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To cite this article: Jonathan Suhl, Paul A. Romitti, Carissa Rocheleau, Yanyan Cao, Trudy L. Burns, Kristin Conway, Erin M. Bell, Patricia Stewart, Peter Langlois & the National Birth Defects Prevention Study (2018) Parental occupational pesticide exposure and nonsyndromic orofacial clefts, Journal of Occupational and Environmental Hygiene, 15:9, 641-653, DOI: [10.1080/15459624.2018.1484127](https://doi.org/10.1080/15459624.2018.1484127)

To link to this article: <https://doi.org/10.1080/15459624.2018.1484127>



Accepted author version posted online: 11 Jul 2018.  
Published online: 04 Oct 2018.



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## Parental occupational pesticide exposure and nonsyndromic orofacial clefts

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### ABSTRACT

Nonsyndromic orofacial clefts are common birth defects. Reported risks for orofacial clefts associated with parental occupational pesticide exposure are mixed. To examine the role of parental pesticide exposure in orofacial cleft development in offspring, this study compared population-based case-control data for parental occupational exposures to insecticides, herbicides, and fungicides, alone or in combinations, during maternal (1 month before through 3 months after conception) and paternal (3 months before through 3 months after conception) critical exposure periods between orofacial cleft cases and unaffected controls. Multivariable logistic regression was used to estimate odds ratios, adjusted for relevant covariables, and 95% confidence intervals for any (yes, no) and cumulative (none, low [ $<$ median exposure level in controls], high [ $\geq$ median exposure level in controls]) occupational pesticide exposures and cleft lip  $\pm$  cleft palate and cleft palate. Associations for cleft lip  $\pm$  cleft palate tended to be near unity for maternal or paternal occupational pesticide exposures, except for low paternal exposure to any pesticide, which produced a statistically significant inverse association with this subtype. Associations for cleft palate tended to be near unity for maternal exposures and mostly positive, but non-significant, for paternal exposures; a significant positive association was observed between paternal low exposure to insecticide + herbicide + fungicide and cleft palate. Combined parental exposure produced non-significant associations near or below unity for all orofacial cleft cases combined and cleft lip  $\pm$  cleft palate and positive, but non-significant, associations for cleft palate. This study observed associations mostly near unity between maternal occupational pesticide exposure and orofacial clefts. Associations for paternal occupational pesticide exposures were mostly near or below unity for cleft lip  $\pm$  cleft palate, and mostly positive for cleft palate. However, due to the limitations of this study, these subtype-specific results should be interpreted cautiously. Future research examining parental occupational pesticide exposure and orofacial clefts should attempt to improve exposure assessment and increase sample size to better facilitate risk estimation.

### KEYWORDS

Cleft lip; cleft palate; herbicides; insecticides; pesticides; pregnancy

## Introduction

Nonsyndromic orofacial clefts (OFC)s – cleft lip  $\pm$  cleft palate (CL/P) and cleft palate only (CP) – fuse due to failure of the lip or palate to completely fuse during development. Prevalence estimates (per 1,000 live births) in the United States for CL/P and CP are 1.06 and 0.64, respectively.<sup>[1]</sup> These subtypes are considered etiologically distinct, based on differences in their development.<sup>[2,3]</sup> Previous studies have reported that OFCs are multifactorial in origin, being associated with several reported gene variants<sup>[reviewed in 4]</sup> and environmental exposures.<sup>[reviewed in 2]</sup>

Pesticides are among the exposures reportedly associated with increased risks for OFCs.<sup>[5]</sup> Several animal studies have demonstrated the teratogenicity of prenatal pesticide exposure in OFC development.<sup>[6–11]</sup> Epidemiologic studies of maternal occupational pesticide exposures suggest positive associations with OFCs,<sup>[12–16]</sup> whereas those for paternal exposures are mixed.<sup>[14–18]</sup> A meta-analysis examining maternal and paternal exposures separately reported positive associations with all OFCs with each parental exposure.<sup>[5]</sup>

Previous epidemiologic studies of parental occupational pesticide exposure and OFCs differed in exposure

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📎 Supplemental data for this article can be accessed at [tandfonline.com/uoeh](http://tandfonline.com/uoeh). AIHA and ACGIH members may also access supplementary material at <http://oeh.tandfonline.com/>.

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assessment approaches used. Three studies used industrial hygienist (IH) review of detailed job descriptions to assess maternal exposure;<sup>[12–14]</sup> one of these studies also used IH review to assess paternal exposure.<sup>[14]</sup> By comparison, two additional studies of maternal exposure<sup>[15,16]</sup> and four studies of paternal exposure<sup>[15–18]</sup> relied only on job title or occupation/industry from self-reports or vital records to assess pesticide exposure. Previous evaluations suggest that use of IH review for assessment of occupational pesticide exposure may help reduce exposure misclassification compared to other methods, such as self-reported exposure.<sup>[19,20]</sup>

Along with the different exposure assessment approaches, most previous epidemiologic studies were limited by additional methodologic weaknesses. These weaknesses included small sample sizes, which diminished statistical power in several studies.<sup>[12,13,15,16,18]</sup> Also, some studies examined pesticide exposures as a summary measure,<sup>[12–14]</sup> precluding investigation of potential differential effects of specific pesticides or pesticide classes.<sup>[21]</sup> Furthermore, most studies examined risk for all OFCs combined; only two studies examined risk by OFC subtypes.<sup>[14,17]</sup> To address these weaknesses, the present study applied IH review of parental occupational data collected in the National Birth Defects Prevention Study (NBDPS) to examine the relationship between parental occupational pesticide exposures and nonsyndromic OFCs in their offspring.

## Methods

NBDPS methods are described elsewhere.<sup>[22–24]</sup> Briefly, the NBDPS examined risk factors for over 30 major structural birth defects among deliveries from October 1997 through December 2011. The NBDPS was conducted in Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah. Cases were identified through active case finding and medical record abstraction by the birth defects surveillance program at each site. Cases were live births, stillbirths, and elective terminations with CL/P or CP; live births were followed for 1 year to confirm diagnosis. Abstracted medical record data were reviewed by clinical geneticists to confirm a nonsyndromic phenotype, and cases with monogenic disorders, chromosome abnormalities, or an OFC secondary to another defect were excluded. Eligible, non-syndromic cases were classified as isolated (no other major defects) or multiple (one or more additional major, unrelated defects). A random sample of live born controls without major defects delivered during the same time frame and in the same geographic

catchment areas was selected from hospital delivery logs or birth certificate files. Approximately 100 controls per year per site were recruited, which permitted a minimum of a 1:1 ratio between controls and each defect type included in the NBDPS.

