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Original Contribution

Residential Agricultural Pesticide Exposures and Risk of Neural Tube Defects and Orofacial Clefts Among Offspring in the San Joaquin Valley of California

Wei Yang, Suzan L. Carmichael, Eric M. Roberts, Susan E. Kegley, Amy M. Padula, Paul B. English, and Gary M. Shaw*

* Correspondence to Dr. Gary M. Shaw, Department of Pediatrics, Stanford University, 1265 Welch Road, Room X159, Stanford, CA 94305-5415 (e-mail: gmshaw@stanford.edu).

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We examined whether early gestational exposures to pesticides were associated with an increased risk of anencephaly, spina bifida, cleft lip with or without cleft palate (CLP), or cleft palate only. We used population-based data along with detailed information from maternal interviews. Exposure estimates were based on residential proximity to agricultural pesticide applications during early pregnancy. The study population derived from the San Joaquin Valley, California (1997–2006). Analyses included 73 cases with anencephaly, 123 with spina bifida, 277 with CLP, and 117 with cleft palate only in addition to 785 controls. A total of 38% of the subjects were exposed to 52 chemical groups and 257 specific chemicals. There were relatively few elevated odds ratios with 95% confidence intervals that excluded 1 after adjustment for relevant covariates. Those chemical groups included petroleum derivatives for anencephaly, hydroxybenzonitrile herbicides for spina bifida, and 2,6-dinitroaniline herbicides and dithiocarbamates-methyl isothiocyanate for CLP. The specific chemicals included 2,4-D dimethylamine salt, methomyl, imidacloprid, and α-(para-nonylphenyl)-ω-hydroxypoly(oxyethylene) phosphate ester for anencephaly; the herbicide bromoxynil octanoate for spina bifida; and trifluralin and maneb for CLP. Adjusted odds ratios ranged from 1.6 to 5.1. Given that such odds ratios might have arisen by chance because of the number of comparisons, our study showed a general lack of association between a range of agricultural pesticide exposures and risks of selected birth defects.

birth defects; congenital abnormalities; endocrine disruptors; environment; pesticides; pregnancy

Abbreviations: aOR, adjusted odds ratio; CEHTP, California Environmental Health Tracking Program; CI, confidence interval; CLP, cleft lip with or without cleft palate; CP, cleft palate alone; EPA, Environmental Protection Agency; NTD, neural tube defects; PLSS, public land survey sections; PM_{10}, particulate matter ≤10 μm in aerodynamic diameter; $PM_{2.5}$, particulate matter ≤2.5 μm in aerodynamic diameter; SB, spina bifida.

Although several pesticide compounds have been shown to be teratogenic in animals (1) and there is substantial concern from the public about potential teratogenic risks, there have been relatively few studies that investigated possible associations between gestational pesticide exposures and specific birth defect phenotypes (2). A few associations have been observed, but the body of data is insufficient to draw clear inferences (2, 3). The multitude of pesticide compounds in use, the difficulty of accurately estimating human exposure to such compounds, and the necessity of obtaining access to geospatially accurate data on birth defects are some of the challenges that have contributed to our knowledge gap.

In particular, women's pesticide exposures have not been sufficiently studied for their contribution to risk of neural tube defects (NTDs) and orofacial clefts in offspring. These birth-defect groups have been studied in relation to women's occupational exposures to pesticides; however, in general, the findings have been inconsistent (4–8). A few studies of residential pesticide exposures have been conducted (4, 9, 10). Although the results have been mixed, these studies are limited by relatively crude measures of exposure, small sample sizes, and investigation of a limited set of specific compounds.

Our objective here was to use population-based data on specific birth defects accompanied by detailed information

from maternal interviews. Exposure estimates were based on residential proximity to agricultural pesticide applications during early pregnancy to extend the limited extant literature on the relationships of pesticides with NTDs and orofacial clefts. The study population was derived from the San Joaquin Valley of California, an area with one of the highest rates of pesticide use in the United States.

METHODS

Study population

The California Center of the National Birth Defects Prevention Study (NBDPS) (11) is a collaborative partnership between Stanford University and the California Birth Defects Monitoring Program in the Department of Public Health. Since 1997, the center has been collecting data from women whose residence at the time of delivery was in 1 of 8 counties in the San Joaquin Valley. The California Birth Defects Monitoring Program is a well-known surveillance program that is population-based (12). 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 1997 to December 2006.

