4-D, cyhalothrin, or glyphosate, but had positive associations with mepiquat, metolachlor, and pendimethalin (Figure 5). There was no evidence of association between non-paraquat pesticide active ingredients and TEF (Figure 6). We observed elevated ORs for choanal atresia with exposure to 2,4-D and metolachlor, while the other pesticide active ingredients showed no evidence of association (Figure 7). ORs for Hirschsprung's are elevated at the higher level of exposure only for 2,4-D, S-metolachlor, and paraquat; however, for cyhalothrin, glyphosate, mepiquat, and pendimethalin ORs are elevated at all levels of exposure (Figure 8). For upper limb defects, ORs are elevated from the null at the higher level of exposure for cyhalothrin, glyphosate, and mepiquat (Figure 9). For the other pesticide active ingredients patterns are less clear. For example, effect estimates for pendimethalin are below the null for <50th percentile exposures compared to unexposed, but elevated at the >=50th percentile exposure level. Associations with lower limb defects are generally elevated at the higher exposure level for all pesticide active ingredients (Figure 10). Paraquat estimates are elevated but highly unstable due to limited numbers of unexposed cases. Because of small case numbers in the exposed groups and the likelihood of sparse data concerns, results for adjustment with an additional pesticide active ingredient with these birth defects are presented only in supplemental material (Supplemental Figures S.5-S.10, Supplemental Tables S.8–13).

Discussion

In our examination of the associations between seven pesticide active ingredients and ten birth defects, we observed several positive associations across birth defects and pesticides, particularly at the higher levels of exposure, with several pesticide-birth defect pairs demonstrating monotonic increasing associations. However, no particular pesticide active ingredient emerged as associated with all birth defects examined and patterns of association vary across both birth defects and pesticide active ingredients. While adjustment for pesticide co-exposures attenuates ORs toward the null, associations that were positive in single-pesticide models generally remain so in models adjusted for an additional pesticide active ingredient.

Comparison with other literature investigating pesticide active ingredients and individual birth defects can be difficult due to differences in ingredients examined, groups or classes of

pesticides examined rather than individual ingredients, or differences in how exposure levels were classified. In several California-based studies, pesticide usage reports were linked to land survey data and pesticide exposures were estimated for 500 to 1000 meter buffers around residences. Carmichael, Yang, et al. (2014) found cyhalothrin exposures associated with increased odds of ASD, similar to what we observed in this analysis, while HLHS was associated with strobin and triazine exposure (not investigated here). While Shaw et al. (2014) examined gastroschisis and observed associations with dichlorophenoxy salt or ester and pendimethalin exposures; in the same study glyphosate and bipyridylium pesticides (which included paraquat) were not associated with increased odds of gastroschisis. In our previous analysis, we observed no evidence of association with total pesticide exposure and gastroschisis, which may be due to the large number of pesticide active ingredients included in that exposure (Rappazzo et al., 2016). Additionally, an examination of neural tube defects found increased ORs with 2,4-D exposure but not with glyphosate (Rull et al., 2006). Generally, information on combinations of specific pesticide active ingredients and specific birth defects is limited.

There are several possible pathways by which pesticides in general, and specific compounds might act on fetal development. Mechanisms studied have been linked to chronic diseases (e.g., cancer, neurodegeneration, cardiometabolic outcomes) (Mostafalou & Abdollahi, 2013), but can potentially disrupt fetal developmental in ways that might lead to birth defect formation. Some of these mechanisms include: endocrine disruption, which could affect hormone-dependent development; oxidative stress and inflammatory response, which could act through maternal systematic processes; or inhibition of electron chain transport activity or mitochondrial dysfunction, which could disrupt signaling or energy generation during growth and organ formation (Abdollahi, Ranjbar, Shadnia, Nikfar, & Rezaie, 2004; Mokarizadeh, Faryabi, Rezvanfar, & Abdollahi, 2015; Mostafalou & Abdollahi, 2013, 2017).

In terms of the specific pesticide active ingredients we examined, though they were chosen for inclusion based on commonality of exposure rather than potential toxicity, there have been associations with reproductive health outcomes, however not necessarily birth defects. 2,4-D, the most commonly applied pesticide, has been linked to persistent sperm abnormalities in occupational farm sprayers (Abdollahi et al., 2004; Lerda & Rizzi, 1991; NIOSH, 2014), poor semen quality in men living in agrarian areas (Swan, 2003; Swan et al., 2003), and with early spontaneous abortion in farm operators and dwellers (Arbuckle, Lin, & Mery, 2001). In rat studies, skeletal abnormalities, pup loss, thyroid dysregulation, and changes to gonad weights were observed at high doses of 2,4-D (EPA, 2007). For glyphosate, research on reproductive health outcomes has been limited. In humans, glyphosate has been variably associated with birth defects, with no clear relationship emerging from the small number of studies (de Araujo, Delgado, & Paumgartten, 2016). In zebrafish, glyphosate exposure caused heart abnormalities (Roy, Ochs, Zambrzycka, & Anderson, 2016), and inhibited carbonic anhydrase activity and led to body malformations (Sulukan et al., 2017). Pyrethroids, of which cyhalothrin is one, have been associated with sperm abnormalities (Koureas, Tsakalof, Tsatsakis, & Hadjichristodoulou, 2012; Saillenfait, Ndiaye, & Sabate, 2015). The other pesticides examined here have limited or no evidence of

reproductive toxicity, and may be acting as proxies for more toxic pesticide active ingredients in our analysis.

A major strength of our study was the ability to examine specific pesticide active ingredients. The pesticide exposure metric used is an improvement of survey data that uses crop phenology, pesticide application records, and residential location, and includes timing of pesticide usage and pregnancy to estimate pesticide active ingredient exposure (Warren et al., 2014). Through this metric we were able to investigate beyond simple exposure to any pesticide or even to a particular class of pesticides. This is of particular benefit in areas where detailed pesticide use and application data are sparse or non-existent. The population-based design and complete case ascertainment through the first year of life reduce the likelihood of selection bias and bias due to misclassification of cases. We were also able to examine individual birth defects, which is beneficial as etiologies may differ even between birth defects within the same organ systems.

