Residential Agricultural Pesticide Exposures and Risks of Selected Birth Defects among Offspring in the San Joaquin Valley of California

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Background: We examined associations of birth defects with residential proximity to commercial agricultural pesticide applications in California. Subjects included 367 cases representing five types of birth defects and 785 nonmalformed controls born 1997 to 2006. **Methods:** Associations with any versus no exposure to physicochemical groups of pesticides and specific chemicals were assessed using logistic regression adjusted for covariates. Overall, 46% of cases and 38% of controls were classified as exposed to pesticides within a 500 m radius of mother's address during a 3-month periconceptional window. **Results:** We estimated odds ratios (ORs) for 85 groups and 95 chemicals with five or more exposed cases and control mothers. Ninety-five percent confidence intervals (CI) excluded 1.0 for 11 ORs for groups and 22 ORs for chemicals, ranging from 1.9 to 3.1 for groups and 1.8 to 4.9 for chemicals except for two that were <1 (noted below). **Conclusion:** For groups, these ORs were for anotia/microtia ($n = 95$ cases) and dichlorophenoxy acids/esters and neonicotinoids; anorectal atresia/stenosis ($n = 77$) and alcohol/ethers and organophosphates (these ORs were < 1.0); transverse limb deficiencies ($n = 59$) and dichlorophenoxy acids/esters, petroleum derivatives, and triazines; and craniosynostosis ($n = 79$) and alcohol/ethers, avermectins, neonicotinoids, and organophosphates. For chemicals, ORs were: anotia/microtia and five pesticides from the groups dichlorophenoxy acids/esters, copper-containing compounds, neonicotinoids, organophosphates, and triazines; transverse limb deficiency and six pesticides – oxyfluorfen and pesticides from the groups copper-containing compounds, 2,6-dinitroanilines, neonicotinoids, petroleum derivatives and polyalkyloxy compounds; craniosynostosis and 10 pesticides – oxyfluorfen and pesticides from the groups alcohol/ethers, avermectins, n-methyl-carbamates, neonicotinoids, ogranophosphates (two chemicals), polyalkyloxy compounds (two chemicals), and pyrethroids; and congenital diaphragmatic hernia ($n = 62$) and a copper-containing compound.

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Key words: birth defects; pesticides; pregnancy

Introduction

Pesticide exposures are ubiquitous and of substantial public health concern. Many are known reproductive toxicants (U.S. Environmental Protection Agency, 2012), and experimental studies suggest that certain pesticides are teratogenic (Kopf and Walker, 2009). Evidence regarding their effects on risks of specific birth defects in humans is sparse, and previous studies tend to rely on self-reported, broad categories of exposure (e.g., use of pesticides at work) or ecologic study designs.

For the current study, we used data on some of the more common birth defects included in a population-based case-control study of more than 30 different types of birth defects (Carmichael et al., 2014; Shaw et al., 2014; Yang et al., 2014). Specifically, we examined anorectal atresia or stenosis, anotia/microtia, transverse limb deficiency, craniosynostosis, and diaphragmatic hernia. Prior evidence regarding their potential association with pesticide exposures varies, and as such we consider this a hypothesis-generating investigation. We are unaware of any previous studies of pesticide exposures and anorectal atresia or stenosis or anotia/microtia in humans. We are aware of one study that examined occupation-related exposure to pesticides and risks of craniosynostosis and congenital diaphragmatic hernia, which found no increased risk (Kielb et al., 2014), and one study that suggested increased risk of craniosynostosis if the father had an agriculture-related occupation (Bradley et al., 1995). A few previous studies have suggested that parental agriculture-related occupations and household use of pesticides are associated with increased risk of limb deficiency defects (Kristensen et al., 1997; Shaw et al., 1999; Engel et al., 2000), while others have not (Lin et al., 1994; Kielb et al., 2014). Experimental data suggest an association of pesticides with the studied birth defects, including microtia (Larsson et al., 1976; Gomes et al., 2008), congenital diaphragmatic hernia (Ambrose et al., 1971; Costlow and Manson, 1981), and limb deficiency defects (Larsson et al., 1976; Varnagy et al., 2000; Farag et al., 2003). These studies tend to focus on only one or at most a few pesticides at a time.

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For the current study, we examined the association of the selected birth defects with residential proximity to commercial agricultural pesticide applications in the San Joaquin Valley, California. The Valley is one of the most intense agricultural production areas in the United States. The exposure assessment incorporated detailed data on pesticide applications, including dates of application, specific types and amounts of chemicals applied, and land-use data to refine the area of application. Using these data, we investigated specific pesticides applied within a 500 m radius of the mother's residential address during early pregnancy, which is when most structures are formed.