Mothers of cases and controls were invited to complete a telephone interview in either English- or Spanish-language from 6 weeks through 24 months after their estimated dates of delivery (EDD)s; overall, 71% of case and 64% of control mothers participated. Mothers were asked to provide information about their medical and prenatal care, diet, lifestyle, and family history of birth defects. Additionally, mothers were asked to provide sociodemographic information and occupational information for jobs held for 1 month or more during the 1-year period prior to their EDDs (3 months before conception [B3] through delivery [P9] or earlier due to fetal loss or termination). For each job reported, mothers were asked to provide the company name, what the company makes/does, job title, typical duties or tasks, any equipment or chemicals that were used on the job, hours, and days worked per week, and the month and year employment began and ended (if applicable). Mothers also were asked to provide the same information for jobs held by the fathers of the case or control children during B3–P9. Fathers were not contacted separately to provide occupational information.

## Exposure assessment

To date, funding has permitted parental occupational pesticide exposure assessment for cases and controls with EDDs from October 1997 through December 2002; during this time frame, data collection occurred at all sites except North Carolina and Utah. Pesticide exposure assessment followed an approach similar to that used by Samanic et al.<sup>[25]</sup> Each maternal or paternal job reported was assigned a 2007 North American Industrial Classification System code and a Standard Occupational Classification code. Each job was first assigned an exposure probability score (0, <1%, 1–33%, 34–66%, 67–89%, ≥90%) based on the National Cancer Institute job-specific task exposure matrix for pesticides. A job assigned an exposure probability >0 was further reviewed using IH judgment and the exposure matrix to assign an exposure intensity rating (<1, 1–9, 10–99, ≥100 mg/hr) and frequency of exposure in an average work week (<2, 2–10, 11–19, ≥20 hrs/week) to each of three pesticide classes (insecticides, herbicides, fungicides). An IH exposure confidence score (very low, low, moderate, high) for each pesticide class was assigned to maternal

jobs; corresponding confidence scores for paternal jobs were not assigned. For paternal occupations, exposure assessment only was completed for a father if the respective mother reported being employed during pregnancy. Additionally, to reduce the potential for confounding through maternal employment status,<sup>[26]</sup> only mothers who reported employment during pregnancy were included in analyses.

For each maternal or paternal job reported, the total number of hours worked per week was calculated by multiplying the reported hours worked per day by the reported days typically worked in a week. For jobs where hours worked per day and/or days worked per week were missing (maternal jobs <1%, paternal jobs <2%), an 8-hr per day and/or 5-day per week schedule was assumed. Job reports that exceeded 12-hr worked per day and/or 7 days worked per week were reviewed; most of these reports were for jobs with 24-hr on-call, but not on-duty time, such as firefighters. These jobs were truncated at 16-hr per day.

The first trimester is the critical period for OFC development.<sup>[2]</sup> Maternal occupational pesticide exposure was estimated for jobs that overlapped all or part of the maternal critical exposure period—defined as 1 month before conception through the first 3 months of pregnancy (B1-P3). To attempt to account for potential paternal adverse spermatogenic effects and take-home exposures, paternal occupational pesticide exposure was estimated for jobs that overlapped all or part of the paternal critical exposure period—defined as 3 months before conception through the first 3 months of pregnancy (B3-P3).

For jobs with an exposure probability >0, cumulative exposure to each pesticide class examined was estimated as: (exposure intensity in mg/hr) × ([exposure frequency in hr/week]/[40 hr/week]) × ([hr worked per week]/[7 days per week]) × (days worked during relevant exposure period). A total cumulative exposure estimate for each pesticide class was generated by summing across all jobs held during the relevant critical exposure period. To account for the potential imprecision of the cumulative exposure estimate, cumulative exposure was categorized as no exposure, low (<median exposure level in controls), or high (≥median exposure level in controls). Mothers or fathers rated with no pesticide exposure in any job in the respective critical exposure period were considered unexposed and included in the referent group in respective analyses.

### Statistical analysis

The final maternal analytic sample consisted of mothers with an EDD from October 1997–December 2002 who

completed the occupational section of the maternal interview and reported employment during B3-P9; mothers who reported not working or the employment status was unknown were not included in the analytic sample. The paternal analytic sample included employment information for those fathers that were reported as employed during the year before the mother's EDD. Descriptive analyses, using the chi-square test or Fisher's exact test (expected cell counts <5), compared child and parental covariables based on previously reported associations with major structural birth defects or OFCs. Child characteristics examined were sex, gestational age, plurality, family history of a first-degree relative with an OFC, and NBDPS site. Self-reported maternal characteristics examined were race/ethnicity; age and education at delivery; parity; pre-pregnancy Body Mass Index (BMI); and alcohol use, cigarette smoke exposure, use of folic acid-containing supplements, and use of vitamin A-containing supplements during the maternal critical exposure period. Because pre-gestational diabetes is a well-known risk factor for birth defects, including OFCs,<sup>[27–30]</sup> mothers who reported diabetes were excluded. Maternal reports of paternal race/ethnicity and age at delivery also were examined.

Crude odds ratios (cOR)s and 95% confidence intervals (CI)s were estimated for any (yes, no) and cumulative (none, low, high) maternal or paternal exposure to pesticides (regardless of pesticide class) during the respective parental critical exposure periods and CL/P and CP. Crude odds ratios and 95% CIs also were estimated for combined parental pesticide exposure, which may increase risk of having a child with an OFC, compared to exposure from either parent singly. For these analyses, maternal (yes, no) and paternal (yes, no) occupational exposures to pesticides were combined. Additional analyses combining maternal cumulative (none, low, high) exposure to pesticides with the respective any (yes, no) paternal exposure to pesticides were conducted; the combination of these exposures produced potential exposure combinations ranging from (no maternal cumulative exposure + no paternal exposure) to (high maternal cumulative exposure + any paternal exposure). Analyses were conducted for a pesticide class or pesticide class combination when at least five case mothers or fathers were rated as exposed to a class or class combination.

