



## Original Contribution

# Neural Tube Defects and Maternal Residential Proximity to Agricultural Pesticide Applications

Rudolph P. Rull<sup>1,2</sup>, Beate Ritz<sup>2,3</sup>, and Gary M. Shaw<sup>4</sup>

<sup>1</sup> Northern California Cancer Center, Berkeley, CA.

<sup>2</sup> Department of Epidemiology, School of Public Health, University of California at Los Angeles, Los Angeles, CA.

<sup>3</sup> Department of Environmental Health Sciences, School of Public Health, University of California at Los Angeles, Los Angeles, CA.

<sup>4</sup> California Birth Defects Monitoring Program, March of Dimes Birth Defects Foundation, Berkeley, CA.

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Residential proximity to applications of agricultural pesticides may be an important source of exposure to agents that have been classified as developmental toxins. Data on two case-control study populations of infants with neural tube defects (NTDs) and nonmalformed controls delivered in California between 1987 and 1991 were pooled to investigate whether maternal residential proximity to applications of specific pesticides or physicochemical groups of pesticides during early gestation increases the risk of these malformations. Maternal residential proximity within 1,000 m of pesticide applications was ascertained by linking mothers' addresses with agricultural pesticide use reports and crop maps. Odds ratios were computed by using conventional single- and multiple-pesticide and hierarchical multiple-pesticide logistic regression. In single-pesticide models, several pesticides were associated with NTDs after adjustment for study population, maternal ethnicity, educational level, cigarette smoking, and vitamin use. In a hierarchical multiple-pesticide model, effect estimates for only benomyl and methomyl suggested a possible association. Elevated risks of NTDs and anencephaly or spina bifida subtypes were also associated with exposures to chemicals classified as amide, benzimidazole, methyl carbamate, or organophosphorus pesticides and with increasing numbers of pesticides. These results suggest that ambient exposure to certain categories of agricultural pesticides may increase the risk of NTDs.

anencephaly; crops, agricultural; environmental exposure; geographic information systems; neural tube defects; maternal exposure; pesticides; spina dysraphism

Abbreviations: CI, confidence interval; NTD, neural tube defect; OR, odds ratio.

Several agricultural pesticides have been evaluated and classified by the US Environmental Protection Agency and the California Environmental Protection Agency as developmental toxins on the basis of animal studies (1, 2). Most epidemiologic research of agricultural pesticides and risks of birth defects has focused on heavily exposed occupational groups such as pesticide applicators, manufacturers, and other agricultural workers (3). Low- and moderate-level exposures may occur in the general population from resi-

dential pesticide use or dietary contamination. Residential proximity to agricultural pesticide applications may be an important source of ambient environmental exposure, particularly in rural communities. Pesticides applied from the air or ground can drift from their intended treatment sites, with measurable concentrations detected several hundred meters away from application sites (4, 5). Epidemiologic evidence suggests that low-level agricultural pesticide exposures may increase the risk of malformations, but these

Correspondence to Dr. Rudolph P. Rull, Northern California Cancer Center, 2001 Center Street, 7th Floor, Berkeley, CA 94704-1204 (e-mail: rull@nccc.org).

studies relied on broad regional indicators of pesticide use or proximity to agricultural activity (6–8) or on self-reports of pesticide use or crop proximity that may be prone to differential reporting between mothers of cases and mothers of controls (9, 10).

Since 1972, California has mandated the filing of detailed pesticide-use reports for commercial applications of restricted-use pesticides (i.e., agents with harmful environmental or toxicologic effects) and has extended this reporting to all pesticides since 1990 (11). These data, which have a spatial resolution of approximately 1 square mile (2.6 square kilometers), have been used in epidemiologic studies to identify population groups residing in high-pesticide-use areas and to examine links between use patterns and adult and childhood cancers (12–14), Parkinson's disease (15), and fetal deaths (16).

Here, we apply a combination of pesticide-use report and land-use data to assess pesticide exposure in two population-based case-control studies of neural tube defects (NTDs) in California infants and fetuses delivered between 1987 and 1991. In this pooled study, we investigate whether maternal residential proximity to specific agricultural pesticide applications is associated with the two most common subtypes of NTDs, anencephaly and spina bifida.