Materials and Methods

STUDY POPULATION

The California Center of the National Birth Defects Prevention Study is a collaborative partnership between Stanford University and the California Birth Defects Monitoring Program in the Department of Public Health (Yoon et al., 2001). Since 1997, the Center has collected data from women whose residences at the time of delivery were one of eight counties in the San Joaquin Valley. The California Birth Defects Monitoring Program is a well-known active surveillance program that identifies malformed fetuses/infants to allow such population-based study composition (Croen et al., 1991). To identify cases with birth defects, data collection staff visit all hospitals with obstetric or pediatric services, cytogenetic laboratories, and all clinical genetics prenatal and postnatal outpatient services. This analysis included study subjects with estimated dates of delivery from October 1, 1997, to December 31, 2006. The study protocol was reviewed and approved by the institutional review boards of Stanford University and the California Department of Public Health.

We included five birth defects for which we had maternal interviews and pesticide exposure data (see below) for at least 50 cases: anotia/microtia (reduction or absence of the external portion of the ear and atretic ear canal), anorectal atresia/stenosis (absence, closure or constriction of the rectum or anus), transverse limb deficiency (limbs with absent distal segments and intact proximal structures), craniosynostosis (premature fusion of one or more cranial sutures, verified by radiographic imaging or surgery), and diaphragmatic hernia (an opening in the diaphragm, through which a portion of the abdominal contents protrudes into the thoracic cavity). Clinicians with expertise in these birth defects reviewed their abstracted medical records to ensure they met eligibility criteria, which have been described in detail previously (Rasmussen et al., 2003). Cases with recognized or strongly suspected single-gene disorders, chromosomal aneuploidy, or identifiable syndromes were ineligible.

Controls included nonmalformed live-born infants randomly selected from birth hospitals to represent the population from which the cases arose. That is, we selected approximately 150 controls per study year, such that their distribution by hospital was proportional to the underlying birth population. Maternal interviews were conducted using a standardized, computer-based questionnaire, primarily by telephone, in English or Spanish, between 6 weeks and 24 months after the infant's estimated date of delivery. Interviews were conducted with mothers of 72% of eligible cases ($n = 480$) and 69% of controls ($n = 974$). Median time to interviews was 11 months for cases and 6 months for controls. Because poorly managed Type I or II diabetes is associated with increased risk of many birth defects (Correa et al., 2008), cases ($n = 18$) and controls ($n = 7$) whose mothers had these types of diabetes were excluded from analyses. Mothers reported their residential history from 3 months before conception through delivery, including dates for all residences occupied for more than 1 month.

SELECTION OF PESTICIDE COMPOUNDS

We assessed exposure to 461 individual chemicals and 62 physicochemical groupings having the same chemical classification and proven or putative mechanism of action (e.g., organophosphates) that were applied at >100 lb in any of eight San Joaquin Valley counties in any year during the study period (1997–2006) (Kegley et al., 2011). Low-toxicity chemicals such as biopesticides (e.g., microbial pesticides, soaps, essential oils), low-toxicity inorganic compounds (e.g., sulfur), and other compounds determined by United States Environmental Protection Agency (U.S. EPA) to have low toxicity, as described in U.S. EPA Risk Assessment documents for each chemical were excluded (U.S. Environmental Protection Agency, 2012). In addition, compounds were designated as having reproductive or developmental toxicity based on the California Proposition 65 list or as endocrine disruptors (Colborn, 1996; Keith, 1997; European-Commission, November 10, 2000; California-Office-of-Environmental-Health-Hazard-Assessment, 2012). Chemicals with a U.S. EPA-determined Reference Dose based on a toxicological study with a reproductive or developmental endpoint as described in EPA risk assessment documents were also included (U.S. Environmental Protection Agency, 2012).

PESTICIDE EXPOSURE ASSESSMENT

Exposure assessment has been described in detail previously and is reviewed in brief here (Carmichael et al., 2014; Shaw et al., 2014; Yang et al., 2014). For this analysis, nearby pesticide applications are considered to be a proxy for exposure by means of spray drift and volatilization drift, which may be inhaled or encountered from contaminated house dust (Koch et al., 2002). For each case or control mother, we estimated pesticide exposure from one month before to two months after her reported date of conception, which is inclusive of the time period of

development of the studied birth defects, with the exception of limb deficiency defects and craniosynostosis, whose developmental time period may also extend beyond the first two months. The California Environmental Health Tracking Program Geocoding Service geocoded subjects' residences during this time window (California-Environmental-Health-Tracking-Program, 2012b). Geocoding was successful for 82% of unique cases (377 of 462) and 83% of controls (807 of 967). Exposure assignments were made for 367 unique cases (95 with anotia/microtia, 77 with anorectal atresia/stenosis, 60 with transverse limb deficiency, 79 with craniosynostosis, and 62 with diaphragmatic hernia) and 785 controls whose mothers lived at the geocoded addresses more than 68 days during the 3-month window (i.e., at least 75% of it). For mothers with multiple addresses, days at each address were used as the weighting for exposure assignment.