Cases included infants or fetuses with anencephaly or spina bifida (SB), which are the 2 most common subtypes of NTDs, as well as those with cleft lip with or without cleft palate (CLP) or cleft palate alone (CP). Diagnoses were confirmed by clinical geneticists to establish study eligibility based on clinical, surgical, or autopsy reports. Cases recognized or strongly suspected to have single-gene conditions or chromosomal abnormalities and those with identifiable syndromes were ineligible (13), given their presumed distinct underlying etiology. One case with anencephaly and SB was counted only in the anencephaly group and 2 cases with anencephaly and orofacial clefts were counted in both case groups.

Controls included nonmalformed live-born infants randomly selected from birth hospitals to represent the population from which the cases arose (approximately 150 per study year). Maternal interviews were conducted using a standardized, computer-based questionnaire administered 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 71% of eligible cases ($n = 763$) and 69% of controls ($n = 974$). Interviews were completed within an average of 10 months from estimated date of delivery for cases and 8 months for controls. Because pregestational diabetes (type I or II) has been associated with birth defects (14), cases ($n = 17$) and controls ($n = 7$) whose mothers had diabetes were excluded from our analyses. Mothers reported their residential history from 3 months before conception through delivery, including dates and residences occupied for more than 1 month.

Pesticide exposure assessment

To estimate pesticide exposures, we assigned a time window of exposure for each case or control mother from 1 month before to 2 months after her reported date of conception. The California Environmental Health Tracking Program (CEHTP) Geocoding Service was used to geocode study subjects' residences corresponding to their exposure time window (15). The CEHTP Geocoding Service standardizes, verifies, and corrects addresses before matching against multiple address-attributed reference databases. Geocoding was successful for 82% cases (613 of 746) and 83% controls (807 of 967). Exposure assignments were made for 589 unique cases (196 with NTDs, 73 with anencephaly, 123 with SB, 117 with CP, and 277 with CLP) and 785 controls whose mothers lived in the geocoded addresses more than 68 days during the window of exposure (i.e., at least 75% of the 3-month window). For those mothers who reported multiple addresses, the number of days during which she lived at each address was 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 that described agricultural pesticide applications that occurred between January 1997 and December 2006. These data are submitted by pesticide applicators to county agriculture commissioners and are spatially referenced to public land survey sections (PLSS). During the 10-year study period, the total number of daily production agricultural-use records with a PLSS specified for the 461 active ingredients in this study was 23,883,704. Following the method of Rull and Ritz (16), 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 (surveys are conducted roughly every 5–7 years in each county in California). Matching was based on location and crop type as specified in the records. Infrequently rotated crops, such as orchard crops and vineyards, were matched one-to-one, whereas frequently rotated crops, such as field and truck crops, were grouped together in a single category, and nonagricultural land uses were subtracted from PLSS polygons when no crop types were matched to available polygons. Of the total applications (and active-ingredient poundage) recorded spanning 1997–2006 for the 461 chemicals of interest, 91.3% (92.1% by poundage) were successfully linked to polygons—31.8% (42.0% by poundage) were matched on individual crop, 56.4% (46.9% by poundage) were under the "frequently rotated" category, and 3.0% (3.1% by poundage) were subtracted for being associated with nonagricultural land-use polygons from PLSS polygons. For the remaining 8.7% of applications (7.9% by poundage), no field polygon was specified and therefore no spatial refinement was possible. Thus, the unrefined PLSS polygon reference was used to link these applications to study subjects. We determined temporal proximity by comparing recorded dates of applications (which are believed to be accurate within a few days) to the time window of exposure for each study subject.

To assign exposure, we utilized the CEHTP Pesticide Linkage Tool, a custom-developed Java (Oracle, Redwood Shores, California) application that incorporates the GeoTools

Java GIS Toolkit, version 2.7.1 (open source; http://geotools.org/) for geographic information systems data management and spatial analysis (17). We calculated pounds of pesticides used during the relevant time window within a 500-m radius of a subject's geocoded address (18), intersecting polygons with the buffer and assuming homogeneous distribution of pesticides within each polygon.

Selection of pesticide compounds

We assessed exposure to 461 individual chemicals and 62 groups of chemicals having the same chemical classification and proven or putative mechanism of action (e.g., organophosphates) that were applied at more than 100 lbs in any of 8 counties (San Joaquin, Stanislaus, Merced, Madera, Fresno, Kings, Tulare, and Kern) in the San Joaquin valley in any year during the study period (1997–2006) (19). We excluded low-toxicity chemicals, such as biopesticides (e.g., microbial pesticides, soaps), low-toxicity inorganic compounds (e.g., sulfur), and other compounds described in risk assessment documents from the US Environmental Protection Agency (EPA) as having low toxicity (20). To create exposure scores, the studied chemicals were flagged as having reproductive or developmental toxicity based on the California Proposition 65 list (21) or as endocrine disruptors (22–24). Chemicals with an EPA-determined reference dose based on an acute toxicological study with a reproductive or developmental endpoint as described in EPA risk-assessment documents were also included (20). We created overall exposure scores by summing the total number of chemical groups, endocrine disruptors, Proposition 65 chemicals, or chemicals in the EPA lists.