Adjusted odds ratios (aOR)s and 95% CIs were estimated for each maternal, paternal, and combined parental occupational exposure-outcome pairing using unconditional logistic regression analysis and two model-building approaches. One was a change-in-parameter estimate approach where each individual covariable was

included in a model with the occupational pesticide exposure of interest; if the covariable altered the unadjusted pesticide cOR estimate by >10%, it was included in the multivariable model. Additionally, any (yes, no) paternal occupational exposure to pesticides was assessed as a possible covariable in models for maternal occupational pesticide exposure; the converse was assessed in models for paternal occupational pesticide exposure. The other model-building approach applied a common set of selected covariables (NBDPS site, maternal race/ethnicity, age at delivery, education at delivery, pre-pregnancy BMI, and cigarette smoke exposure during the critical exposure period) to each individual pesticide exposure model; these covariables were selected based on their use in models in previous studies of pesticide exposure and OFCs or previous NBDPS literature for OFCs. Because results from each model-building approach were similar, only results of the common covariable set are presented. In addition to examining OFC subtypes for maternal and paternal analyses, aORs were estimated for any maternal and any paternal pesticide exposure and all OFCs combined for comparison with previous literature.

Several child-level sub-analyses were conducted examining: (1) cleft lip and cleft lip with cleft palate, separately, compared to all controls, as there may be etiologic differences between these two subtypes, which compose the CL/P subtype; (2) only cases with isolated defects, compared to all controls, as there may be etiologic differences between isolated and multiple cases; and (3) cases and controls without a family history of an OFC to examine risk among cases, independent of potential increased hereditary risk. Several exposure-level sub-analyses also were conducted. These sub-analyses included comparing mothers with unexposed jobs to those with (1) jobs rated as exposed with the highest two IH confidence levels (moderate, high) and (2) jobs with an exposure probability score  $\geq 90\%$ , as well as comparing mothers or fathers with unexposed jobs with the respective (3) mothers and fathers with jobs highly exposed (highest 25%) among the high exposure group and (4) mothers and fathers with jobs with high exposure intensity ratings ( $\geq 100$  mg/hr), as cumulative exposure may dilute the effect of high intensity exposure. All analyses were conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC).

## Results

For NBDPS OFC cases and controls, interview data were collected from 5,880 mothers (cases = 1,763,

controls = 4,117) with an EDD from October 1997–December 2002. Of these mothers, 1,596 (cases = 510, controls = 1,086) reported not working during pregnancy, and the occupational status during pregnancy was unknown for 64 (cases = 17, controls = 47) mothers. The remaining 4,220 (71.7%; cases = 1,236, controls = 2,984) mothers who responded to the occupational section of the maternal interview and reported employment during B3-P9 were included in the maternal analytic sample. Of these 4,220 employed mothers, the paternal analytic sample included employment information for 3,877 fathers (66%; cases = 1,127, controls = 2,750) who were reported as employed sometime during the year before the mothers' EDDs.

Of the 4,220 mothers who reported employment during pregnancy, 183 (cases = 45, controls = 138) reported a job that did not overlap with the maternal critical exposure period and 20 (cases = 6, controls = 14) provided insufficient occupational information or did not provide dates of employment, leaving 4,017 mothers (95.2%; cases = 1,185, controls = 2,832) in the maternal analytic sample.

Among the 3,877 fathers who were reported as employed during pregnancy, 71 (cases = 22, controls = 49) did not have a reported job that overlapped the paternal critical exposure period. Because mothers provided proxy reports for fathers, insufficient occupational information or missing information for dates of employment, days worked per week, or hours worked per week were more common for fathers than mothers. Fifty-four fathers (cases = 8, controls = 46) rated as exposed but with missing information for dates, days, or hours worked were excluded, because applying assumptions for these variables could result in questionable exposure classifications. Fathers rated as unexposed to pesticides, but with missing information for dates, days, or hours worked, were not excluded, because these assumptions would not influence exposure classification. Following exclusions, the paternal analytic sample was composed of 3,752 (97.8%; case = 1,097, controls = 2,655) fathers. For analysis of combined parental exposure, maternal self-reports and reports of paternal occupation were available for 1,052 cases and 2,541 control parental pairs.

## Descriptive analysis

Compared to controls, CL/P cases were more likely to be male, from a multiple pregnancy, positive for a first-degree family history of OFCs, or delivered preterm; differences in proportions of cases and controls



**Table 1.** Selected characteristics of children and birth mothers for controls and orofacial clefts cases, National Birth Defects Prevention Study, 1997–2002.