To estimate pesticide applications, we obtained statewide Pesticide Use Reporting records from the California Department of Pesticide Regulation describing agricultural pesticide applications from 1997 to 2006, which are recorded by specific dates. These data are submitted by county agriculture commissioners and are spatially referenced to public land survey sections (PLSS). Following the method of Rull and Ritz (2003), we spatially refined PLSS polygons through overlay of matched land-use survey field polygons provided by the California Department of Water Resources; that is, we refined the pesticide application to a specific polygon, which is smaller than the 1-square-mile area of the PLSS polygon. We matched each Pesticide Use Reporting record to the land-use survey conducted closest in time to the application date (Department of Water Resources surveys are conducted roughly every 5–7 years in each California county). Spatial refinement by means of matching was successful for 91% of applications (92% by poundage).

To assign exposure, we used the California Environmental Health Tracking Program Pesticide Linkage Tool, a custom-developed Java (Oracle, Redwood Shores, CA) application which incorporates the GeoTools Java GIS Toolkit, version 2.7.1 (open source, http://geotools.codehaus.org/) for GIS data management and spatial analysis (California-Environmental-Health-Tracking-Program, 2012a). We calculated pounds of pesticides used during the relevant time window within a 500 m radius of a geocoded point, intersecting polygons with the buffer, and assuming homogeneous distribution of pesticides within each polygon (Roberts et al., 2007).

STATISTICAL ANALYSIS

We used logistic regression to estimate odds ratios and 95% confidence intervals (CI) reflecting associations between any versus no exposure to each pesticide or pesticide group of interest and birth defect group. We examined bivariate associations of any versus no pesticide exposure with numerous covariates (maternal race-ethnicity, education, age, prepregnancy body mass index, use of folic acid-containing supplements, smoking, drinking, parity, plurality) among the controls; no substantial associations were observed (results not shown). However, based on known variability in prevalence by socio-demographic variables, we adjusted odds ratios for race/ethnicity (non-Hispanic white, U.S.-born Hispanic, foreign-born Hispanic, other), education (less than high school, high school, more than high school), and age at delivery (year, continuous).

To focus on comparisons likely to have the most precise risk estimates and to fully use available data, we did the following. Adjusted odds ratios (AORs) were estimated only for pesticide chemicals or chemical groups that had at least 5 exposed cases and 5 exposed controls. To focus on the most important results, we only report odds ratios that: (1) had confidence intervals excluding 1.0; or (2) were less than or equal to 0.5 or greater than or equal to 2.0. Results that did not meet these criteria are available upon request. In addition, we created overall exposure scores by summing the total number of chemicals or groups, endocrine disruptors, Proposition 65 chemicals, or EPA reproductive or developmental toxicants to which each case or control was exposed. We examined the association of specific birth defects with these scores specified as categorical variables (exposed subjects were divided into tertiles based on the control distributions). Analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, 2014–2015).

Results

Comparisons of descriptive factors between the 785 control mothers and the mothers of 367 unique case infants indicated that case mothers tended to be more likely to be foreign-born Hispanic, greater than 30 years of age, and have lower education compared with control mothers (Table 1).

Women in this study were exposed to 53 groups of chemicals and 248 individual chemicals during the month before or first two months of pregnancy based on residential proximity within 500 m of pesticide applications. Overall, 38.1% of control mothers (299/785) and 45.5% of unique case mothers (167/367) had any periconceptional pesticide exposure.

The number of cases of each type of birth defect ranged from 60 to 95. Sixteen of the 85 physicochemical groups and 29 of the 95 individual chemicals that met our criteria for odds ratio estimation (i.e., at least five case mothers and five control mothers were exposed, Table 2) also met our criteria for presentation (i.e., odds ratio [ORs] were ≥ 2.0 or ≤ 0.5 *or* had confidence intervals that excluded 1.0) and are shown in Tables 3 and 4 (other results are available upon request).

AORs with 95% CIs excluding 1.0 were observed for 11 comparisons at the physicochemical group level and

TABLE 1. *Characteristics of Study Subjects, San Joaquin Valley of California, 1997 to 2006*

	Percent of cases[a] ($n = 367$)	Percent of controls[a] ($n = 785$)
Maternal race/ethnicity		
White	27	33
U.S.-born Hispanic	27	25
Foreign-born Hispanic	36	28
Other	10	14
Maternal age at delivery (years)		
<20	11	17
20-24	27	28
25-29	23	26
30-34	27	18
≥35	13	10
Maternal education (years)		
<12	34	30
12	26	28
>12	39	41

[a]Percentages may not equal 100 owing to rounding or missing data.

22 at the individual chemical level; these AORs ranged from 1.8 to 4.9, with the exception of two that were less than 1.0. Physicochemical groups with 95% CIs that excluded 1.0 were observed for: anotia/microtia with dichlorophenoxy acid or esters and neonicotinoids; anorectal atresia/stenosis and alcohol/ethers and organophosphates (these were the only two reported AORs that were <1.0); transverse limb deficiencies and dichlorophenoxy acids or esters, petroleum derivatives, and triazines; and craniosynostosis and alcohol/ethers, avermectins, neonicotinoids, and organophosphates (Table 3). Individual chemicals that had AORs with confidence intervals excluding 1.0 were observed for: anotia/microtia and 5 specific pesticides from the physicochemical groups dichlorophenoxy acid or esters, copper-containing compounds, neonicotinoids, organophosphates, and triazines; transverse limb deficiency and 6 specific pesticides – oxyfluorfen (a specific chemical, without a group affiliation) and pesticides from the groups copper-containing compounds, 2,6-dinitroanilines, neonicotinoids, petroleum derivatives and polyalkyloxy compounds; craniosynostosis and 10 specific pesticides – oxyfluorfen and pesticides from the groups alcohol/ethers, avermectins, n-methyl-carbamates, neonicotinoids, ogranophosphates (2 chemicals), polyalkyloxy compounds (2) and pyrethroids; and congenital diaphragmatic hernia and a copper-containing compound (specific chemicals are shown in Table 4).