## MATERIALS AND METHODS

### Study population

The study population was pooled from two case-control studies conducted by the California Birth Defects Monitoring Program; the studies have been described previously (9, 17). Eligible NTD cases, including elective terminations, had confirmed diagnoses of anencephaly, spina bifida cystica, craniorrhachischisis, and iniencephaly. Unmatched controls were randomly sampled from all liveborn infants (without structural congenital anomalies diagnosed before their first birthday). The first study population involved 315 ascertained NTD cases and 652 controls sampled from 344,214 singleton liveborn infants and fetal deaths delivered between January 1987 and December 1988 by women residing in most California counties (9). The second study population included 613 ascertained NTD cases and 611 controls sampled from 708,129 singleton births and fetal deaths delivered between June 1989 and May 1991 in all California counties except Los Angeles, Ventura, and Riverside (17).

Information on residential, medical, reproductive, occupational, nutritional, and family history and on various sociodemographic and lifestyle factors was elicited from interviews in English or Spanish with mothers of cases and mothers of controls (women who did not speak either of these languages were excluded). In the first study, 265 (84 percent of those eligible) mothers of NTD cases and 481 (74 percent of those eligible) mothers of controls were interviewed over the telephone (91 percent in English) on average 3.8 years after the date of delivery. These interviews focused on the 4-month periconceptional period from 1 month before to 3 months after conception. In the second study, in-person interviews (74 percent in English) were con-

ducted with 538 (88 percent of those eligible) mothers of NTD cases and 539 (88 percent of those eligible) mothers of controls. Interviews occurred an average of 5 months after the date of delivery and focused on the 6-month periconceptional period from 3 months before to 3 months after conception.

Mothers in both studies were asked for all addresses at which they resided for 2 weeks or more during the periconceptional periods. These addresses were geocoded to latitude and longitude coordinates by using the Dynamap/2000 street centerline database (GDT, Inc., Lebanon, New Hampshire) derived from 1990 US Census data (18). Our objective was to estimate exposures during the period between conception and closure of the neural tube (up to the 30th day of gestation (19)), but data for only the estimated month of conception were available. To adequately cover the embryonic neurulation period, we selected for each subject the addresses lived at during the calendar month of conception or the month after conception and ascertained exposures for these addresses during each month of this 2-month period. Addresses for this period were successfully geocoded for 739 (92 percent of those interviewed) mothers of NTD cases and 959 (94 percent of those interviewed) mothers of controls. Eight cases and 19 controls were excluded from the analysis because of missing data on maternal characteristics, resulting in a pooled study population of 731 cases (307 with anencephaly, 390 with spina bifida, and 34 with other subtypes) and 940 controls (table 1).

### Exposure assessment

Potential exposures to specific restricted-use agricultural pesticides were evaluated by using a geographic metric based on linking pesticide-use reports with land-use survey maps of crops; this metric has been described previously (20). Pesticide-use report data for agricultural applications were obtained from the California Department of Pesticide Regulation. Each pesticide-use report provides detailed information on the name of the active ingredient in the pesticide and the crop treated, the amount applied, the acreage treated, and the date and location of the application (11). Locations are reported according to the Public Land Survey System, a grid that parcels land into sections of approximately 1 square mile. Using an algorithm developed by the California Department of Pesticide Regulation, we edited the pesticide-use reports to remove data entry errors such as those in reports including invalid sections of the Public Land Survey System and to adjust the number of pounds of pesticides applied in records with extremely high application rates (pounds applied ÷ acres treated; 1 pound = 0.454 kg) (11). We aggregated individual reports according to a pesticide's active ingredient, the crop to which it was applied, the month in which it was applied, and the Public Land Survey System section (20).