Characteristic	Controls (n = 2,832) N* (%) <sup>†</sup>	CL/P Cases (n = 765) N* (%) <sup>†</sup>	CP Cases (n = 420) N* (%) <sup>†</sup>
<i>Child</i>			
<b>Phenotype</b>			
Isolated	NA	672 (87.8)	346 (82.4)
Multiple	NA	93 (12.2)	74 (17.6)
<b>Sex<sup>a</sup></b>			
Male	1,407 (49.7)	520 (68.0)	189 (45.0)
Female	1,423 (50.2)	242 (31.6)	230 (54.8)
<b>Plurality<sup>a</sup></b>			
Singleton	2,736 (96.6)	721 (94.3)	402 (95.7)
Multiple	94 (3.3)	43 (5.6)	18 (4.3)
<b>First Degree Family History of OFCs<sup>a,b</sup></b>			
Yes	7 (0.2)	48 (6.3)	20 (4.8)
No	2,825 (99.8)	717 (93.7)	400 (95.2)
<b>Gestational Age (weeks)<sup>a,b</sup></b>			
Preterm: ≤36 weeks	247 (8.7)	119 (15.6)	84 (20.0)
Term: >36 weeks	2,585 (91.3)	646 (84.4)	336 (80.0)
<b>NBDPS Site<sup>a,b</sup></b>			
Arkansas	353 (12.5)	80 (10.5)	43 (10.2)
California	323 (11.4)	99 (12.9)	38 (9.1)
Georgia	332 (11.7)	86 (11.2)	61 (14.5)
Iowa	398 (14.1)	111 (14.5)	47 (11.2)
Massachusetts	422 (14.9)	112 (14.6)	86 (20.5)
New Jersey	391 (13.7)	79 (10.3)	47 (11.2)
New York	318 (11.3)	85 (11.1)	50 (11.9)
Texas	295 (10.5)	113 (14.8)	48 (11.4)
<i>Maternal</i>			
<b>Race/Ethnicity<sup>a,b</sup></b>			
Non-Hispanic White	1,872 (66.1)	519 (67.8)	312 (74.3)
Non-Hispanic Black	358 (12.6)	44 (5.8)	27 (6.4)
Hispanic	476 (16.8)	156 (20.4)	60 (14.3)
Other	126 (4.5)	46 (6.0)	21 (5.0)
<b>Age at Delivery (years)<sup>a</sup></b>			
<20	204 (7.2)	75 (9.8)	32 (7.6)
20–24	605 (21.4)	198 (25.9)	87 (20.7)
25–29	768 (27.1)	203 (26.5)	113 (26.9)
30–34	825 (29.1)	181 (23.7)	109 (26.0)
35–39	365 (12.9)	88 (11.5)	63 (15.0)
≥40	65 (2.3)	20 (2.6)	16 (3.8)
<b>Education at Delivery (years)<sup>a</sup></b>			
0–8	65 (2.3)	29 (3.8)	9 (2.1)
9–11	198 (7.0)	78 (10.2)	32 (7.6)
12	685 (24.2)	209 (27.3)	103 (24.5)
13–15	861 (30.5)	220 (28.8)	146 (34.8)
≥16	1,018 (36.0)	228 (29.8)	130 (31.0)
<b>Pre-pregnancy BMI (kg/m<sup>2</sup>)<sup>a</sup></b>			
Underweight (<18.5)	144 (5.1)	60 (7.8)	23 (5.5)
Normal Weight (18.5–24.9)	1,595 (56.3)	403 (52.7)	226 (53.8)
Overweight (25–<30.0)	622 (22.0)	151 (19.7)	92 (21.9)
Obese (≥30.0)	419 (14.8)	131 (17.1)	73 (17.4)
<b>Parity<sup>a</sup></b>			
Nulliparous	1,233 (43.5)	370 (48.4)	197 (46.9)
Primiparous	988 (34.9)	241 (31.5)	141 (33.6)
Multiparous	610 (21.5)	154 (20.1)	82 (19.5)
<b>Use of Folic Acid-Containing Supplements<sup>**</sup></b>			
Yes	2,508 (88.6)	671 (87.7)	373 (88.9)
No	283 (10.0)	87 (11.4)	41 (9.8)
<b>Alcohol w/ Binge Events<sup>**</sup></b>			
No Drinking	1,566 (55.3)	425 (55.6)	226 (53.8)
Drinking and Binge Event (≥4 drinks on one occasion)	432 (15.3)	110 (14.4)	56 (13.3)
Drinking but no Binge Events	808 (28.5)	222 (29.0)	132 (31.4)
<b>Cigarette Smoking<sup>** a,b</sup></b>			
No Active or Passive Smoking	1,762 (62.2)	439 (57.4)	239 (56.9)
Active Smoking Only	188 (6.6)	60 (7.8)	43 (10.2)
Passive Smoking Only	491 (17.3)	126 (16.5)	70 (16.7)
Active and Passive Smoking	384 (13.6)	136 (17.8)	67 (16.0)
<b>Use of Vitamin A-Containing Supplements<sup>**</sup></b>			
Yes	1,464 (51.7)	371 (48.5)	210 (50.0)
No	1,348 (47.6)	391 (51.1)	209 (49.8)

(Continued)

Table 1. Continued.

Characteristic	Controls (n = 2,832) N* (%) <sup>†</sup>	CL/P Cases (n = 765) N* (%) <sup>†</sup>	CP Cases (n = 420) N* (%) <sup>†</sup>
<i>Paternal</i>			
<b>Race/Ethnicity</b>			
Non-Hispanic White	1,824 (64.4)	500 (65.4)	297 (70.7)
Non-Hispanic Black	383 (13.5)	53 (6.9)	43 (10.2)
Hispanic	463 (16.4)	155 (20.3)	57 (13.6)
Other	134 (4.7)	43 (5.6)	20 (4.8)
<b>Age at delivery (years)</b>			
<20	81 (2.9)	32 (4.2)	13 (3.1)
20–24	430 (15.2)	153 (20.0)	59 (14.1)
25–29	725 (25.6)	180 (23.5)	98 (23.3)
30–34	760 (26.8)	187 (24.4)	124 (29.5)
35–39	494 (17.44)	116 (15.2)	72 (17.1)
>40	247 (8.7)	72 (9.4)	43 (10.2)

CL/P: cleft lip with or without cleft palate; CP: cleft palate; OFC: orofacial cleft; NA: not applicable; BMI: body mass index.

\*Numbers may vary due to incomplete or missing data.

<sup>†</sup>Due to rounding, percentages may not total 100.

\*\*During the maternal critical exposure period (1 month before conception through the first 3 months of pregnancy).

<sup>°</sup>CL/P: 499 CL with CP cases; 266 CL without CP cases.

<sup>a</sup>*p* < 0.05 for CL/P vs. controls.

<sup>b</sup>*p* < 0.05 for CP vs. controls.

recruited across NBDPS sites also were observed (Table 1). CL/P case mothers tended to be Hispanic or other race/ethnicity, younger, less educated, underweight or obese, nulliparous, or cigarette smokers, compared to controls; CL/P case fathers were more frequently reported to be Hispanic or other race/ethnicity and younger. Compared to controls, CP cases were more frequently positive for a first-degree family history of OFCs or be delivered preterm; differences also were observed for NBDPS site. CP case mothers tended to be non-Hispanic White or cigarette smokers, compared to controls. Differences observed for child, maternal, and paternal characteristics between

all OFCs combined and controls were similar to those for CL/P (data not shown).

### Pesticide exposure

Overall, mothers of 35.4% of CL/P cases, 32.1% of CP cases, and 32.3% of controls were rated as potentially occupationally exposed to pesticides during the maternal critical exposure period (Table 2). Likewise, similar proportions of case and control mothers were rated as occupationally exposed to individual or combinations of pesticide classes examined, except

Table 2. Parental occupational pesticide exposure during the critical exposure period for controls and orofacial cleft subtypes, National Birth Defects Prevention Study, 1997–2002.