Scores reflecting the number of pesticides to which each woman was exposed suggested increasing risk associated with increasing numbers of pesticides for transverse limb deficiency and craniosynostosis (Table 5). For example, the OR for exposure to the highest category of reproductive or developmental toxicants (i.e., 7–21 chemicals) was 2.1 for transverse limb deficiency (95% CI, 1.1–4.3) and 2.0 for craniosynostosis (95% CI, 1.1–3.9).

Discussion

We examined the association of residential proximity to commercial applications of agricultural pesticides with risks of specific birth defects among offspring born to women living in the San Joaquin Valley of California, one of the highest pesticide-use areas in the U.S. Overall, 46% of mothers of cases and 38% of mothers of controls lived within 500 m of pesticide applications during the periconceptional period. Most of the individual pesticides were not associated with increased risk. For those pesticides that were associated, results should be interpreted with caution, given the novelty and hypothesis-generating nature of our investigation and potential for false-positive results due to multiple testing.

Although our results were primarily negative, a wide variety of mechanisms could potentially contribute to associations between pesticides and the birth defects studied here. For example, anorectal atresia and craniosynostosis have been associated with maternal thyroid disease, and many pesticides are known to affect thyroid hormones (Rasmussen et al., 2007; Browne et al., 2009; Boas et al., 2012; Bhaskar and Mohanty, 2014). Risks of many birth defects are increased in the presence of maternal diabetes, including anorectal atresia/stenosis, anotia/microtia and limb defects, and some pesticides may affect glycemic control (Saldana et al., 2007; Correa et al., 2008; Udeigwe et al., 2015). The herbicide nitrofen is thought to induce congenital diaphragmatic hernia by means of interruption of retinoid signaling, based on experimental models (Greer, 2013). A more exhaustive review of potential mechanisms is beyond the scope of this study and premature, given the uniqueness of our findings.

As noted above, few previous studies have examined the association of the studied birth defects with specific pesticide exposures. Results and methods have varied widely. In the few human studies that have been conducted, occupational categories (e.g., agriculture) were most often used as the proxy of exposure. Experimental studies, on the other hand, tend to examine specific chemicals, but are limited to examining one to at most a few at a time, and birth defect definitions and doses of exposure vary widely. Given these differences, we hesitate to draw more direct comparisons between our study and previous literature.

TABLE 2. *Percent of Cases Exposed to Pesticides and Number of Chemical Groups and Specific Chemicals That Were Tested for Association with Birth Defects,[a] San Joaquin Valley of California, 1997 to 2006*

Birth defect group	No. of cases	No. (percent) of cases with any pesticide exposure	No. of physico-chemical groups tested	No. of specific chemicals tested
Anotia/microtia	95	43 (45.3%)	20	25
Anorectal atresia/stenosis	77	30 (39.0%)	14	10
Transverse limb deficiency	60	32 (53.3%)	19	20
Craniosynostosis	79	37 (46.8%)	17	24
Diaphragmatic hernia	62	28 (45.2%)	15	16

[a]For each birth defect, the table shows the number of physicochemical groups and specific chemicals that met sample size criteria for risk estimation, i.e., had at least five exposed cases and five exposed controls. Physicochemical groupings included pesticides having the same chemical classification and proven or putative mechanism of action. Risks were only estimated for these physicochemical groups and specific chemicals. For reference, the study included 785 controls, 299 (38.1%) of whom were exposed to pesticides, and a total of 367 unique cases (6 had more than one of the studied birth defects).

We made 180 comparisons (the number per type of birth defect ranged from 24 to 45). Given the hypothesis-generating nature of our investigation, we did not adjust results for multiple comparisons or restrict presentation of results to criteria of statistical significance. Among the 180 physicochemical groups and individual chemicals that had sufficiently common exposure to merit risk estimation, 45 had ORs that were greater than 2.0 or less than 0.5 or had 95% confidence intervals that excluded 1.0. Anorectal atresia/stenosis and congenital diaphragmatic hernia had the fewest findings that met these criteria for presentation (two and one, respectively), and craniosynostosis had the most (17). Most of the individual chemicals or physicochemical groups only met these criteria for one of the

TABLE 3. *ORs for Pesticide Physicochemical Groups and Birth Defects, San Joaquin Valley of California, 1997 to 2006[a]*