As a corollary to the use of individual birth defects and specific pesticide active ingredients, a limitation is small numbers of cases and of exposed cases, which may result in unstable effect measure estimates. Confounding by unexamined pesticides is a possibility as well; without larger numbers of subjects and more variability in exposures, this is difficult to fully investigate, though we have attempted to at least examine potential confounding by including adjustment for additional pesticide active ingredients in this analysis. Relatedly, pesticide active ingredients examined had moderate to high correlations and though we examined two pesticide models to evaluate confounding, small numbers of exposed cases in any given category of multiple pesticide exposures mean results of those analyses should be interpreted with caution.

Limitations from the previous study of total pesticide exposures (Rappazzo et al., 2016) and the creation of the exposure metric (Warren et al., 2014) persist and merit discussion. Among these are the use of live births occurring after 20 weeks of completed gestation as the cohort base rather than the true at-risk population of conceptions reaching implantation, which may attenuate observed effects and is a common limitation to many birth defect studies, as conceptions themselves are often unrecorded. There is also the potential for unmeasured confounding from unmeasured variables such as parental occupational exposures, which we were unable to control for in this analysis as parental occupation is not recorded on birth records. Exclusions due to geocoding and concerns of residential mobility during pregnancy are concerns because it is possible that individuals excluded due to rural route addresses have more potential to be exposed to agricultural pesticides, and residential mobility may introduce exposure misclassification. Research on other environmental exposures, such as air pollution, has shown that effects of residential mobility are limited and most within-pregnancy moves are over short distances (Bell & Belanger, 2012; Lupo et al., 2010), and misclassification from this would likely attenuate effect estimates toward the null. For the exposure metric, tobacco data was unavailable and so was excluded from the exposure metric. However previously published total pesticide sensitivity analyses results were unchanged for individuals with and without tobacco within their 500m buffer (Rappazzo et al., 2016). In addition, crop map data were not available for the years of birth data examined and so crop information was interpolated across years (Warren et al., 2014).

Results from the Bayesian sensitivity analysis suggest that the complexities of mixtures analysis remain in populations that are potentially exposed to many pesticide active ingredients simultaneously, and that while one pesticide ingredient may have a positive association in a single exposure model, it may be acting as a representative of another pesticide active ingredient or as a surrogate for a mixture of pesticide active ingredients.

It is important to note that the exposure metric used herein is an estimate of exposure and should not be used as a surrogate for dose. Specifically, we used a novel metric of residential proximity to agricultural pesticides that balances computational difficulty and expense with information on temporal and spatial specificity of pesticide application and timing of exposure (Warren et al., 2014) to further investigate our initial results informing the relationship between pesticide exposure and birth defects (Rappazzo et al., 2016). We observed several positive associations, particularly at the higher levels of pesticide exposure, with several pesticide-birth defect pairs demonstrating stronger associations as estimates of pesticide exposure increased. Our results provide preliminary evidence for positive associations between some pesticide active ingredients and birth defect phenotypes. Additional studies using improved exposure assessment (e.g., biomarkers of exposure) will be useful in building a body of evidence that is sufficient for making determinations about the causal nature of the associations we observed.

Overall, this work represents an improvement in the ability to examine specific pesticide active ingredients in geographically-based epidemiologic studies of pesticides and birth defects (outside of California). It also highlights the ongoing issues with these analyses, mainly small numbers and the potential for confounding by joint pesticide exposures. We observed positive associations between several birth defects across organ systems and pesticide active ingredients commonly applied in NC agriculture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Literature Cited

Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, & Rezaie A (2004). Pesticides and oxidative stress: a review. Med Sci Monit, 10(6), RA141–147. [PubMed: 15173684]

Agopian AJ, Cai Y, Langlois PH, Canfield MA, & Lupo PJ (2013). Maternal residential atrazine exposure and risk for choanal atresia and stenosis in offspring. J Pediatr, 162(3), 581–586. doi: 10.1016/j.jpeds.2012.08.012 [PubMed: 23036484]

Agopian AJ, Langlois PH, Cai Y, Canfield MA, & Lupo PJ (2013). Maternal residential atrazine exposure and gastroschisis by maternal age. Matern Child Health J, 17(10), 1768–1775. doi: 10.1007/s10995-012-1196-3 [PubMed: 23184502]

Agopian AJ, Lupo PJ, Canfield MA, & Langlois PH (2013). Case-control study of maternal residential atrazine exposure and male genital malformations. Am J Med Genet A, 161A(5), 977–982. doi: 10.1002/ajmg.a.35815 [PubMed: 23494929]

Arbuckle TE, Lin Z, & Mery LS (2001). An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ Health Perspect, 109(8), 851–857. [PubMed: 11564623]

Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, . . . Robson MG (2010). Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. Science of the total environment, 408(4), 790–795. [PubMed: 19900697]

Bell ML, & Belanger K (2012). Review of research on residential mobility during pregnancy: consequences for assessment of prenatal environmental exposures. Journal of Exposure Science and Environmental Epidemiology, 22(5), 429–438. [PubMed: 22617723]

Bradman A, Barr DB, Claus Henn BG, Drumheller T, Curry C, & Eskenazi B (2003). Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study. Environ Health Perspect, 111(14), 1779–1782. [PubMed: 14594631]

Bradman MA, Harnly ME, Draper W, Seidel S, Teran S, Wakeham D, & Neutra R (1997). Pesticide exposures to children from California's Central Valley: results of a pilot study. J Expo Anal Environ Epidemiol, 7(2), 217–234. [PubMed: 9185013]

Carmichael SL, Ma C, Tinker S, Rasmussen SA, Shaw GM, & National Birth Defects Prevention, S. (2014). Maternal stressors and social support as risks for delivering babies with structural birth defects. Paediatr Perinat Epidemiol, 28(4), 338–344. doi:10.1111/ppe.12123 [PubMed: 24697924]

Carmichael SL, Yang W, Roberts E, Kegley SE, Padula AM, English PB, . . . Shaw GM (2014). Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. Environ Res, 135, 133–138. doi:10.1016/j.envres.2014.08.030 [PubMed: 25262086]

Carmichael SL, Yang W, Roberts EM, Kegley SE, Wolff C, Guo L, . . . Shaw GM (2013). Hypospadias and residential proximity to pesticide applications. Pediatrics, 132(5), e1216–1226. doi:10.1542/peds.2013-1429 [PubMed: 24167181]