	Controls			CL/P			CP		
	N	% <sup>†</sup>	median (mg)	N	% <sup>†</sup>	median (mg)	N	% <sup>†</sup>	median (mg)
<b>Maternal Pesticide Exposure</b>									
Pesticide exposure									
No exposure	1,915	67.6	N/A	494	64.6	N/A	285	67.9	N/A
Exposed to any pesticide*	917	32.3	100.0	271	35.4	130	135	32.1	69.5
Pesticide exposure by class or class combination among exposed									
Insecticide only	632	22.3	62.0	166	21.7	96.0	88	21.0	69.5
Insecticide + Herbicide	54	1.9	12.4	21	2.7	10.8	11	2.6	6.8
Insecticide + Fungicide	6	0.2	53.0	2	0.3	134.0	3	0.7	76.0
Insecticide + Herbicide + Fungicide	222	7.8	300.0	80	10.5	270.0	33	7.9	271.4
<b>Paternal Pesticide Exposure</b>									
Pesticide exposure									
No exposure	2,380	89.6	N/A	634	90.7	N/A	348	87.4	N/A
Exposed to any pesticide*	275	10.4	2,169.6	65	9.3	2,892.9	50	12.6	2,314.3
Pesticide exposure by class or class combination among exposed									
Insecticide only	32	1.2	964.3	7	1.0	642.9	9	2.3	723.2
Fungicide only	61	2.3	1,928.6	13	1.9	1,928.6	10	2.5	1,928.6
Insecticide + Herbicide	15	0.6	2,169.6	4	0.6	1,366.1	4	1.0	2,571.4
Insecticide + Fungicide	67	2.5	1,928.6	12	1.7	3,857.1	7	1.8	1,285.7
Insecticide + Herbicide + Fungicide	85	3.2	32,785.7	27	3.9	21,214.3	19	4.8	13,725.0

CL/P: cleft lip with or without cleft palate; CP: cleft palate.

Critical exposure period for mothers corresponds to 1 month before conception through the first 3 months of pregnancy.

Critical exposure period for fathers corresponds to 3 months before conception through the first 3 months of pregnancy.

<sup>†</sup>Due to rounding, percentages may not total 100.

\*Exposed to any pesticide = exposure to insecticide, herbicide, or fungicide.

**Table 3.** Combined parental occupational pesticide exposure during the critical exposure period for controls and orofacial cleft subtypes, National Birth Defects Prevention Study, 1997–2002.

	Controls (n = 2,541)		CL/P (n = 668)		CP (n = 384)	
	N	% <sup>†</sup>	N	% <sup>†</sup>	N	% <sup>†</sup>
Any Maternal Exposure + Any Paternal Exposure						
No Pesticide Exposure	1,591	62.6	415	62.1	230	59.9
Paternal Exposure Only	160	6.3	31	4.6	32	8.3
Maternal Exposure Only	683	26.9	195	29.2	107	27.9
Both Parents Exposed	107	4.2	27	4.0	15	3.9
Maternal Cumulative Exposure + Any Paternal Exposure						
No Pesticide Exposure	1,591	62.6	415	62.1	230	59.9
Paternal Exposure Only	160	6.3	31	4.6	32	8.3
Maternal Low + No Paternal Exposure	365	14.4	96	14.4	56	14.6
Maternal High + No Paternal Exposure	318	12.5	99	14.8	51	13.3
Maternal Low + Any Paternal Exposure	48	1.9	7	1.0	10	2.6
Maternal High + Any Paternal Exposure	59	2.3	20	3.0	5	1.3

CL/P: cleft lip with or without cleft palate; CP: cleft palate.

Critical exposure period for mothers corresponds to 1 month before conception through the first 3 months of pregnancy.

Critical exposure period for fathers corresponds to 3 months before conception through the first 3 months of pregnancy.

<sup>†</sup>Due to rounding, percentages may not total 100.**Table 4.** Adjusted odds ratio estimates for orofacial cleft subtypes associated with maternal occupational exposure to pesticides during the maternal critical exposure period, National Birth Defects Prevention Study, 1997–2002.

Pesticide Exposures During the Maternal Critical Exposure Period	Controls (n = 2,832)		CL/P (n = 765)		CP (n = 420)	
	N		N	aOR <sup>a</sup> (95% CI)	N	aOR <sup>a</sup> (95% CI)
Any (yes, no) pesticide exposure						
No Exposure	1,915		494	Ref	285	Ref
Exposure to any pesticide*	917		271	1.0 (0.9, 1.2)	135	1.1 (0.8, 1.3)
Insecticide Only	632		166	1.0 (0.8, 1.2)	88	1.0 (0.8, 1.3)
Insecticide + Herbicide	54		21	1.2 (0.7, 2.1)	11	1.3 (0.6, 2.7)
Insecticide + Fungicide	6		2	NC	3	NC
Insecticide + Herbicide + Fungicide	222		80	1.1 (0.8, 1.5)	33	1.1 (0.7, 1.6)
Cumulative pesticide exposure						
No Exposure	1,915		494	Ref	285	Ref
Exposed to any pesticide*						
Low (>0 and <100.0 mg)	456		120	1.0 (0.8, 1.3)	77	1.2 (0.9, 1.6)
High (≥100.0 mg)	461		151	1.0 (0.8, 1.3)	58	0.9 (0.7, 1.2)
Insecticide Only						
Low (>0 and <62.0 mg)	315		70	0.9 (0.7, 1.2)	48	1.2 (0.9, 1.6)
High (≥62.0 mg)	317		96	1.0 (0.8, 1.4)	40	0.9 (0.6, 1.3)
Insecticide + Herbicide						
Low (>0 and <12.4 mg)	27		11	0.9 (0.4, 2.3)	6	1.6 (0.6, 4.0)
High (≥12.4 mg)	27		10	1.4 (0.6, 3.0)	5	1.1 (0.4, 3.2)
Insecticide + Fungicide						
Low (>0 and <53.0 mg)	4		0	NC	2	NC
High (≥53.0 mg)	2		2	NC	1	NC
Insecticide + Herbicide + Fungicide						
Low (>0 and <300.0 mg)	110		44	1.3 (0.9, 1.9)	17	1.1 (0.6, 1.9)
High (≥300.0 mg)	112		36	0.9 (0.6, 1.4)	16	1.0 (0.6, 1.8)

CL/P: cleft lip with or without cleft palate; CP: cleft palate; aOR: adjusted odds ratio; Ref: reference; NC: not calculated; Low: &lt;median exposure level in controls; High: ≥median exposure level in controls.

Critical exposure period for mothers corresponds to 1 month before conception through the first 3 months of pregnancy.

\*Exposed to any pesticide = exposure to insecticide, herbicide, or fungicide.

<sup>a</sup>ORs adjusted for NBDPS site, maternal race/ethnicity, age at delivery, education at delivery, pre-pregnancy body mass index, cigarette smoke exposure during the maternal critical exposure period.

exposure to insecticide + herbicide + fungicide, which was higher among CL/P cases than controls. For cumulative exposures, estimated median values were highest for mothers rated as exposed to insecticide + herbicide + fungicide (range 270.0–300.0 mg) and lowest for insecticide + herbicide (range 6.8–12.4 mg).