Assessment of exposure to air pollutants

In a previous investigation (25), we evaluated daily metrics of the following air pollutants: carbon monoxide, nitrogen oxide, nitrogen dioxide, ozone, particulate matter 10 μm or less in aerodynamic diameter (PM_{10}), and particulate matter 2.5 μm or less in aerodynamic diameter ($PM_{2.5}$). We further considered these measures in the present analysis as a means of capturing a more comprehensive environmental exposure burden. Briefly, using ambient air-quality data collected routinely at more than 20 locations in the San Joaquin Valley by the EPA's Air Quality System database (26), we estimated quartile levels of carbon monoxide, nitrogen oxide, nitrogen dioxide, ozone, PM_{10}, and $PM_{2.5}$ as determined by the distribution in the controls. We also estimated traffic-density measures from distance-decayed annual average daily traffic volumes within a 300-m radius of geocoded maternal residences using the CEHTP web-based traffic volume linkage tool (27).

Statistical analysis

Risks associated with pesticide exposures were estimated using logistic regression. Univariate analyses were conducted to estimate crude odds ratios and 95% confidence intervals that reflected the associations between pesticide exposures and selected birth defects. Associations between pesticide exposure (any vs. none) and numerous covariates (maternal educational level, prepregnancy body mass index (weight (kg)/height $(m)^2$), use of supplements containing folic acid, smoking, alcohol drinking, parity, plurality, and infant sex) were examined in bivariate analyses among 785 controls with no substantial associations observed (results not shown). However, based on previous reported risk factors for selected birth defects, we performed multivariable analyses that were adjusted for race/ethnicity (non-Hispanic white, US-born Hispanic, foreign-born Hispanic, other), educational level (less than a high school diploma, high school diploma, more than high school), prepregnancy body mass index (continuous), parity (0, 1, >1), any (vs. none) intake of supplements containing folic acid, and smoking during the month before and the first 2 months of pregnancy. Analyses of clefts were further stratified by infant sex because of the known association of sex with CP and CLP (28–30).

To focus on comparisons likely to have the most precise estimates and to fully utilize the available data, we did the following. For pesticides that had 5 or more exposed cases and controls for each phenotype, risks were estimated to compare any versus no exposure. Risks were not estimated for pesticides that had fewer than 5 exposed cases or controls. For the exposure scores, we examined the associations of specific phenotypes with these scores specified as categorical variables; that is, exposed subjects were divided into tertiles based on the control distributions.

Analyses were conducted using SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina). The study protocol was reviewed and approved by the institutional review boards of Stanford University and the California Department of Public Health.

RESULTS

Compared with control mothers, mothers of infants with anencephaly were more likely to be foreign-born Hispanic, older, less educated, multiparous, and taking folic acid–containing supplements and were less likely to drink alcohol; mothers of infants with SB were more likely to be foreign-born Hispanic and 25–29 years of age; mothers of infants with CLP were more likely to deliver male infants; and mothers of infants with CP were more likely to be older and to deliver female infants (Table 1). Study subjects were exposed (based on residential proximity) to 52 groups of chemicals and 257 individual chemicals within 500 m of their residence during the month before or first 2 months of pregnancy. Overall, 38.1% of control mothers (299 of 785) and 46.6% of anencephaly (34 of 73), 32.5% of SB (40 of 123), 38.5% of CP (45 of 117), and 37.9% of CLP (105 of 277) case mothers had any pesticides applied near their residence. For controls, the 5 most frequently applied chemical groups were polyalkyloxy compounds (polymers used as spray adjuvants made by condensation of ethylene oxide and an alcohol) (25%), glyphosate and its salts (22%), organophosphorus insecticides (17%), simple alcohols/ethers (17%), and pyrethroid insecticides (14%).