CDC. (2018). Facts about birth defects. Retrieved from http://www.cdc.gov/ncbddd/birthdefects/facts.html

de Araujo JS, Delgado IF, & Paumgartten FJ (2016). Glyphosate and adverse pregnancy outcomes, a systematic review of observational studies. BMC Public Health, 16, 472. doi:10.1186/s12889-016-3153-3 [PubMed: 27267204]

Decoufle P, Boyle CA, Paulozzi LJ, & Lary JM (2001). Increased risk for developmental disabilities in children who have major birth defects: a population-based study. Pediatrics, 108(3), 728–734. [PubMed: 11533343]

EPA. (2007). 2,4-Dichlorophenoxyacetic Acid (2,4-D) TEACH Chemical Summary. Retrieved from https://nepis.epa.gov/Exe/ZyNET.exe/P100BNRV.txt?ZyActionD=ZyDocument&Client=EPA&Index=2006%20Thru%202010&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C06THRU10%5CTXT%5C00000028%5CP100BNRV.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1

EPA. (2018). Paraquat Dichloride. Retrieved from https://www.epa.gov/ingredients-used-pesticide-products/paraquat-dichloride

Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, & Burroughs BL (2002). Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red

River Valley of Minnesota, USA. Environ Health Perspect, 110 Suppl 3, 441–449. [PubMed: 12060842]

Heeren GA, Tyler J, & Mandeya A (2003). Agricultural chemical exposures and birth defects in the Eastern Cape Province, South Africa: a case-control study. Environ Health, 2(1), 11. doi: 10.1186/1476-069X-2-11 [PubMed: 14613490]

Kielb C, Lin S, Herdt-Losavio M, Bell E, Chapman B, Rocheleau CM, . . . National Birth Defects Prevention, S. (2014). Maternal periconceptional occupational exposure to pesticides and selected musculoskeletal birth defects. Int J Hyg Environ Health, 217(2–3), 248–254. doi:10.1016/j.ijheh. 2013.06.003 [PubMed: 23871272]

Koureas M, Tsakalof A, Tsatsakis A, & Hadjichristodoulou C (2012). Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett, 210(2), 155–168. doi:10.1016/j.toxlet.2011.10.007 [PubMed: 22020228]

Kristensen P, Irgens LM, Andersen A, Bye AS, & Sundheim L (1997). Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology, 8(5), 537–544. [PubMed: 9270956]

Lerda D, & Rizzi R (1991). Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). Mutat Res, 262(1), 47–50. [PubMed: 1986284]

Lupo PJ, Symanski E, Chan W, Mitchell LE, Waller DK, Canfield MA, & Langlois PH (2010). Differences in exposure assignment between conception and delivery: the impact of maternal mobility. Paediatr Perinat Epidemiol, 24(2), 200–208. [PubMed: 20415777]

MacLehose RF, Dunson DB, Herring AH, & Hoppin JA (2007). Bayesian methods for highly correlated exposure data. Epidemiology, 18(2), 199–207. doi:10.1097/01.ede. 0000256320.30737.c0 [PubMed: 17272963]

Mai CT, Kucik JE, Isenburg J, Feldkamp ML, Marengo LK, Bugenske EM, . . . National Birth Defects Prevention, N. (2013). Selected birth defects data from population-based birth defects surveillance programs in the United States, 2006 to 2010: featuring trisomy conditions. Birth Defects Res A Clin Mol Teratol, 97(11), 709–725. doi:10.1002/bdra.23198 [PubMed: 24265125]

Matthews TJ, & MacDorman MF (2013). Infant mortality statistics from the 2010 period linked birth/infant death data set. Natl Vital Stat Rep, 62(8), 1–26.

Meyer KJ, Reif JS, Veeramachaneni DN, Luben TJ, Mosley BS, & Nuckols JR (2006). Agricultural pesticide use and hypospadias in eastern Arkansas. Environ Health Perspect, 114(10), 1589–1595. [PubMed: 17035148]

Mokarizadeh A, Faryabi MR, Rezvanfar MA, & Abdollahi M (2015). A comprehensive review of pesticides and the immune dysregulation: mechanisms, evidence and consequences. Toxicol Mech Methods, 25(4), 258–278. doi:10.3109/15376516.2015.1020182 [PubMed: 25757504]

Mostafalou S, & Abdollahi M (2013). Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol Appl Pharmacol, 268(2), 157–177. doi:10.1016/j.taap.2013.01.025 [PubMed: 23402800]

Mostafalou S, & Abdollahi M (2017). Pesticides: an update of human exposure and toxicity. Arch Toxicol, 91(2), 549–599. doi:10.1007/s00204-016-1849-x [PubMed: 27722929]

NCSCHS. (2018). Birth Defects Monitoring Program. Retrieved from http://www.schs.state.nc.us/units/bdmp/

NIOSH. (2014). The Effects of Workplace Hazards on Male Reproductive Health. Retrieved from https://www.cdc.gov/niosh/docs/96-132/default.html

Ochoa-Acuna H, & Carbajo C (2009). Risk of limb birth defects and mother's home proximity to cornfields. Sci Total Environ, 407(15), 4447–4451. doi:10.1016/j.scitotenv.2009.04.028 [PubMed: 19427676]

Rappazzo KM, Warren JL, Meyer RE, Herring AH, Sanders AP, Brownstein NC, & Luben TJ (2016). Maternal residential exposure to agricultural pesticides and birth defects in a 2003 to 2005 North Carolina birth cohort. Birth Defects Res A Clin Mol Teratol, 106(4), 240–249. doi:10.1002/bdra.23479 [PubMed: 26970546]

Roy NM, Ochs J, Zambrzycka E, & Anderson A (2016). Glyphosate induces cardiovascular toxicity in Danio rerio. Environ Toxicol Pharmacol, 46, 292–300. doi:10.1016/j.etap.2016.08.010 [PubMed: 27525560]

Rull RP, & Ritz B (2003). Historical pesticide exposure in California using pesticide use reports and land-use surveys: an assessment of misclassification error and bias. Environ Health Perspect, 111(13), 1582–1589. [PubMed: 14527836]

Rull RP, Ritz B, & Shaw GM (2006). Neural tube defects and maternal residential proximity to agricultural pesticide applications. Am J Epidemiol, 163(8), 743–753. doi:10.1093/aje/kwj101 [PubMed: 16495467]