Maternal occupational exposures to insecticide only and insecticide + herbicide were most often rated as low intensity and low frequency, with waitress (13.4%)

and janitorial service (46.4%), respectively, the most frequently reported jobs. Maternal occupational exposures to insecticide + herbicide + fungicide were most often rated as low intensity and high frequency, with supermarket/grocery store employee (15.8%) the most frequently reported job (data not shown).

Overall, fathers of 9.3% of CL/P cases, 12.6% of CP cases, and 10.4% of controls were rated as potentially occupationally exposed to pesticides during the paternal



**Table 5.** Adjusted odds ratio estimates for orofacial cleft subtypes associated with paternal occupational exposure to pesticides during the paternal critical exposure period, National Birth Defects Prevention Study, 1997–2002.

Pesticide Exposures During the Paternal Critical Exposure Period	Controls ( <i>n</i> = 2,655)	CL/P ( <i>n</i> = 699)		CP ( <i>n</i> = 398)	
	N	N	aOR <sup>a</sup> (95% CI)	N	aOR <sup>a</sup> (95% CI)
Any (yes, no) pesticide exposure					
No Exposure	2,380	634	Ref	348	Ref
Exposed to any pesticide*	275	65	0.8 (0.6, 1.0)	50	1.3 (0.9, 1.8)
Insecticide Only	32	7	0.8 (0.3, 1.8)	9	1.9 (0.9, 4.0)
Fungicide Only	61	13	0.7 (0.4, 1.3)	10	1.1 (0.5, 2.1)
Insecticide + Herbicide	15	4	NC	4	NC
Insecticide + Fungicide	67	12	0.5 (0.3, 1.1)	7	0.7 (0.3, 1.6)
Insecticide + Herbicide + Fungicide	85	27	1.1 (0.7, 1.7)	19	1.6 (0.9, 2.8)
Cumulative Exposure					
No exposure	2,380	634	Ref	348	Ref
Exposed to any pesticide*					
Low (>0 and <2,169.6 mg)	137	24	0.6 (0.4, 0.9)	25	1.2 (0.8, 1.9)
High (≥2,169.6 mg)	138	41	0.9 (0.6, 1.3)	25	1.3 (0.8, 2.0)
Insecticide Only					
Low (>0 and <964.3 mg)	18	5	1.0 (0.4, 2.8)	6	2.1 (0.8, 5.7)
High (≥964.3 mg)	14	2	NC	3	NC
Fungicide Only					
Low (>0 and <1,928.6 mg)	30	6	0.7 (0.3, 1.6)	3	NC
High (≥1,928.6 mg)	31	7	0.7 (0.3, 1.7)	7	1.6 (0.7, 3.7)
Insecticide + Herbicide					
Low (>0 and <2,169.6 mg)	8	3	NC	2	NC
High (≥2,169.6 mg)	7	1	NC	2	NC
Insecticide + Fungicide					
Low (>0 and <1,928.6 mg)	33	4	NC	4	NC
High (≥1,928.6 mg)	34	8	0.6 (0.2, 1.3)	3	NC
Insecticide + Herbicide + Fungicide					
Low (>0 and <32,785.7 mg)	42	16	1.3 (0.7, 2.4)	15	2.6 (1.4, 4.8)
High (≥32,785.7 mg)	43	11	1.0 (0.5, 1.9)	4	NC

CL/P: cleft lip with or without cleft palate; CP: cleft palate; aOR: adjusted odds ratio; Ref: reference; NC: not calculated; Low: <median exposure level in controls; High: ≥median exposure level in controls.

Critical exposure period for fathers corresponds to 3 month before conception through the first 3 months of pregnancy.

\*Exposed to any pesticide = exposure to insecticide, herbicide, or fungicide.

<sup>a</sup>ORs adjusted for NBDPS site, maternal race/ethnicity, age at delivery, education at delivery, pre-pregnancy body mass index, cigarette smoke exposure during the maternal critical exposure period (1 month before conception through the first 3 months of pregnancy).

critical exposure period (Table 2). Similar proportions of case and control fathers were rated as occupationally exposed to individual or combinations of pesticide classes examined. For paternal cumulative exposures, estimated median values were higher than those for maternal cumulative exposures and were highest for fathers rated as exposed to insecticide + herbicide + fungicide (range 13,725.0–32,785.7 mg) and lowest for insecticide only (642.9–964.3 mg).

Paternal occupational exposures to insecticide only, fungicide only, insecticide + herbicide, and insecticide + fungicide were most often rated as low intensity and low frequency with janitorial service (16.0%), painting/wall covering contractor (29.2%), landscaping service (44.0%), and grape farmer (11.0%), respectively, the most frequently reported jobs. Paternal occupational exposures to insecticide + herbicide + fungicide varied, with intensity and frequency ratings ranging from low to high; crop production and landscaping service (23.0% each) were the most frequently reported jobs (data not shown).

For cases and controls with both parents employed, the proportion of children with maternal exposure only to pesticides was modestly higher among CL/P

and CP than controls, whereas proportions of children with both parents exposed were similar among CL/P and CP and controls (Table 3). Exposure proportions combining categories of maternal cumulative pesticide exposures with the corresponding any paternal pesticide exposure were mostly similar among CL/P and CP and controls.

### Multivariable analysis

Compared to controls, maternal associations, adjusted for selected covariables (described in Methods), for any exposure to pesticides, pesticide classes, or pesticide class combinations and CL/P and CP were mostly near unity and statistically non-significant; the highest associations observed for both CL/P and CP were for insecticide + herbicide (Table 4). Several associations for cumulative exposures (none, low, high) also tended to be near unity. Similarly, the association for any pesticide exposure and all OFCs combined was near unity and statistically non-significant (data not shown).

Paternal associations, adjusted for selected covariables, for any exposures to pesticides, pesticide classes, or

**TABLE 6.** Adjusted odds ratio estimates for orofacial cleft subtypes associated with combined parental occupational exposure to pesticides during the critical exposure period, National Birth Defects Prevention Study, 1997–2002.