As noted above, we used a minimum sample size criterion for risk estimation; that is, pesticides (groups or specific chemicals) that had 5 or more exposed cases and controls for each phenotype. Table 2 shows the number of chemical

Table 1. Descriptive Characteristics of Case and Control Infants From 8 Counties in the San Joaquin Valley of California, 1997–2006

Characteristic	Percentage[a]				
	Controls (*n* = 785)	Anencephaly (*n* = 73)	Spina Bifida (*n* = 123)	CLP (*n* = 277)	CP (*n* = 117)
Maternal race/ethnicity					
White	33	29	26	33	29
US-born Hispanic	25	23	30	26	24
Foreign-born Hispanic	28	36	36	31	31
Other	14	11	8	9	16
Maternal age at delivery, years					
<20	17	15	12	12	10
20–24	28	19	28	32	27
25–29	26	30	35	28	29
30–34	18	22	17	18	19
≥35	10	14	8	10	15
Maternal education, years					
<12	30	38	29	31	32
12	28	36	33	30	32
>12	41	26	37	40	37
Parity					
0	37	19	33	31	34
1	31	40	28	33	27
≥2	32	41	39	35	38
Multivitamin use[b]					
Yes	64	73	63	66	60
No	34	22	33	32	39
Smoking[b]					
None	85	85	91	83	83
Any	15	15	9	17	17
Drinking[b]					
None	69	77	72	72	68
Any	31	23	28	28	32
Plurality					
Singletons	99	95	98	97	98
Infant sex					
Male	53	48	49	66	43
Female	47	45	49	34	57
Prepregnancy body mass index[c,d]	25.6 (6.0)	25.3 (6.9)	26.1 (6.2)	26.5 (6.6)	25.8 (5.6)

Abbreviations: CLP, cleft lip with or without cleft palate; CP, cleft palate alone.
[a] Percentages may not equal 100 because of rounding or missing data.
[b] During the month before and the first 2 months of pregnancy.
[c] Values are expressed as mean (standard deviation).
[d] Weight (kg)/height $(m)^2$.

groups and specific chemicals that met the sample size criterion for risk estimation by phenotype. Tables 3 and 4 and Web Tables 1 and 2 show adjusted odds ratios (crude estimates were similar) for the associations of each phenotype with chemical groups (Tables 3 and 4) and specific chemicals (Web Tables 1 and 2).

As shown in Tables 3 and 4, the chemical groups that had confidence intervals excluding 1 were petroleum derivatives (for any vs. no exposure, adjusted odds ratio (aOR) = 2.0, 95% confidence interval (CI): 1.0, 4.0) for anencephaly; bromoxynil (hydroxybenzonitriles) (aOR = 5.1, 95% CI: 1.7, 15.6) and silicone (aOR = 0.4, 95% CI: 0.1, 0.9) for

Table 2. Number of Chemical Groups and Specific Chemicals That Met Case and Control Count Criteria for Risk Estimation[a], 8 Counties in the San Joaquin Valley of California, 1997–2006

Phenotype	No. of Chemical Groups	No. of Specific Chemicals
Anencephaly	18	18
Spina bifida	21	25
CLP	32	67
CP	20	26

Abbreviations: CLP, cleft lip with or without cleft palate; CP, cleft palate alone.

[a] Criteria required 5 or more exposed cases and controls out of a total of 52 chemical groups and 257 individual chemicals to which any study subjects were exposed. Risks were only estimated for these chemical groups and specific chemicals.

SB; 2,6-dinitroaniline herbicides (aOR = 1.6, 95% CI: 1.0, 2.5) and dithiocarbamate-methyl isothiocyanate (aOR = 3.1, 95% CI: 1.0, 8.9) for CLP; and none for CP. Stratification by infant sex (male vs. female) did not change the results substantially (data not shown) among CP and CLP cases, with the exception of the chemical groups 2,6-dinitroaniline herbicides (aOR = 2.7, 95% CI: 1.4, 5.3) among females with CLP and monochlorophenoxy salt or ester herbicides (aOR = 3.8, 95% CI: 1.0, 14.4) among males with CLP.

We also examined the specific chemicals (full results are shown in Web Tables 1 and 2). Those chemicals that had confidence intervals excluding 1 were 2,4-D, dimethylamine salt (aOR = 3.3, 95% CI: 1.2, 8.7), methomyl (aOR = 3.2, 95% CI: 1.0, 10.2), imidacloprid (aOR = 2.9, 95% CI: 1.0, 8.2), and α-(para-nonylphenyl)-ω-hydroxypoly(oxyethylene), phosphate ester (aOR = 3.4, 95% CI: 1.2, 9.9) for anencephaly;

Table 3. Odds Ratios for Anencephaly and Spina Bifida Associated With Residential Proximity to Pesticide Applications by Chemical Groups, 8 Counties in the San Joaquin Valley of California, 1997–2006