Saillenfait AM, Ndiaye D, & Sabate JP (2015). Pyrethroids: exposure and health effects--an update. Int J Hyg Environ Health, 218(3), 281–292. doi:10.1016/j.ijheh.2015.01.002 [PubMed: 25648288]

Shaw GM, Wasserman CR, O'Malley CD, Nelson V, & Jackson RJ (1999). Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiology, 10(1), 60–66. [PubMed: 9888281]

Shaw GM, Yang W, Roberts E, Kegley SE, Padula A, English PB, & Carmichael SL (2014). Early pregnancy agricultural pesticide exposures and risk of gastroschisis among offspring in the San Joaquin Valley of California. Birth Defects Res A Clin Mol Teratol, 100(9), 686–694. doi:10.1002/bdra.23263 [PubMed: 24910073]

Sulukan E, Kokturk M, Ceylan H, Beydemir S, Isik M, Atamanalp M, & Ceyhun SB (2017). An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (Daino rerio). Chemosphere, 180, 77–85. doi:10.1016/j.chemosphere.2017.04.018 [PubMed: 28391155]

Swan SH (2003). Semen quality in relation to pesticide exposure in Missouri males. Mo Med, 100(6), 554. [PubMed: 14699813]

Swan SH, Kruse RL, Liu F, Barr DB, Drobnis EZ, Redmon JB, . . . Study for Future Families Research, G. (2003). Semen quality in relation to biomarkers of pesticide exposure. Environ Health Perspect, 111(12), 1478–1484. [PubMed: 12948887]

Team, R. C. (2017). A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria Retrieved from https://www.R-project.org

Team R. S. (2015). RStudio: Integrated Development for R. Boston, MA Retrieved from http://www.rstudio.com

Warren JL, Luben TJ, Sanders AP, Brownstein NC, Herring AH, & Meyer RE (2014). An evaluation of metrics for assessing maternal exposure to agricultural pesticides. J Expo Sci Environ Epidemiol, 24(5), 497–503. doi:10.1038/jes.2013.75 [PubMed: 24149974]

Wickham H (2009). ggplot2: Elegant Graphics for Data Analysis. New York, NY: Springer-Verlag

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(6), 740–748. doi:10.1093/aje/kwt324 [PubMed: 24553680]

Figure 1:
Odds ratios and 95% confidence intervals for atrial septal defects (ASD) with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.

Figure 2:
Odds ratios and 95% confidence intervals for Patent Ductus Arteriosus (PDA) with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.

Figure 3:
Odds ratios and 95% confidence intervals for hypertrophic pyloric stenosis (HPS) with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
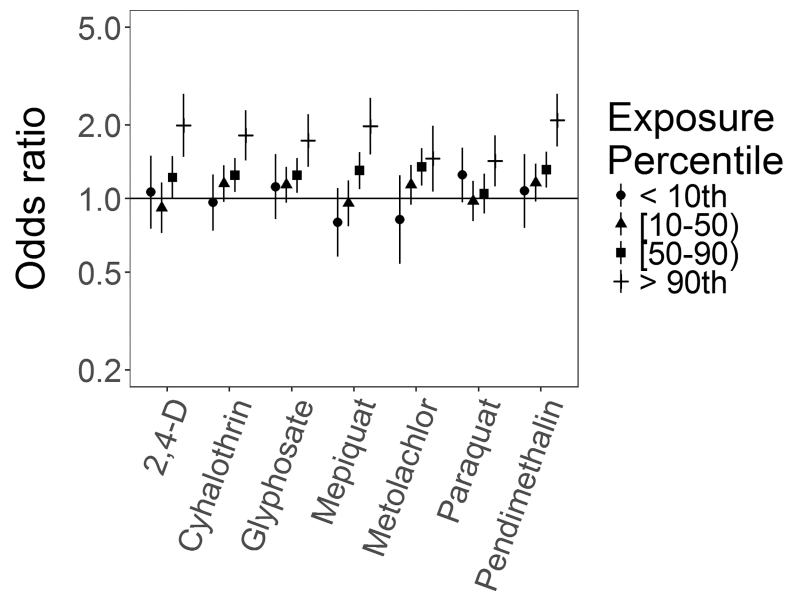
Figure 4:
Odds ratios and 95% confidence intervals for hypospadias with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.