Pesticide Exposures During the Critical Exposure Period	Controls ( <i>n</i> = 2,541)	CL/P ( <i>n</i> = 668)		CP ( <i>n</i> = 384)	
	N	N	aOR <sup>a</sup> (95% CI)	N	aOR <sup>a</sup> (95% CI)
Any Maternal Exposure + Any Paternal Exposure					
No Pesticide Exposure	1,591	415	Ref	230	Ref
Paternal Exposure Only	160	31	0.7 (0.5, 1.0)	32	1.4 (0.9, 2.1)
Maternal Exposure Only	683	195	1.0 (0.8, 1.2)	107	1.2 (0.9, 1.5)
Both Parents Exposed	107	27	0.7 (0.4, 1.1)	15	1.0 (0.5, 1.8)
Maternal Cumulative Exposure + Paternal Exposure					
No Pesticide Exposure	1,591	415	Ref	230	Ref
Paternal Exposure Only	160	31	0.7 (0.5, 1.0)	32	1.4 (0.9, 2.1)
Maternal Low + No Paternal Exposure	365	96	1.0 (0.8, 1.3)	56	1.2 (0.8, 1.6)
Maternal High + No Paternal Exposure	318	99	1.0 (0.7, 1.3)	51	1.2 (0.8, 1.7)
Maternal Low + Any Paternal Exposure	48	7	0.5 (0.2, 1.1)	10	1.3 (0.6, 2.9)
Maternal High + Any Paternal Exposure	59	20	0.9 (0.5, 1.6)	5	0.7 (0.3, 1.8)

OFCs: orofacial clefts; CL/P: cleft lip with or without cleft palate; CP: cleft palate; aOR: adjusted odds ratio; Ref: reference; Low: <median exposure level in controls; High: ≥median exposure level in controls.

Critical exposure period for mothers corresponds to 1 month before conception through the first 3 months of pregnancy.

Critical exposure period for fathers corresponds to 3 months before conception through the first 3 months of pregnancy.

<sup>a</sup>ORs adjusted for NBDPS site, maternal race/ethnicity, age at delivery, education at delivery, pre-pregnancy body mass index, cigarette smoke exposure during the maternal critical exposure period (1 month before conception through the first 3 months of pregnancy).

pesticide class combinations and CL/P were mostly below unity, whereas those for CP were mostly above unity (Table 5); no associations were statistically significant. Findings tended to be similar for cumulative exposures with most associations for CL/P below unity and associations for CP above unity; a statistically significant, inverse association was observed for low exposure to pesticides and CL/P and a significant, positive association was observed for low exposure to insecticide + herbicide + fungicide and CP. The association for any paternal occupational pesticide exposure and all OFCs combined was near unity (data not shown).

Combined parental analyses, adjusted for selected covariables, produced null (maternal exposure only) or inverse (paternal exposure only and both parents exposed) associations for CL/P, and null (both parents exposed) or positive (maternal exposure only and paternal exposure only) associations for CP; no associations were statistically significant (Table 6). In similar analyses, associations for combining categories of maternal cumulative exposures and any paternal exposure and CL/P tended to be near unity for categories with only one parent exposed (paternal exposure only, maternal low or high exposure without paternal exposure), but below unity for both parents exposed; no associations were significant. Positive, but non-significant, associations for CP were observed for most maternal cumulative and paternal exposure combinations. Associations for combined parental exposure and all OFCs combined were mostly near unity (data not shown).

### Sub-analyses

Results for child-level sub-analyses examining (1) cleft lip (*n* = 266) and cleft lip with cleft palate (*n* = 499) separately, compared to all controls; (2) only cases with isolated defects (*n* = 1,018), compared to all

controls; and (3) cases (*n* = 1,117) and controls (*n* = 2,825) without a family history of an OFC were similar to those of the respective main analyses (data not shown). Exposure-level sub-analyses comparing (1) mothers with jobs (3,782 mothers, 94.2%) rated with the two highest IH confidence levels and (2) mothers with jobs (75 mothers, 1.9%) with high probability scores to mothers with unexposed jobs, and (3) comparing the most highly exposed mothers (181 mothers, 4.5%) and fathers (47 fathers, 1.3%) with unexposed parents also produced results similar to those of the respective, main analyses (data not shown). The comparison of maternal (38 mothers, 1.0%) jobs with high intensity ratings to jobs with no exposure was limited to examination of any pesticide exposure and insecticide exposure only. Comparison of paternal (14 fathers, 0.4%) jobs with high intensity ratings to jobs with no exposure was limited to examination of any pesticide exposure. Although no statistically significant results were observed, maternal aORs for these analyses, ranged from 1.7–2.0; results of paternal analyses were similar to the main analyses.

### Discussion

Compared to controls, maternal associations for any or cumulative occupational exposures to pesticides, pesticide classes, or pesticide class combinations during the maternal critical exposure period were mostly near unity for CL/P and CP and non-significant; the association for any exposure and all OFCs combined also was near unity and non-significant. The highest associations observed were for insecticide + herbicide for both CL/P and CP, although these estimates were imprecise. Paternal analyses for any exposure produced

non-significant associations below and above unity for CL/P, but mostly above unity for CP; the association for any pesticide exposure and all OFCs combined was near unity. Directions of associations for cumulative paternal occupational pesticide exposure were mixed, including a significant, inverse association observed between low exposure to pesticides and CL/P and a significant, positive association between low exposure to insecticide + herbicide + fungicide and CP. Associations estimated for combined parental pesticide exposure tended to be below unity for CL/P, above unity for CP, and near unity for all OFCs combined; no associations were significant.

The findings for any maternal occupational pesticide exposure were consistent with those in some previous studies,<sup>[13,14,16]</sup> although two case-control studies<sup>[12,15]</sup> and one meta-analysis<sup>[5]</sup> reported significant, positive associations. In both case-control studies, small sample sizes limited results to all OFCs combined<sup>[12]</sup> or all birth defects combined,<sup>[15]</sup> and reported estimates were imprecise. In contrast, the present study had 406 mothers exposed to any pesticides, although the numbers were much smaller for some classes or combinations of classes. Among most previous studies, small sample sizes and limited exposure assessment did not allow risk estimation for specific pesticides or pesticide classes.<sup>[12–16]</sup> Additionally, significant positive results reported in one study<sup>[15]</sup> were based on self-reported pesticide exposures, rather than the likely lower biased IH exposure assessment.

The findings for any paternal occupational exposure to pesticides tended to parallel those in previous studies<sup>[14–18]</sup> and one meta-analysis.<sup>[5]</sup> Exposure assessment in most previous studies was limited to self-reported exposures or use of occupational titles.<sup>[15–18]</sup> Findings of an inverse association between low cumulative exposure to any pesticide and CL/P, and a positive association between low cumulative exposure to insecticide + herbicide + fungicide and CP could not be compared with previous studies, as no studies examined pesticide classes or cumulative exposure to pesticides. Likewise, no studies were identified that examined combined parental occupational pesticide exposure and OFCs, although one study examined combined parental occupational pesticide exposure and any birth defect.<sup>[15]</sup> The study compared self-reported agricultural work with nonagricultural work and reported a positive association for all birth defects combined.