Chemical Group	Controls ($n = 785$)		Anencephaly ($n = 73$)				Spina Bifida ($n = 123$)			
	Any Exposed	None Exposed	Any Exposed	None Exposed	OR[a]	95% CI[a]	Any Exposed	None Exposed	OR[a]	95% CI[a]
Alcohol/ether	131	654	16	57	1.5	0.8, 2.8	23	100	1.1	0.6, 1.9
Avermectin	31	754	6	67	2.5	1.0, 6.4	7	116	0.7	0.2, 2.3
Azole	61	724	5	68	1.1	0.4, 2.9	7	116	0.7	0.3, 1.6
Bipyridylium	89	696	9	64	1.4	0.6, 3.1	11	112	0.8	0.4, 1.6
Bromoxynil (hydroxybenzonitrile)	10	775	0	73	NC	NC	7	116	5.1[b]	1.7, 15.6[b]
Copper-containing compound	98	687	9	64	0.9	0.4, 2.1	12	111	0.8	0.4, 1.6
Dichlorophenoxy salt or ester (2,4-D and dichlorprop)	41	744	7	66	2.0	0.8, 5.1	4	119	NC	NC
2,6-Dinitroaniline	70	715	5	68	0.7	0.2, 2.1	11	112	1.2	0.6, 2.4
Dicarboximide	49	736	3	70	NC	NC	6	117	0.6	0.2, 1.6
Dithiocarbamate-ETU	52	733	5	68	1.0	0.3, 3.0	5	118	0.4	0.1, 1.3
N-methyl carbamate	61	724	9	64	1.5	0.6, 3.8	10	113	0.8	0.4, 1.8
Neonicotinoid	35	750	6	67	2.5	0.9, 7.1	3	120	NC	NC
Organophosphate	137	648	15	58	1.2	0.6, 2.4	17	106	0.8	0.4, 1.4
Petroleum derivative	102	683	16	57	2.0[b]	1.0, 4.0[b]	17	106	0.9	0.5, 1.7
Phosphonoglycine	169	616	15	58	0.9	0.5, 1.9	23	100	0.9	0.5, 1.4
Polyalkyloxy compound	194	591	24	49	1.7	0.9, 3.0	29	94	0.9	0.5, 1.5
Pyrethroid	108	677	11	62	1.1	0.5, 2.3	15	108	0.8	0.4, 1.5
Pyridazinone	23	762	3	70	NC	NC	5	118	1.4	0.5, 4.2
Silicone	95	690	8	65	0.9	0.4, 2.2	7	116	0.4[b]	0.1, 0.9[b]
Strobin	33	752	3	70	NC	NC	5	118	0.6	0.2, 2.2
Triazine	57	728	5	68	0.5	0.1, 2.1	15	108	1.6	0.8, 3.1
Urea	53	732	7	66	1.0	0.3, 2.9	8	115	1.1	0.5, 2.3
Zinc-inorganic	26	759	1	72	NC	NC	5	118	1.2	0.4, 3.3

Abbreviations: CI, confidence interval; ETU, ethylene thiourea; NC, not calculated; OR, odds ratio.

[a] Odds ratio adjusted for maternal race/ethnicity, educational level, prepregnancy body mass index (weight (kg)/height $(m)^2$), parity, intake of folic acid–containing supplements, and smoking during the month before and the first 2 months of pregnancy. Odds ratios were not calculated for those with less than 5 exposed cases or controls.

[b] Confidence interval excluded 1.0 before rounding to one decimal.

Table 4. Odds Ratios for Cleft Lip With or Without Cleft Palate and Cleft Palate Alone Associated With Residential Proximity to Pesticide Applications by Chemical Groups, 8 Counties in the San Joaquin Valley of California, 1997–2006