Figure 5:
Odds ratios and 95% confidence intervals for hypoplastic left heart syndrome (HLHS) with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
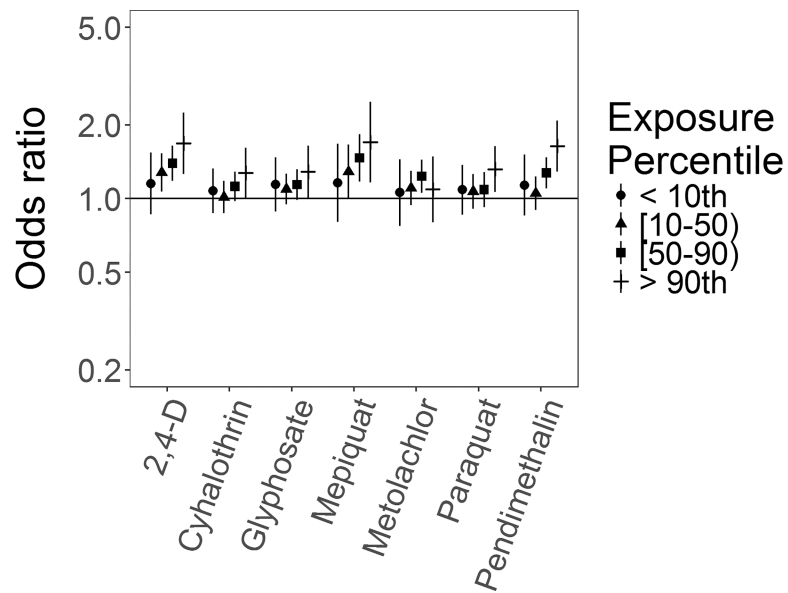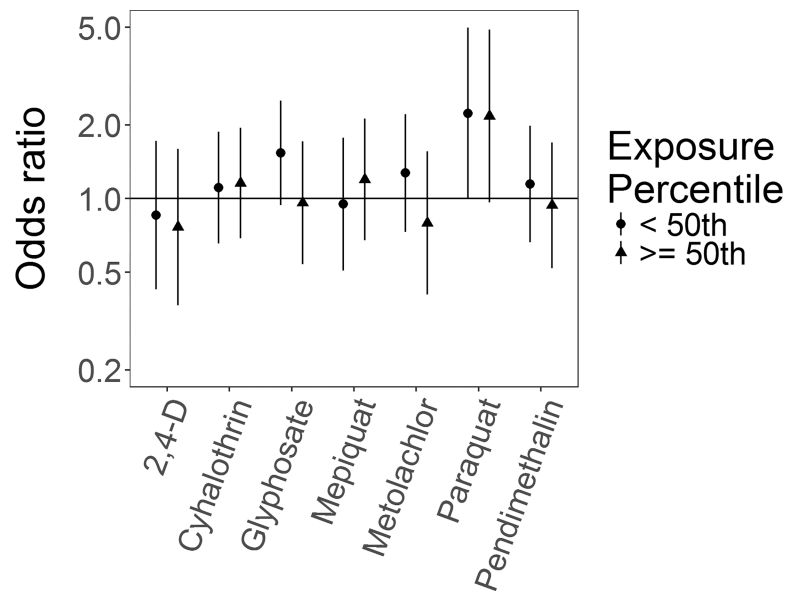
Figure 6:
Odds ratios and 95% confidence intervals for tracheal/esophageal fistula (TEF) with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
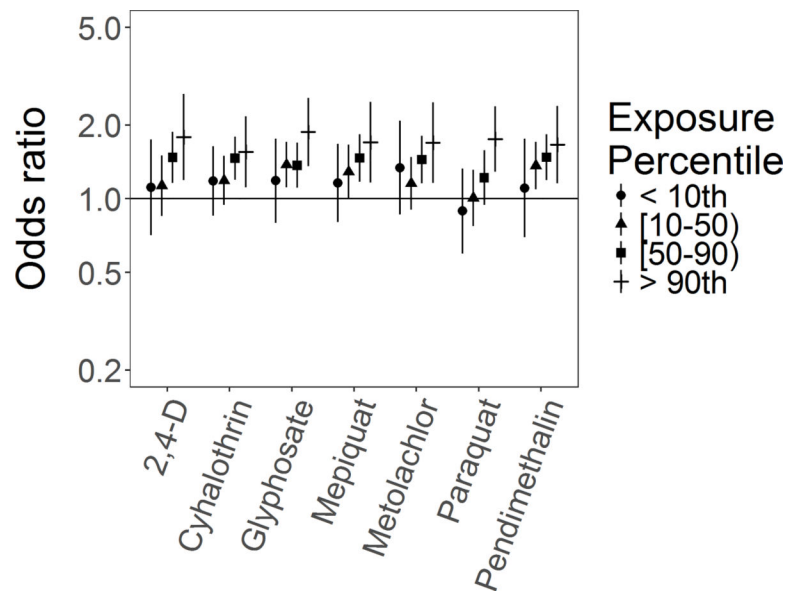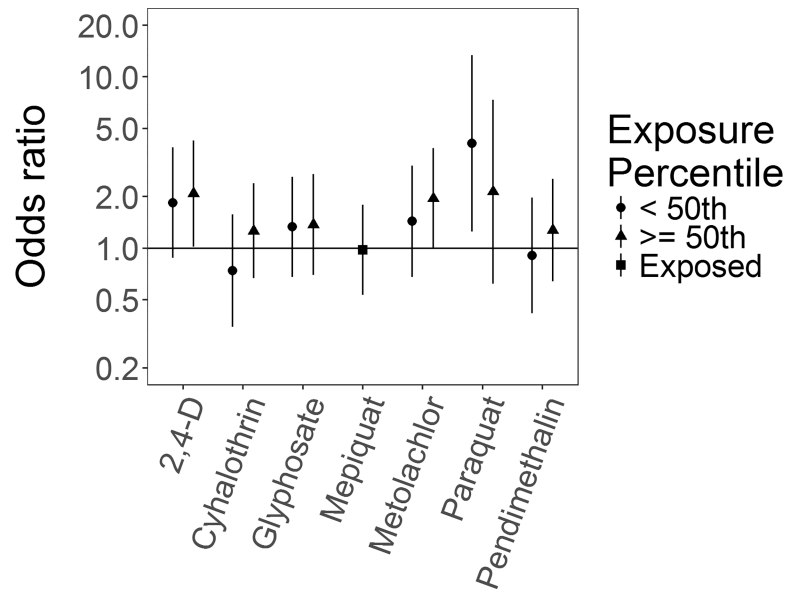
Figure 7:
Odds ratios and 95% confidence intervals for choanal atresia with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
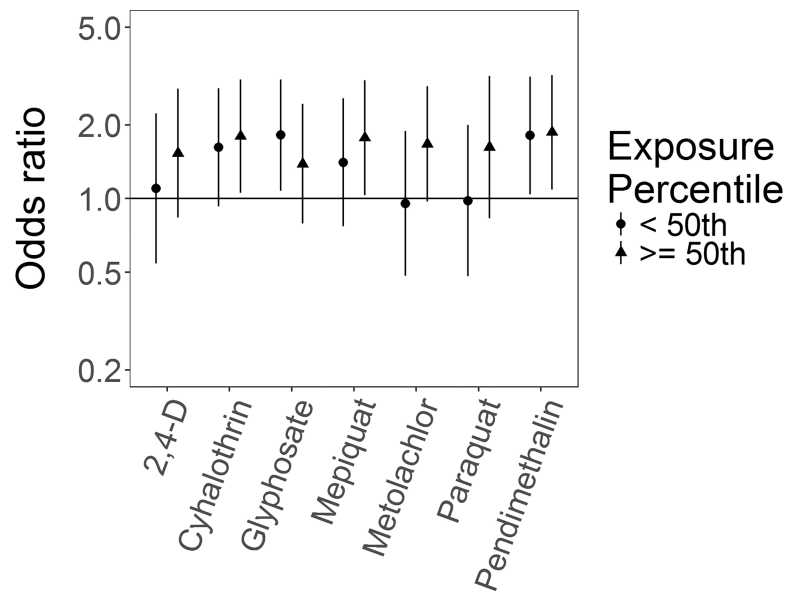
Figure 8:
Odds ratios and 95% confidence intervals for Hirschsprung's disease with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
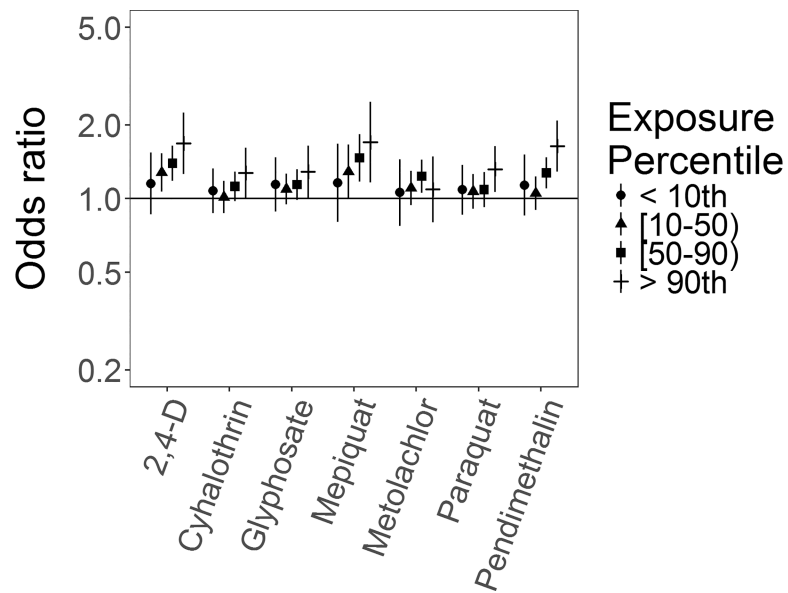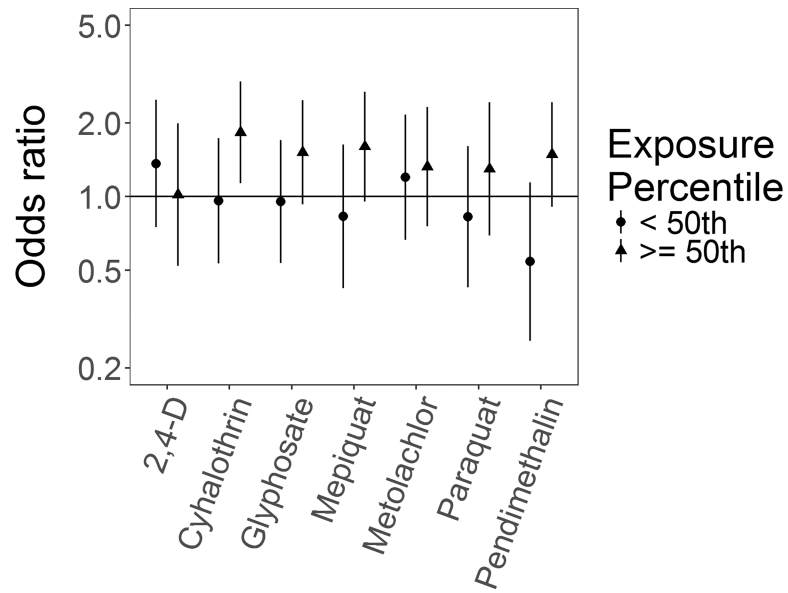
Figure 9:
Odds ratios and 95% confidence intervals for upper limb defects with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
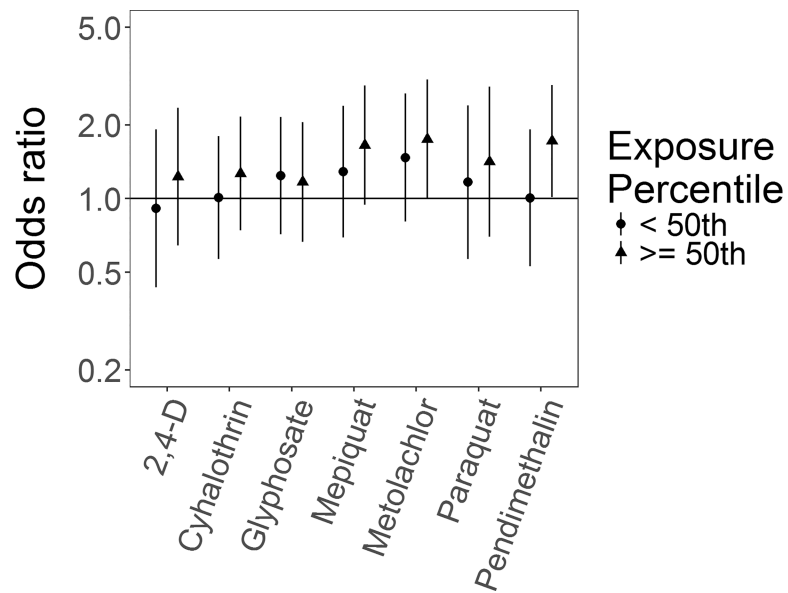
Figure 10:
Odds ratios and 95% confidence intervals for lower limb defects with exposure to pesticide active ingredients compared to unexposed, adjusted for maternal race/ethnicity, age at delivery, education, marital status, and smoking status. Numerical ORs and 95% CIs are reported in Supplementary Table S.2.
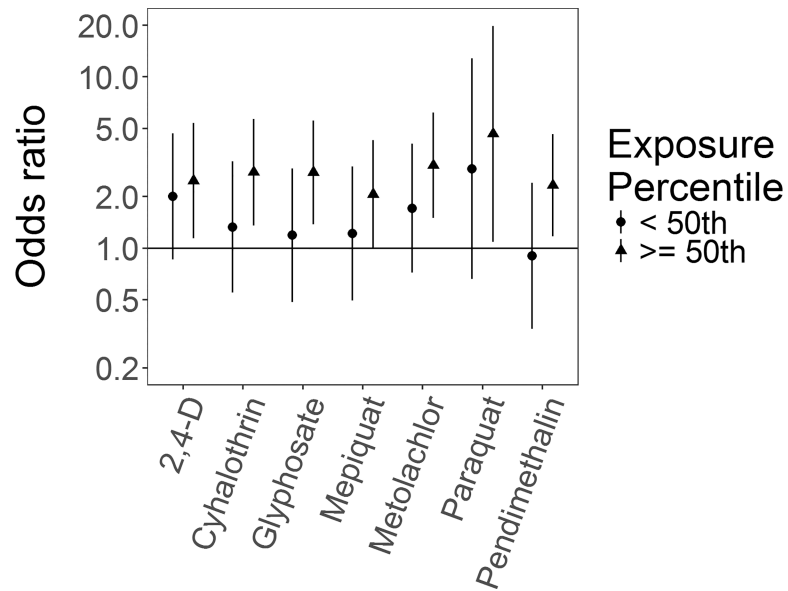
Table 1:

Descriptive statistics by case status for included births in North Carolina 2003–2005. N (%)

	Control	ASD	PDA	HPS	Hypospadias	HLHS	TEF	Choanal Atresia	Hirschsprungs	Upper limb	Lower limb
N	298548	1020	1364	592	936	75	84	50	77	84	39
Race/ethnicity											
White, non-Hispanic	179,856 (60)	541 (53)	796 (58)	396 (67)	670 (72)	47 (63)	54 (64)	33 (66)	34 (44)	51 (61)	20 (51)
Black, non-Hispanic	65,284 (22)	303 (30)	339 (25)	71 (12)	194 (21)	18 (24)	18 (21)	12 (24)	34 (44)	15 (18)	11 (28)
Hispanic	41,527 (14)	135 (13)	186 (14)	102 (17)	48 (5)	10 (13)	11 (13)	5 (10)	8 (10)	14 (17)	6 (15)
Other, non-Hispanic	11,881 (4)	41 (4)	43 (3)	23 (4)	24 (3)	0 (0)	1 (1)	0 (0)	1 (1)	4 (5)	2 (5)
Sex											
Female	146,146 (49)	494 (48)	641 (47)	129 (22)	0 (0)*	32 (43)	34 (40)	24 (48)	20 (26)	38 (45)	18 (46)
Male	152,402 (51)	526 (52)	723 (53)	463 (78)	936 (100)	43 (57)	50 (60)	26 (52)	57 (74)	46 (55)	21 (54)
Maternal education											
Greater than high school	146,522 (49)	439 (43)	676 (50)	200 (34)	526 (56)	30 (40)	40 (48)	27 (54)	30 (39)	34 (40)	14 (36)
High school	85,791 (29)	306 (30)	393 (29)	216 (36)	261 (28)	26 (35)	27 (32)	13 (26)	28 (36)	25 (30)	12 (31)
Less than high school	66,235 (22)	275 (27)	295 (22)	176 (30)	149 (16)	19 (25)	17 (20)	10 (20)	19 (25)	25 (30)	13 (33)
Marital status											
Married	191,015 (64)	621 (61)	881 (65)	330 (56)	620 (66)	44 (59)	49 (58)	34 (68)	39 (51)	46 (55)	18 (46)
Unmarried	107,533 (36)	399 (39)	483 (35)	262 (44)	316 (34)	31 (41)	35 (42)	16 (32)	38 (49)	38 (45)	21 (54)
Smoking status											
Non-smoker	261,483 (88)	877 (86)	1,226 (90)	474 (80)	831 (89)	70 (93)	69 (82)	43 (86)	65 (84)	59 (70)	24 (62)
Smoker	37,065 (12)	143 (14)	138 (10)	118 (20)	105 (11)	5 (7)	15 (18)	7 (14)	12 (16)	25 (30)	15 (38)
Diabetes status											
No	290,340 (97)	968 (95)	1,278 (94)	569 (96)	907 (97)	73 (97)	79 (94)	46 (92)	77 (100)	83 (99)	36 (92)
Yes	8,208 (3)	52 (5)	86 (6)	23 (4)	29 (3)	2 (3)	5 (6)	4 (8)	0 (0)	1 (1)	3 (8)
Parity											
Primiparous	123,055 (41)	379 (37)	525 (38)	242 (41)	458 (49)	31 (41)	39 (46)	20 (40)	21 (27)	42 (50)	18 (46)
Multiparous	175,493 (59)	641 (63)	839 (62)	350 (59)	478 (51)	44 (59)	45 (54)	30 (60)	56 (73)	42 (50)	21 (54)
Age group											
Less than 20	33,851 (11)	115 (11)	131 (10)	100 (17)	100 (11)	11 (15)	8 (10)	4 (8)	7 (9)	8 (10)	6 (15)
20 – less than 35	228,261 (76)	745 (73)	999 (73)	450 (76)	697 (74)	57 (76)	60 (71)	37 (74)	63 (82)	68 (81)	27 (69)

	Control	ASD	PDA	HPS	Hypospadias	HLHS	TEF	Choanal Atresia	Hirschsprungs	Upper limb	Lower limb
35+	36,436 (12)	160 (16)	234 (17)	42 (7)	139 (15)	7 (9)	16 (19)	9 (18)	7 (9)	8 (10)	6 (15)

*
Hypospadias only occurs in male infants, so female infants were not used in this analysis

ASD: atrial septal defects

PDA: patent ductus arteriosus

HPS: hypertrophic pyloric stenosis

HLHS: hypoplastic left heart syndrome

TEF: tracheal esophageal fistula

Table 2a:

Exposure distributions by case status for included births in North Carolina 2003–2005, N (%)