Although the teratogenicity of prenatal pesticide exposure for OFCs has been demonstrated in several animal studies,<sup>[6–11]</sup> mechanisms by which pesticides affect lip and palate development are not well understood. Pesticides may mediate alterations to retinoic

acid (RA) signaling in the developing embryo; RA affects proliferation and differentiation of cranial neural crest cells,<sup>[31]</sup> which give rise to the lip and palate.<sup>[32]</sup> Notably, expression of some genes, including Sonic hedgehog (*Shh*)<sup>[33]</sup> and *Msx2*,<sup>[34]</sup> mediated by RA and involved in lip and palate development were downregulated in rat embryos exposed to the fungicide, Triadimefon.<sup>[35]</sup> This fungicide also was shown to inhibit activity of RA degrading enzymes, leading to downregulation of transforming growth factor beta 1 (*TGFB1*) and *TGFB2* expression in rats;<sup>[36]</sup> *TGFB1*, *TGFB2* are involved in the reorientation and fusion of the palatal shelves.<sup>[reviewed in 4]</sup> Additionally, glyphosate-based herbicides were found to alter RA signaling in African clawed frog embryos, leading to elevated blood levels of RA<sup>[37]</sup> and subsequent downregulation of *Shh* and Orthodenticle homeobox 2 (*Otx2*) genes and disruption of cranial neural crest cell development. The role of RA in disrupting neural crest cell development was further supported by prevention of craniofacial defects through resumption of normal *Shh* expression following administration of an RA antagonist.<sup>[37]</sup>

In the present study, a statistically significant, positive association was observed with paternal, but not maternal occupational pesticide exposures, possibly reflecting the differences in magnitude of paternal versus maternal exposures. The highest median exposure was observed among fathers potentially exposed to insecticide + herbicide + fungicide. Given that the positive associations were observed among fathers rated with exposure to insecticide + herbicide + fungicide, this pattern of exposure may have been sufficient to produce adverse spermatogenic effects or represent a considerable source of take-home exposures that contributed to maternal exposure; notably, most mothers were rated as unexposed to pesticides when the respective fathers were rated as exposed to insecticide + herbicide + fungicide. Other explanations may be exposure misclassification resulting from maternal reports of paternal occupational information, and exposure misclassification produced by IH review. The present study also observed a significant inverse association between low exposure to pesticides and CL/P. It is difficult to imagine a biologically plausible mechanism by which pesticide exposure would impart a reduced risk; as such, this association should be interpreted cautiously as it may be a consequence of exposure misclassification or a chance finding.

The present study analyzed data from the NBDPS, one of the largest population-based case-control studies of birth defects. Medical record data for cases were reviewed by clinical geneticists, helping to reduce the

potential for case misclassification. Also, control participants were observed to be similar on several characteristics to mothers of all live births in the corresponding study areas,<sup>[23]</sup> helping to reduce the potential for selection bias. Additionally, occupational information collected allowed exclusion of non-working mothers, helping to reduce the potential for confounding through factors related to employment status.<sup>[26]</sup> Furthermore, the NBDPS collected employment information for both mothers and fathers, which allowed us to control for potential adverse spermatogenic effects or take-home exposures in adjusted analytic models. It also allowed examination of combined parental occupational pesticide exposure, unexplored previously for OFCs.

Another strength of the present study was use of IH review of detailed job descriptions to estimate pesticide exposure. This approach may decrease exposure misclassification and increase precision of estimates compared to the use of a job exposure matrix only, job title only, or self-reported exposure.<sup>[19,20]</sup> Pesticide exposure also was examined by pesticide classes, an approach not used in previous studies of OFCs; with differences in the mechanisms of action of pesticides, examining pesticides as a summary measure may mask potential teratogenic effects of specific pesticides or pesticide classes.<sup>[21]</sup> Lastly, exposures during the critical period of lip and palate development were examined, rather than at any time point during pregnancy.

Even with improved methods, the present study has several limitations. Although the sample size in the present study was larger than those in previous studies, it was still modest for examining risk by OFC subtypes, producing imprecise associations or the inability to estimate risk for some subtype comparisons. Nonetheless, associations between OFC subtypes and several pesticide classes and class combinations not reported in previous studies, including for CL, were estimated. Use of IH review of reported jobs in the present study improved upon the methods used in several previous studies; however, this review was based on maternal self-reports and may have produced non-differential exposure misclassification. Also, although analysis by pesticide class may indicate general pesticide class effects, these classes are made up of a multitude of individual pesticides with different active and inactive ingredients. A review of job titles among maternal and paternal exposures revealed many different jobs held across each pesticide class or class combinations; different jobs within a pesticide class or class combination may be exposed to different pesticides, which may mask pesticide-specific effects.

Additional limitations included paternal occupational information provided by maternal reports, and paternal occupational pesticide exposure only assessed for a father if the mother was employed during pregnancy, potentially producing selection bias among paternal data analyzed. Maternal reports of paternal occupational information produced more missing information than maternal self-reports and may have introduced exposure misclassification among fathers. Despite these limitations, occupational data for >3,700 fathers were available, making the present study among the largest conducted to date for paternal occupational pesticide exposure and OFCs. Also, data regarding occupational factors that could modify maternal or paternal pesticide exposure, such as use of personal protective equipment, were not available for analysis; it is possible that highly exposed workers may have had better exposure controls than those exposed infrequently or at low levels, possibly attenuating the effects of high exposures. Additionally, information on environmental or residential pesticide exposures was not available for analysis, which could produce exposure misclassification. Lastly, given the number of associations estimated and lack of control for multiple comparisons, significant associations observed may be due to chance.

## Conclusions

Using NBDPS data, associations mostly near unity and statistically non-significant for maternal occupational pesticide exposure and OFCs were observed. A statistically significant, inverse association between paternal low exposure to any pesticide and CL/P was observed, as well as a significant, positive association between paternal low exposure to insecticide + herbicide + fungicide and CP; these results should be interpreted cautiously. Future studies using increased sample sizes to facilitate better risk estimation for OFC subtypes are recommended. Improved exposure assessments, where possible, by using direct measurements, examining specific pesticides, and collecting paternal self-reports of occupational information and characterization of other factors, such as use of personal protective equipment and residential and environmental exposures, which may influence pesticide exposure, also are recommended.

## Acknowledgments

We thank the study participants and study staff at each site who contributed to the NBDPS. This work was supported by funding from the Centers for Disease Control and



Prevention (U01DD001035). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

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