Birth defect group	Physicochemical pesticide group	No. cases exposed/ not exposed	No. controls exposed/ not exposed	AOR (95% CI)
Anotia/microtia	Dichlorophenoxy acid or ester	12/83	41/738	2.8 (1.4–5.6)
	Benzoic acid	5/90	17/762	2.1 (0.7–6.0)
	Neonicotinoid	12/83	35/744	3.0 (1.4–6.1)
	Triazine	13/82	57/722	2.0 (1.0–3.9)
Anorectal atresia/stenosis	Alcohol/ether	5/72	131/648	0.3 (0.1–0.9)
	Organophosphate	6/71	137/642	0.4 (0.2–0.9)
Transverse limb deficiency	Dichlorophenoxy acid or ester	7/52	41/738	2.5 (1.1–6.0)
	Neonicotinoid	6/53	35/744	2.4 (1.0–6.1)
	Petroleum derivative	15/44	102/677	2.1 (1.1–3.9)
	Strobin	6/53	33/746	2.6 (1.0–6.6)
	Triazine	9/50	57/722	2.3 (1.1–5.0)
Craniosynostosis	Alcohol/ether	22/57	131/648	2.0 (1.2–3.4)
	Avermectin	7/72	31/748	2.7 (1.1–6.6)
	Neonicotinoid	8/71	35/744	3.1 (1.3–7.1)
	Organophosphate	21/58	137/642	1.9 (1.1–3.2)
	Urea	10/69	53/726	2.1 (1.0–4.3)

[a]AORs are presented for chemicals for which the AOR was ≤ 0.5 or ≥ 2.0 *or* for which the confidence interval excluded 1.0. AORs were adjusted for maternal race/ethnicity, education, and age (continuous). Analyses included 366 cases and 779 controls with complete data on adjusted covariates. Physicochemical groupings included pesticides having the same chemical classification and proven or putative mechanism of action.

AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

TABLE 4. *ORs for Specific Pesticide Chemicals and Birth Defects, San Joaquin Valley of California, 1997 to 2006*[a]

Birth defect	Pesticide name	Physicochemical pesticide group	No. cases exposed/ not exposed	No. controls exposed/ not exposed	AOR (95% CI)
Anotia	2,4-D,dimethylamine salt	Dichlorophenoxy acid or ester	10/85	26/753	3.4 (1.6–7.6)
	Copper hydroxide	Copper-containing compound	17/78	78/701	2.0 (1.1–3.6)
	Imidacloprid	Neonicotinoid	10/85	31/748	3.0 (1.4–6.6)
	Chlorpyrifos	Organophosphate	15/80	67/712	2.0 (1.1–3.8)
	Petroleum oil, unclassified	Petroleum derivative	14/81	52/727	2.0 (1.0–3.9)
	Polyoxyethylenepolyoxypropylene	Polyalkyloxy Compound	5/90	17/762	2.1 (0.7–6.1)
	Simazine	Triazine	13/82	50/729	2.3 (1.1–4.4)
Transverse limb deficiency	Oxyfluorfen	n.a.	12/47	80/699	2.2 (1.1–4.3)
	Copper sulfate (Basic)	Copper-containing compound	6/53	23/756	3.9 (1.5–10.1)
	Oryzalin	2,6-Dinitroaniline	6/53	26/753	3.8 (1.4–9.8)
	Imidacloprid	Neonicotinoid	6/53	31/748	2.9 (1.1–7.4)
	Petroleumoil, unclassified	Petroleum derivative	9/50	52/727	2.3 (1.1–5.0)
	Alpha-Alkylaryl-Omega-Hydroxypoly(Oxyethylene)	Polyalkyloxy Compound	9/50	48/731	2.6 (1.2–5.7)
	Simazine	Triazine	8/51	50/729	2.3 (1.0–5.3)
Craniosynostosis	Oxyfluorfen	n.a.	15/64	80/699	2.0 (1.1–3.7)
	Isopropylalcohol	Alcohol/Ether	22/57	125/654	2.1 (1.2–3.6)
	Abamectin	Avermectin	7/72	31/748	2.7 (1.1–6.6)
	Oryzalin	2,6-Dinitroaniline	6/73	26/753	2.4 (0.9–6.0)
	Methomyl	N-Methyl-Carbamate	6/73	23/756	3.0 (1.1–7.8)
	Imidacloprid	Neonicotinoid	8/71	31/748	3.5 (1.5–8.3)
	Dimethoate	Organophosphate	5/74	16/763	3.8 (1.3–11.2)
	Chlorpyrifos	Organophosphate	13/66	67/712	2.4 (1.2–4.6)
	Petroleum oil, unclassified	Petroleum derivative	10/69	52/727	2.1 (1.0–4.4)
	Petroleum oil, paraffin-based	Petroleum derivative	5/74	25/754	2.4 (0.8–6.6)
	Alpha-Octylphenyl-Omega-Hydroxypoly(Oxyethylene)	Polyalkyloxy Compound	10/69	50/729	2.2 (1.1–4.6)
	Alpha-(Para-Nonylphenyl)-Omega-Hydroxypoly(Oxyethylene)	Polyalkyloxy Compound	23/56	147/632	1.8 (1.1–3.2)
	Cyfluthrin	Pyrethroid	5/74	13/766	4.6 (1.5–14.0)
	Diuron	Urea	10/69	51/728	2.2 (1.0–4.6)
Diaphragmatic hernia	Copper oxide(ous)	Copper-containing compound	5/57	14/765	4.9 (1.7–14.4)