Chemical Group	Controls (*n* = 785)		CLP (*n* = 277)				CP (*n* = 117)			
	Any Exposed	None Exposed	Any Exposed	None Exposed	OR[a]	95% CI[a]	Any Exposed	None Exposed	OR[a]	95% CI[a]
Alcohol/ether	131	654	41	236	0.9	0.6, 1.3	21	96	1.1	0.6, 1.9
Avermectin	31	754	13	264	1.2	0.6, 2.5	6	111	1.2	0.5, 3.2
Azole	61	724	18	259	0.8	0.4, 1.4	12	105	1.3	0.6, 2.7
Bipyridylium	89	696	26	251	0.8	0.5, 1.4	13	104	0.9	0.5, 1.8
Bromoxynil (hydroxybenzonitrile)	10	775	7	270	1.9	0.7, 5.7	0	117	NC	NC
Copper-containing compound	98	687	39	238	1.2	0.8, 1.8	12	105	0.9	0.4, 1.7
Cyclohexenone derivative	14	771	5	272	1.0	0.3, 2.8	0	117	NC	NC
Dichlorophenoxy salt or ester (2,4-D and dichlorprop)	41	744	16	261	1.1	0.6, 2.1	4	113	NC	NC
2,6-Dinitroaniline	70	715	36	241	1.6[b]	1.0, 2.5[b]	10	107	0.8	0.4, 1.8
Diacylhydrazine	16	769	6	271	1.3	0.5, 3.5	4	113	NC	NC
Dicamba (benzoicacid)	17	768	2	275	NC	NC	5	112	1.4	0.4, 5.1
Dicarboximide	49	736	17	260	1.0	0.6, 1.8	7	110	0.8	0.3, 2.0
Dithiocarbamate-ETU	52	733	24	253	1.5	0.9, 2.5	6	111	0.9	0.4, 2.2
Halogenatedorganic	32	753	8	269	0.8	0.3, 1.8	3	114	NC	NC
Monochlorophenoxy salt or ester	16	769	8	269	1.5	0.6, 3.6	1	116	NC	NC
Dithiocarbamate-MITC	9	776	8	269	3.1[b]	1.0, 8.9[b]	1	116	NC	NC
N-methyl carbamate	61	724	21	256	1.0	0.6, 1.7	11	106	1.2	0.6, 2.5
Neonicotinoid	35	750	17	260	1.4	0.7, 2.7	7	110	1.6	0.7, 3.7
Organophosphate	137	648	51	226	1.1	0.7, 1.5	22	95	1.1	0.6, 1.8
Petroleum derivative	102	683	38	239	1.1	0.7, 1.7	10	107	0.5	0.2, 1.1
Phosphonoglycine	169	616	60	217	0.9	0.7, 1.3	24	93	0.9	0.5, 1.5
Polyalkyloxy compound	194	591	60	217	0.8	0.6, 1.2	30	87	1.1	0.7, 1.7
Pyrethroid	108	677	38	239	1.1	0.7, 1.7	15	102	1.0	0.5, 1.8
Pyridazinone	23	762	12	265	1.6	0.7, 3.3	1	116	NC	NC
Silicone	95	690	31	246	1.0	0.6, 1.5	13	104	0.8	0.4, 1.6
Strobin	33	752	12	265	1.0	0.5, 2.1	5	112	1.1	0.4, 3.0
Sulfonylurea	9	776	6	271	1.7	0.5, 5.1	2	115	NC	NC
Thiocarbamate	15	770	7	270	1.5	0.6, 3.9	1	116	NC	NC
Thiophthalimide	17	768	9	268	1.8	0.7, 4.2	1	116	NC	NC
Triazine	57	728	20	257	1.0	0.6, 1.8	7	110	0.8	0.3, 2.0
Urea	53	732	20	257	1.1	0.6, 1.9	8	109	1.2	0.5, 2.6
Xylylalanine	16	769	11	266	1.8	0.8, 4.3	1	116	NC	NC
Zinc-inorganic	26	759	6	271	0.6	0.3, 1.6	2	115	NC	NC

Abbreviations: CI, confidence interval; CLP, cleft lip with or without cleft palate; CP, cleft palate alone; ETU, ethylene thiourea; MITC, methyl isothiocyanate; NC, not calculated; OR, odds ratio.

[a] Odds ratio adjusted for maternal race/ethnicity, educational level, prepregnancy body mass index (weight (kg)/height $(m)^2$), parity, intake of folic acid–containing supplements, and smoking during the month before and the first 2 months of pregnancy. Odds ratios were not calculated for those with less than 5 exposed cases or controls.

[b] Confidence interval excluded 1.0 before rounding to one decimal.

bromoxynil octanoate (aOR = 5.1, 95% CI: 1.7, 15.6) and dimethylpolysiloxane (aOR = 0.3, 95% CI: 0.1, 1.0) for SB; and trifluralin (aOR = 2.2, 95% CI: 1.2, 4.0) and maneb (aOR = 2.3, 95% CI: 1.1, 4.8) for CLP. None of the 26 chemicals had an odds ratio with an associated confidence interval excluding 1 for CP. Stratification by infant sex (male vs. female) revealed additional elevated risks for female infants with CLP when the mother was exposed to trifluralin (aOR = 3.1, 95% CI: 1.3, 7.7), for male infants with CLP exposed to MCPA, dimethylamine salt (aOR = 3.8, 95% CI: 1.0, 4.4), for

Table 5. Adjusted Odds Ratios for Sums of Specific Classifications of Pesticide Exposures and Selected Phenotypes, 8 Counties in the San Joaquin Valley of California, 1997–2006