Pesticide active ingredient	Exposure level	Control	ASD	PDA	HPS	Hypospadias
N		298548	1020	1364	592	936
2, 4-D	Unexposed	227,481 (76)	750 (74)	964 (71)	413 (70)	676 (72)
	<10th Percentile	9,858 (3)	34 (3)	48 (4)	20 (3)	36 (4)
	10 – <50th	25,732 (9)	76 (7)	138 (10)	54 (9)	95 (10)
	50 – <90th	28,399 (10)	113 (11)	165 (12)	80 (14)	98 (10)
	>= 90th	7,078 (2)	47 (5)	49 (4)	25 (4)	31 (3)
Glyphosate	Unexposed	165,996 (56)	514 (50)	718 (53)	275 (46)	493 (53)
	<10th Percentile	13,282 (4)	45 (4)	65 (5)	27 (5)	45 (5)
	10 – <50th	53,432 (18)	186 (18)	252 (18)	122 (21)	163 (17)
	50 – <90th	52,642 (18)	203 (20)	257 (19)	124 (21)	182 (19)
	>= 90th	13,196 (4)	72 (7)	72 (5)	44 (7)	53 (6)
Cyhalothrin	Unexposed	154,407 (52)	472 (46)	678 (50)	260 (44)	464 (50)
	<10th Percentile	21,388 (7)	63 (6)	101 (7)	42 (7)	59 (6)
	10 – <50th	50,256 (17)	177 (17)	223 (16)	101 (17)	163 (17)
	50 – <90th	58,158 (19)	226 (22)	283 (21)	149 (25)	195 (21)
	>= 90th	14,339 (5)	82 (8)	79 (6)	40 (7)	55 (6)
Mepiquat chloride	Unexposed	206,678 (69)	666 (65)	916 (67)	364 (61)	634 (68)
	<10th Percentile	15,181 (5)	39 (4)	72 (5)	31 (5)	41 (4)
	10 – <50th	30,687 (10)	95 (9)	129 (9)	70 (12)	103 (11)
	50 – <90th	36,864 (12)	160 (16)	189 (14)	98 (17)	121 (13)
	>= 90th	9,138 (3)	60 (6)	58 (4)	29 (5)	37 (4)
S, Metolachlor	Unexposed	208,762 (70)	668 (65)	915 (67)	370 (63)	636 (68)
	<10th Percentile	8,860 (3)	23 (2)	41 (3)	21 (4)	39 (4)
	10 – <50th	36,067 (12)	131 (13)	174 (13)	75 (13)	113 (12)
	50 – <90th	35,908 (12)	155 (15)	192 (14)	97 (16)	110 (12)
	>= 90th	8,951 (3)	43 (4)	42 (3)	29 (5)	38 (4)
Paraquat	Unexposed	50,214 (17)	165 (16)	215 (16)	82 (14)	137 (15)
	<10th Percentile	22,599 (8)	91 (9)	104 (8)	34 (6)	62 (7)
	10 – <50th	101,702 (34)	318 (31)	460 (34)	175 (30)	317 (34)
	50 – <90th	99,300 (33)	331 (32)	451 (33)	219 (37)	331 (35)
	>= 90th	24,733 (8)	115 (11)	134 (10)	82 (14)	89 (10)
Pendimethalin	Unexposed	190,766 (64)	583 (57)	813 (60)	327 (55)	568 (61)
	<10th Percentile	10,327 (3)	34 (3)	50 (4)	19 (3)	29 (3)
	10 – <50th	43,357 (15)	154 (15)	194 (14)	101 (17)	146 (16)
	50 – <90th	43,364 (15)	178 (17)	233 (17)	113 (19)	155 (17)
	>= 90th	10,734 (4)	71 (7)	74 (5)	32 (5)	38 (4)

ASD: atrial septal defects

PDA: patent ductus arteriosus

HPS: hypertrophic pyloric stenosis

HLHS: hypoplastic left heart syndrome

TEF: tracheal esophageal fistula

Table 2b:

Exposure distributions by case status, N (%)

Pesticide active ingredient	Exposure level	HLHS	TEF	Choanal Atresia	Hirschsprung's	Upper Limb Defects	Lower Limb Defects
N		75	84	50	77	84	39
2, 4-D	Unexposed	56 (75)	67 (80)	31 (62)	55 (71)	61 (73)	23 (59)
	<50th Percentile	8 (11)	9 (11)	9 (18)	9 (12)	13 (15)	7 (18)
	>= 50th	11 (15)	8 (10)	10 (20)	13 (17)	10 (12)	9 (23)
Glyphosate	Unexposed	38 (51)	42 (50)	24 (48)	34 (44)	42 (50)	15 (38)
	<50th Percentile	19 (25)	26 (31)	13 (26)	24 (31)	16 (19)	7 (18)
	>= 50th	18 (24)	16 (19)	13 (26)	19 (25)	26 (31)	17 (44)
Cyhalothrin	Unexposed	36 (48)	41 (49)	26 (52)	29 (38)	36 (43)	13 (33)
	<50th Percentile	17 (23)	21 (25)	9 (18)	22 (29)	16 (19)	8 (21)
	>= 50th	22 (29)	22 (26)	15 (30)	26 (34)	32 (38)	18 (46)
Mepiquat chloride	Unexposed	45 (60)	57 (68)	35 (70)	44 (57)	54 (64)	22 (56)
	<50th Percentile	13 (17)	12 (14)	4 (8) *	14 (18)	10 (12)	6 (15)
	>= 50th	17 (23)	15 (18)	11 (22)	19 (25)	20 (24)	11 (28)
S, Metolachlor	Unexposed	44 (59)	58 (69)	29 (58)	49 (64)	54 (64)	19 (49)
	<50th Percentile	14 (19)	16 (19)	9 (18)	10 (13)	14 (17)	7 (18)
	>= 50th	17 (23)	10 (12)	12 (24)	18 (23)	16 (19)	13 (33)
Paraquat	Unexposed	10 (13)	7 (8)	3 (6)	11 (14)	13 (15)	2 (5)
	<50th Percentile	29 (39)	39 (46)	31 (62)	25 (32)	27 (32)	14 (36)
	>= 50th	36 (48)	38 (45)	16 (32)	41 (53)	44 (52)	23 (59)
Pendimethalin	Unexposed	42 (56)	53 (63)	31 (62)	37 (48)	53 (63)	20 (51)
	<50th Percentile	12 (16)	17 (20)	8 (16)	19 (25)	8 (10)	5 (13)
	>= 50th	21 (28)	14 (17)	11 (22)	21 (27)	23 (27)	14 (36)

ASD: atrial septal defects

PDA: patent ductus arteriosus

HPS: hypertrophic pyloric stenosis

HLHS: hypoplastic left heart syndrome

TEF: tracheal esophageal fistula

*
exposure categories were further collapsed to unexposed/exposed for analysis

Table 3:

Spearman correlations between pesticide active ingredients using 5 level categories of exposure

	Glyphosate	Cyhalothrin	Mepiquat chloride	S, Metolachlor	Paraquat	Pendimethalin
2, 4-D	0.44	0.17	0.31	0.67	0.31	0.28
Glyphosate	1	0.60	0.24	0.53	0.50	0.71
Cyhalothrin		1	0.71	0.31	0.68	0.78
Mepiquat chloride			1	0.49	0.55	0.56
S, Metolachlor				1	0.41	0.40
Paraquat					1	0.55