[a]AORs are presented for chemicals for which the AOR was ≤ 0.5 or ≥ 2.0 *or* for which the confidence interval excluded 1.0. ORs were adjusted for maternal race/ethnicity, education, and age (continuous). Analyses included 366 cases and 779 controls with complete data on adjusted covariates. Physicochemical groupings included pesticides having the same chemical classification and proven or putative mechanism of action.

AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

TABLE 5. *AORs for Sums of Specific Classifications of Pesticide Exposures and Selected Birth Defects, San Joaquin Valley of California, 1997 to 2006*

		Anotia/microtia		Anorectal atresia/ stenosis		Transverse limb deficiency		Craniosynostosis		Diaphragmatic hernia	
	Controls	Cases	OR (95%CI)[a]	Cases	OR (95%CI)[a]	Cases	OR (95%CI)[a]	Cases	OR (95%CI)[a]	Cases	OR (95% CI)[a]
No. of chemical groups with any exposure											
0	482	52	Reference	47	Reference	29	Reference	42	Reference	35	Reference
1–3	89	15	1.6 (0.9–3.0)	12	1.4 (0.7–2.7)	9	1.7 (0.8–3.7)	11	1.3 (0.6–2.6)	3	0.4 (0.1–1.5)
4–8	114	9	0.7 (0.3–1.5)	12	1.1 (0.6–2.2)	8	1.2 (0.5–2.8)	11	1.1 (0.6–2.3)	14	1.7 (0.9–3.4)
9–22	94	19	1.7 (0.9–3.0)	6	0.6 (0.3–1.5)	13	2.1 (1.0–4.3)	15	1.9 (1.0–3.7)	10	1.4 (0.7–3.0)
Continuous	779	95	1.04 (0.99–1.08)	77	0.97 (0.91–1.03)	59	1.05 (1.00–1.11)	79	1.05 (1.00–1.10)	62	1.02 (0.97–1.08)
No. of endocrine disruptors with any exposure											
0	514	59	Reference	51	Reference	34	Reference	47	Reference	39	Reference
1	70	7	0.9 (0.4–2.0)	9	1.3 (0.6–2.8)	5	1.1 (0.4–2.9)	5	0.8 (0.3–2.0)	3	0.5 (0.2–1.8)
2–3	90	9	0.8 (0.4–1.7)	10	1.1 (0.6–2.3)	9	1.5 (0.7–3.3)	9	1.1 (0.5–2.4)	8	1.2 (0.5–2.6)
4–13	105	20	1.5 (0.9–2.7)	7	0.7 (0.3–1.5)	11	1.5 (0.7–3.1)	18	2.0 (1.1–3.7)	12	1.5 (0.7–2.9)
Continuous	779	95	1.06 (0.98–1.16)	77	0.92 (0.81–1.04)	59	1.07 (0.97–1.19)	79	1.10 (1.01–1.20)	62	1.05 (0.94–1.16)
No. of Prop. 65 reproductive toxicants with any exposure											
0	634	75	Reference	68	Reference	43	Reference	60	Reference	52	Reference
1	93	13	1.1 (0.6–2.1)	7	0.7 (0.3–1.6)	8	1.3 (0.6–2.8)	7	0.8 (0.4–1.9)	7	0.9 (0.4–2.1)
2–6	52	7	1.0 (0.4–2.3)	2	NC	8	2.1 (0.9–4.9)	12	2.6 (1.3–5.3)	3	0.7 (0.2–2.3)
Continuous	779	95	1.07 (0.83–1.38)	77	0.68 (0.43–1.09)	59	1.19 (0.89–1.59)	79	1.27 (0.99–1.63)	62	0.81 (0.52–1.25)
No. of reproductive or developmental toxicants with any exposure											
0	488	53	Reference	48	Reference	30	Reference	42	Reference	36	Reference
1–2	85	12	1.4 (0.7–2.8)	10	1.3 (0.6–2.6)	9	1.7 (0.8–3.8)	10	1.2 (0.6–2.6)	4	0.6 (0.2–1.8)
3–6	114	14	1.1 (0.6–2.0)	14	1.2 (0.7–2.3)	7	1.0 (0.4–2.3)	12	1.2 (0.6–2.4)	16	1.9 (1.0–3.5)
7–21	92	16	1.4 (0.8–2.6)	5	0.5 (0.2–1.4)	13	2.1 (1.1–4.3)	15	2.0 (1.1–3.9)	6	0.9 (0.3–2.1)
Continuous	779	95	1.03 (0.98–1.09)	77	0.96 (0.90–1.04)	59	1.06 (1.00–1.12)	79	1.07 (1.01–1.12)	62	1.01 (0.94–1.08)

[a]ORs not calculated (NC) if cell count was less than three cases or controls. ORs were adjusted for maternal race/ethnicity, education, and age (continuous). Analyses included 366 cases and 779 controls with complete data on adjusted covariates.