Sum	Controls	Anencephaly			Spina Bifida			CLP			CP		
		Cases	OR[a]	95% CI[a]	Cases	OR[a]	95% CI[a]	Cases	OR[a]	95% CI[a]	Cases	OR[a]	95% CI[a]
No. of chemical groups with any exposure													
0	487	39	1.0	Referent	84	1.0	Referent	173	1.0	Referent	72	1.0	Referent
1–3	90	9	1.7	0.8, 3.9	11	0.7	0.3, 1.5	32	0.9	0.6, 1.5	16	1.3	0.7, 2.4
4–8	114	14	2.1[b]	1.1, 4.3[b]	13	0.8	0.4, 1.5	39	1.0	0.6, 1.5	15	1.0	0.5, 1.8
9–24	94	11	1.2	0.5, 3.0	15	0.8	0.4, 1.5	33	1.0	0.6, 1.6	14	0.9	0.5, 1.9
No. of endocrine disruptors with any exposure													
0	519	43	1.0	Referent	91	1.0	Referent	188	1.0	Referent	79	1.0	Referent
1	70	7	1.7	0.7, 4.2	7	0.6	0.3, 1.5	20	0.8	0.4, 1.4	7	0.8	0.3, 1.8
2–3	91	12	2.1[b]	1.0, 4.4[b]	8	0.5	0.2, 1.2	28	0.9	0.5, 1.4	16	1.4	0.8, 2.5
4–14	105	11	1.0	0.4, 2.4	17	0.8	0.5, 1.6	41	1.1	0.7, 1.7	15	0.9	0.5, 1.7
No. of proposition 65 reproductive or developmental toxicants with any exposure													
0	640	57	1.0	Referent	103	1.0	Referent	227	1.0	Referent	98	1.0	Referent
1	93	12	1.8	0.8, 3.7	12	0.7	0.4, 1.6	34	1.1	0.7, 1.7	7	0.6	0.3, 1.3
2–6	52	4	0.9	0.2, 2.9	8	1.0	0.4, 2.3	16	1.0	0.5, 1.8	12	1.6	0.8, 3.3
No. of EPA reproductive or developmental toxicants with any exposure													
0	493	39	1.0	Referent	84	1.0	Referent	176	1.0	Referent	73	1.0	Referent
1–2	86	10	1.9	0.9, 4.3	14	1.0	0.5, 2.0	29	0.9	0.5, 1.5	14	1.2	0.6, 2.3
3–6	114	13	1.9	0.9, 3.9	9	0.6	0.3, 1.2	40	1.0	0.7, 1.6	15	1.1	0.6, 2.0
7–22	92	11	1.5	0.6, 3.4	16	0.8	0.4, 1.6	32	1.0	0.6, 1.6	15	0.9	0.5, 1.9

Abbreviations: CI, confidence interval; CLP, cleft lip with or without cleft palate; CP, cleft palate alone; EPA, Environmental Protection Agency; OR, odds ratio.

[a] Odds ratio adjusted for maternal race/ethnicity, educational level, prepregnancy body mass index (weight (kg)/height (m)^2), parity, intake of folic acid–containing supplements, and smoking during the month before and the first 2 months of pregnancy.

[b] Confidence interval excluded 1.0 before rounding to one decimal.

female infants with CP exposed to tebuconazole (aOR = 3.9, 95% CI: 1.0, 15.6), and for male infants with CP exposed to silicone defoamer (aOR = 3.3, 95% CI: 1.0, 11.4).

To estimate potential associations associated with cumulative exposures, we scored the number of chemical groups, endocrine disruptors, and reproductive or developmental toxicants to which each subject was exposed. Increasing scores were not consistently associated with increasing risk for any phenotype. Of note, however, some categories of higher scores were associated with increased risk of anencephaly (Table 5).

We also explored potential risks associated with the combination of pesticide and air pollutant exposures. Mothers for whom we had data on both classes of exposure were a subset of the overall study, that is, 328 cases (36 with anencephaly, 69 with SB, 157 with CLP, and 66 with CP) and 452 controls. There were no statistically precise associations observed among these phenotypes except for anencephaly (data not shown). For anencephaly, exposure to any pesticides (vs. none) had an adjusted odds ratio of 1.6 (95% CI: 0.7, 3.4), exposure to the highest quartile of any 5 primary air pollutants (i.e., carbon monoxide, nitrogen oxide, nitrogen dioxide, PM_{10}, or $PM_{2.5}$) versus none had an adjusted odds ratio of 3.6 (95% CI: 1.5, 9.0), and exposure to any pesticides and the highest quartile of any 5 pollutants (vs. neither) had an adjusted odds ratio of 6.6 (95% CI: 1.9, 23.4). These estimates, however, were quite imprecise because of the small sample size.