Because thousands of pesticide active ingredients have been registered for use in California (11), we selected those organic pesticides used most frequently (i.e., total crop acres treated) in Public Land Survey System sections near maternal residences between 1986 and 1991. Krieger's *Handbook of Pesticide Toxicology* (21), the *Compendium of Pesticide*

**TABLE 1. Characteristics of mothers of NTD\* cases and mothers of controls (%),† California, 1987–1991**

	NTD cases (n = 731)	Controls (n = 940)	OR*,‡	95% CI*
Study population by month of delivery				
January 1987–December 1988	32.8	46.6	N/A*	N/A
June 1989–May 1991	67.2	53.4		
Ethnicity				
White (reference)	47.5	54.5	1.0	
US-born Latina	13.4	15.3	1.0	0.7, 1.3
Foreign-born Latina	28.7	16.8	1.8	1.4, 2.3
Black	4.2	3.9	1.1	0.7, 1.9
Other	6.2	9.5	0.8	0.5, 1.1
Age at conception (years)				
14–19	11.2	9.0	1.2	0.8, 1.7
20–24	27.1	26.7	1.0	0.8, 1.3
25–29 (reference)	31.6	31.2	1.0	
30–34	21.5	23.5	0.9	0.7, 1.2
≥35	8.6	9.4	0.9	0.6, 1.2
Education completed				
>High school graduate (reference)	29.6	38.4	1.0	
High school graduate	37.4	40.0	1.2	1.0, 1.6
<High school graduate	33.1	25.6	1.5	1.2, 2.0
Periconceptional§ employment				
Unemployed (reference)	37.4	34.9	1.0	
Employed	62.7	65.1	0.8	0.7, 1.0
Periconceptional§ vitamin use				
No (reference)	36.8	29.3	1.0	
Yes	63.2	70.7	0.7	0.6, 0.9
Periconceptional§ cigarette smoking				
None (reference)	82.8	77.2	1.0	
1–19 cigarettes/day	12.9	17.3	0.7	0.5, 0.9
≥20 cigarettes/day	4.4	5.4	0.8	0.5, 1.2
Infant's NTD subtype				
Anencephaly	42.0	N/A	N/A	N/A
Spina bifida	53.4			
Other NTD	4.6			

\* NTD, neural tube defect; OR, odds ratio; CI, confidence interval; N/A, not applicable.

† Some percentages do not total 100 because of rounding.

‡ Adjusted for study population.

§ The period between 1) 1 month before conception and 3 months after conception for the 1987–1988 study population and 2) 3 months before conception and 3 months after conception for the 1989–1991 study population.

*Common Names* (22), and the Pesticide Action Network Pesticides Database (23) were consulted to categorize agents into groups with similar physicochemical properties. We identified developmental toxins based on listings by the US and the California Environmental Protection Agencies (1, 2). We used classifications by Colborn et al. (24) and the Illinois Environmental Protection Agency (25) to identify suspected and known endocrine disruptors.

Countywide land-use surveys, which map the locations of specific orchard, vineyard, and nonpermanent crops, were obtained from the California Department of Water Resources (26). Because surveys are conducted every 7–10 years, we matched residential addresses to the appropriate county surveys conducted in the survey year closest to the year of conception. Eighty-six percent of the conception years for subjects' residences were within 3 years of when

**TABLE 2. Effect estimates\* for agricultural pesticides applied within 1,000 m of maternal residences on neural tube defects, by physicochemical category, California, 1987–1991**