OR, odds ratio; CI, confidence interval; NC, not calculated.

studied outcomes. The physicochemical group neonicotinoids and the individual chemical imidacloprid (a neonicotinoid), however, each met the criteria for three outcomes. Imidacloprid is used in agriculture and also as an active ingredient in widely used flea and tick medications for household pets. It affects a variety of physiologic pathways, for example thyroid-related signaling, glycemic control, immune response and neurodevelopment, and it was recently reported to be associated with autism spectrum disorder (Moser et al., 2012; Tanaka, 2012; Gawade et al., 2013; EFSA-European-Food-Safety-Authority, 2013; Bhaskar and Mohanty, 2014; Keil et al., 2014). We are unaware of other previous studies examining the studied birth defects and neonicotinoids, with the possible exception of a study we conducted in California that suggested increased risk of limb defects (transverse or longitudinal) associated with flea collars on household pets (OR 1.5, 95% CI 0.8–2.6) (Shaw et al., 1999).

Strengths of the current study include the population-based design, carefully defined birth defects, and pesticide exposure assessment that was spatially and temporally specific and encompassed a broad spectrum of specific pesticide compounds. Exposure assessment did not, however, take into account other factors that may affect actual exposures, such as chemical half-lives and vapor pressure, wind patterns, individual variability in chemical metabolism, time spent at home, and other potential sources of pesticide exposure like occupational or household use. These factors likely contribute to errors in exposure assessment but are unlikely to have varied systematically by case status. Based on prior characterization of occupational exposures for the early birth years of National Birth

Defects Prevention Study, we expect few women to have been exposed to pesticides by means of their occupations (Rocheleau et al., 2015). Selection bias is a possible contributor to results, but based on prior analysis comparing characteristics of participants and nonparticipants, its impact is likely to be minimal (Cogswell et al., 2009). We used a common time window of exposure for all birth defects being studied. We acknowledge that the relevant time window does vary somewhat, but it was not feasible to examine a different time window for each different birth defect (and multiple windows for the controls). If this decision biased results, we expect the bias would be toward the null.

Results of this study do not indicate strong associations of the studied birth defects with residential proximity to agricultural pesticide applications, even though we investigated risk for birth defects within an area of high pesticide use. Our study is the first to investigate a wide range of potential exposures to specific chemicals and groups of chemicals in relation to the studied birth defects. Results, therefore, require further inquiry and verification before firm conclusions can be reached regarding potential teratogenicity of the studied pesticides.

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References

Ambrose AM, Larson PS, Borzelleca JF, et al. 1971. Toxicologic studies on 2,4-dichlorophenyl-p-nitrophenyl ether. Toxicol Appl Pharmacol 19:263–275.

Bhaskar R, Mohanty B. 2014. Pesticides in mixture disrupt metabolic regulation: in silico and in vivo analysis of cumulative toxicity of mancozeb and imidacloprid on body weight of mice. Gen Comp Endocrinol 205:226–234.

Boas M, Feldt-Rasmussen U, Main KM. 2012. Thyroid effects of endocrine disrupting chemicals. Mol Cell Endocrinol 355:240–248.

Bradley CM, Alderman BW, Williams A, et al. 1995. Parental occupations as risk factors for craniosynostosis in offspring. Epidemiology 6:306–310.

Browne ML, Rasmussen SA, Hoyt AT, et al. 2009. Maternal thyroid disease, thyroid medication use, and selected birth defects in the National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol 85:621–628.

California-Environmental-Health-Tracking-Program. 2012a. Agricultural pesticide mapping tool. Available at: http://cehtp.org/p/tools_pesticide. Accessed July 7, 2015.

California-Environmental-Health-Tracking-Program. 2012b. Geocoding Service. Available at: http://www.ehib.org/page.jsp?page_key=137. Accessed July 7, 2015.

California-Office-of-Environmental-Health-Hazard-Assessment. 2012. Proposition 65. Available at: http://www.oehha.ca.gov/prop65.html. Accessed July 7, 2015.

Carmichael SL, Yang W, Roberts E, et al. 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.

Cogswell ME, Bitsko RH, Anderka M, et al. 2009. Control selection and participation in an ongoing, population-based, case-control study of birth defects: the National Birth Defects Prevention Study. Am J Epidemiol 170:975–985.

Colborn T. 1996. Widespread pollutants with reproductive and endocrine-disrupting effects. Our Stolen Future web site. Available at: http://www.ourstolenfuture.org/basics/chemlist.htm. Accessed July 7, 2015.

Correa A, Gilboa SM, Besser LM. 2008. Diabetes mellitus and birth defects. Am J Obstet Gynecol 199:231–239.

Costlow RD, Manson JM. 1981. The heart and diaphragm: target organs in the neonatal death induced by nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether). Toxicology 20:209–227.