DISCUSSION

We examined potential associations between women's residential proximity to agricultural pesticide applications in the San Joaquin Valley of California during early pregnancy and risk of anencephaly, SB, CLP, and CP. Despite consideration of a variety of exposure classifications, such as chemical

groups, specific chemicals, and cumulative pesticides as well as air pollution exposures, there was a general lack of association between pesticide exposures and risk of the studied birth defects. A few chemical groups or specific chemicals showed associations with certain defects. However, because of the sizable number of comparisons made here, such associations may have emerged by chance alone.

Like ours, another study also showed a general lack of association between residential exposure to pesticides and orofacial clefts (4). However, our study is the first of which we are aware that has estimated the risk of orofacial clefts on the basis of individual exposure assessment to pesticides as a result of residential proximity to agricultural use. Very few chemical groups or specific chemicals were associated with an elevated risk of CLP. For example, trifluralin (an herbicide) was associated with CLP risk (aOR = 2.2, 95% CI: 1.2, 4.0), and this association was slightly stronger among female infants (aOR = 3.1, 95% CI: 1.3, 7.7). Results from experimental studies have shown that trifluralin influences serum concentrations of reproductive and metabolic hormones in ewes (31), but it has not been observed to have teratogenic effects in rats and rabbits (32, 33).

Previous research on exposures to residential pesticides and risks of birth-defect phenotypes is not extensive. One previous study reported that methomyl had a moderate association with NTDs (odds ratio = 1.6, 95% CI: 1.1, 2.3) (10). This association was also observed in the present study for anencephaly (aOR = 3.2, 95% CI: 1.0, 10.2). The insecticide methomyl is an endocrine disruptor and reproductive/developmental toxicant that has been reported in EPA registration documents and given a moderate volatility ranking (23,24).

One notable set of findings in the present work pertains to anencephaly. In general, cumulative exposures appeared to be associated with this birth defect. Exposures to a higher number of chemical groups or to specific chemicals classified as endocrine disruptors were associated with an increased risk of anencephaly, although this was not observed at the highest levels of exposure (Table 5). The biologic underpinnings for this association are not easily derived from experimental studies because of the fact that few studies on animals have reported potential cumulative effects among specific pesticides. One study showed that mixtures of pesticides may increase the percentage of apoptosis and reduce development to blastocyst and mean cell number in murine preimplantation embryos (34). A recent study found that 2 mixtures of 7 N-methyl carbamates produced a moderate synergistic response on brain cholinesterase inhibition in adult rats (35). However, little or no information is available about combined teratogenicity of pesticides in humans.

A further cumulative exposure and anencephaly risk involved both pesticides and air pollutants. We recently observed that higher levels of exposure to carbon monoxide, nitrogen oxide, nitrogen dioxide, PM_{10}, or $PM_{2.5}$ during the first 2 months of pregnancy were positively associated with anencephaly in the same study area (25). These exposures in combination with pesticide exposures suggested an even larger (>6-fold) risk of anencephaly. Although clearly such composite exposures are more indicative of the human condition in terms of exposure, our estimated associations for these combined exposures need be interpreted with caution because of small sample size.

Our study has several strengths, including its population-based design, complete case ascertainment by a well-established active birth defects monitoring program, residential history for the relevant embryonic period, and an exposure assessment that was highly detailed and spatially and temporally specific and that captured a broad spectrum of pesticide compounds. Our study also had challenges. Sample sizes for many comparisons were modest, contributing to imprecision in potential risk estimation. Cases and controls with successful geocoding tended to have somewhat higher educational levels than did subjects with unsuccessful geocoding. However, we expect any potential selection bias to be minimal, as both cases and controls had such similar patterns. Our assessment of residential proximity to pesticide applications was thorough, but it does not take into account other factors, such as qualities of the pesticides and individuals' metabolism or behaviors that would affect actual exposures (e.g., chemical half-lives and vapor pressure, wind patterns, cumulative exposures over time, an individual's ability to metabolize the various types of chemicals, and other sources of pesticide exposure such as occupation or home use). However, it is also notable that most pesticides are prone to drift and detectable in air samples at locations beyond the application site (36), and residential proximity to pesticide-treated fields has been associated with household dust and urine levels (37, 38). These factors would be nondifferential with respect to case and control status and would therefore bias results toward the null.

Our study rigorously adds to the scant literature on this topic, particularly in its effort to investigate multiple environmental exposures. Because of sample size limitations and multiple comparisons, our positive findings should be interpreted with caution and need to be replicated in other populations.

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Author affiliations: Department of Pediatrics, Division of Neonatology, Stanford University School of Medicine, Stanford, California (Wei Yang, Suzan L. Carmichael, Amy M. Padula, Gary M. Shaw); Public Health Institute, Oakland, California (Eric M. Roberts); Pesticide Research Institute, Berkeley, California (Susan E. Kegley); and California Department of Public Health, Richmond, California (Paul B. English).

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