Croen LA, Shaw GM, Jensvold NG, Harris JA. 1991. Birth defects monitoring in California: a resource for epidemiological research. Paediatr Perinat Epidemiol 5:423–427.

EFSA-European-Food-Safety-Authority. 2013. Scientific opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid. EFSA J 11:3471–3518.

Engel LS, O'Meara ES, Schwartz SM. 2000. Maternal occupation in agriculture and risk of limb defects in Washington State, 1980-1993. Scand J Work Environ Health 26:193–198.

European-Commission. November 10, 2000. Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption, Appendix 1. Available at: http://ec.europa.eu/environment/archives/docum/pdf/bkh_annex_01.pdf. Accessed July 7, 2015.

Farag AT, El Okazy AM, El-Aswed AF. 2003. Developmental toxicity study of chlorpyrifos in rats. Reprod Toxicol 17:203–208.

Gawade L, Dadarkar SS, Husain R, Gatne M. 2013. A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. Food Chem Toxicol 51:61–70.

Gomes J, Lloyd OL, Hong Z. 2008. Oral exposure of male and female mice to formulations of organophosphorous pesticides: congenital malformations. Hum Exp Toxicol 27:231–240.

Greer JJ. 2013. Current concepts on the pathogenesis and etiology of congenital diaphragmatic hernia. Respir Physiol Neurobiol 189:232–240.

Kegley S, Hill B, Orme S, Choi A. 2011. PAN pesticide database. San Francisco, CA: Pesticide Action Netowrk.

Keil AP, Daniels JL, Hertz-Picciotto I. 2014. Autism spectrum disorder, flea and tick medication, and adjustments for exposure misclassification: the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Environ Health 13:3.

Keith LH. 1997. Environmental endocrine disruptors: a handbook of property data. New York: Wiley Interscience.

Kielb C, Lin S, Herdt-Losavio M, et al. 2014. Maternal periconceptional occupational exposure to pesticides and selected musculoskeletal birth defects. Int J Hyg Environ Health 217:248–254.

Koch D, Lu C, Fisker-Andersen J, et al. 2002. Temporal association of children's pesticide exposure and agricultural spraying: report of a longitudinal biological monitoring study. Environ Health Perspect 110:829–833.

Kopf PG, Walker MK. 2009. Overview of developmental heart defects by dioxins, PCBs, and pesticides. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27:276–285.

Kristensen P, Irgens LM, Andersen A, et al. 1997. Birth defects among offspring of Norwegian farmers, 1967-1991. Epidemiology 8:537–544.

Larsson KS, Arnander C, Cekanova E, Kjellberg M. 1976. Studies of teratogenic effects of the dithiocarbamates maneb, mancozeb, and propineb. Teratology 14:171–183.

Lin S, Marshall EG, Davidson GK. 1994. Potential parental exposure to pesticides and limb reduction defects. Scand J Work Environ Health 20:166–179.

Moser VC, Padilla S, Simmons JE, et al. 2012. Impact of chemical proportions on the acute neurotoxicity of a mixture of seven carbamates in preweanling and adult rats. Toxicol Sci 129:126–134.

Rasmussen SA, Olney RS, Holmes LB, et al. 2003. Guidelines for case classification for the National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol 67:193–201.

Rasmussen SA, Yazdy MM, Carmichael SL, et al. 2007. Maternal thyroid disease as a risk factor for craniosynostosis. Obstet Gynecol 110(Pt 1):369–377.

Roberts EM, English PB, Grether JK, et al. 2007. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ Health Perspect 115:1482–1489.

Rocheleau CM, Bertke SJ, Lawson CC, et al. 2015. Maternal occupational pesticide exposure and risk of congenital heart defects in the National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol [Epub ahead of print].

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: 1582–1589.

Saldana TM, Basso O, Hoppin JA, et al. 2007. Pesticide exposure and self-reported gestational diabetes mellitus in the Agricultural Health Study. Diabetes Care 30:529–534.

Shaw GM, Wasserman CR, O'Malley CD, et al. 1999. Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiology 10:60–66.

Shaw GM, Yang W, Roberts E, et al. 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 100:686–694.

Tanaka T. 2012. Effects of maternal clothianidin exposure on behavioral development in F(1) generation mice. Toxicol Ind Health 28:697–707.

Udeigwe TK, Teboh JM, Eze PN, et al. 2015. Implications of leading crop production practices on environmental quality and human health. J Environ Manage 151:267–279.

U.S. Environmental Protection Agency. 2012. Pesticide chemical search. Washington, DC: U.S. Environmental Protection Agency, Office of Pesticide Programs.

Varnagy L, Budai P, Molnar E, et al. 2000. Interaction of Dithane M-45 (mancozeb) and lead acetate during a teratogenicity test in rats. Acta Vet Hung 48:113–124.

Yang W, Carmichael SL, Roberts EM, et al. 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.

Yoon PW, Rasmussen SA, Lynberg MC, et al. 2001. The National Birth Defects Prevention Study. Public Health Rep 116(Suppl 1): 32–40.