Physicochemical category and pesticide	No. of cases (n = 731)	No. of controls (n = 940)	Conventional logistic regression				Hierarchical logistic regression (multiple pesticide model)†	
			Single-pesticide models		Multiple-pesticide model		OR	95% CI
			OR‡	95% CI‡	OR	95% CI		
<b>Amide</b>								
Napropamide	10	4	3.4	1.0, 11.2	3.1	0.7, 13.4	2.1	0.8, 5.6
Propyzamide	18	13	1.7	0.8, 3.7	1.4	0.4, 4.7	1.7	0.7, 4.2
<b>Benzimidazole</b>								
Benomyl§,¶,#	35	18	2.2	1.2, 4.0	2.3	0.9, 5.6	2.0	0.9, 4.3
Thiophanate-methyl	6	5	1.1	0.3, 3.9	1.2	0.2, 6.7	1.8	0.6, 5.6
<b>Dicarboximide</b>								
Iprodione#	30	27	1.3	0.7, 2.2	0.9	0.4, 1.9	1.0	0.5, 2.0
Vinclozolin§,¶,#	17	10	1.8	0.8, 4.1	1.5	0.5, 5.1	1.1	0.5, 2.6
<b>Dithiocarbamate</b>								
Mancozeb#	6	11	0.7	0.2, 1.9	0.5	0.1, 2.3	0.5	0.2, 1.4
Maneb#	18	21	1.1	0.6, 2.1	0.6	0.2, 2.4	0.6	0.2, 1.4
Ziram#	10	8	1.4	0.5, 3.6	1.1	0.3, 4.9	0.7	0.3, 1.7
<b>Halogenated organic</b>								
1,3-dichloropropene	24	14	2.1	1.0, 4.1	1.8	0.8, 4.3	1.3	0.7, 2.6
Dicofol#	12	15	1.0	0.4, 2.1	0.3	0.1, 0.9	0.7	0.3, 1.5
Endosulfan#	31	27	1.6	0.9, 2.7	1.5	0.7, 3.3	1.1	0.6, 2.0
Methyl bromide¶	33	50	0.8	0.5, 1.3	0.7	0.4, 1.3	0.8	0.5, 1.2
<b>Methyl carbamate</b>								
Aldicarb#,**	10	12	1.1	0.5, 2.6	0.9	0.3, 2.6	1.2	0.6, 2.4
Carbaryl#,**	25	19	1.7	0.9, 3.2	1.7	0.8, 3.9	1.4	0.8, 2.6
Carbofuran**	5	4	1.7	0.4, 6.4	2.1	0.5, 9.1	1.5	0.7, 3.6
Methomyl#,**	63	53	1.6	1.1, 2.3	1.4	0.8, 2.5	1.4	0.9, 2.2
Oxamyl**	19	12	1.9	0.9, 3.9	1.5	0.6, 4.1	1.4	0.7, 2.8
<b>Organophosphorus</b>								
Acephate**	33	26	1.7	1.0, 2.8	1.4	0.6, 3.4	1.2	0.7, 2.2
Azinphos methyl**	12	14	1.2	0.5, 2.7	1.0	0.4, 2.5	1.1	0.6, 2.0
Chlorpyrifos**	49	43	1.5	1.0, 2.3	1.3	0.7, 2.3	1.2	0.7, 1.9
Demeton**	6	5	1.7	0.5, 5.6	0.7	0.1, 4.6	1.0	0.5, 2.3
Diazinon§,**	38	49	0.9	0.6, 1.4	0.4	0.2, 0.8	0.6	0.4, 1.1
Dimethoate§,**	36	29	1.7	1.0, 2.9	1.6	0.7, 3.7	1.3	0.7, 2.3
Disulfoton**	13	4	4.0	1.3, 12.6	2.4	0.5, 10.7	1.3	0.6, 2.8
Fosetyl-al	7	5	1.3	0.4, 4.1	1.5	0.3, 8.4	1.2	0.4, 3.1
Glyphosate	45	33	1.5	1.0, 2.4	1.5	0.8, 2.9	1.4	0.8, 2.5
Malathion#,**	13	13	1.0	0.5, 2.3	1.0	0.4, 2.7	0.9	0.5, 1.9
Methamidophos**	13	9	2.0	0.8, 4.7	1.5	0.4, 5.5	1.2	0.6, 2.4
Methidathion**	17	15	1.5	0.7, 3.0	1.5	0.6, 3.7	1.2	0.7, 2.3
Mevinphos**	25	32	1.0	0.6, 1.7	0.3	0.1, 1.0	0.8	0.4, 1.5
Naled**	23	11	2.8	1.3, 5.8	2.7	0.9, 8.2	1.4	0.7, 2.7

Table continues

the respective county land-use survey was conducted, with an average difference of 1.8 years. To account for uncertainty due to seasonal or annual rotation of nonpermanent field, vegetable, grain, and pasture crops (e.g., cotton and

tomatoes) occurring between survey years, we combined these crop categories into a class of nonpermanent crops. Thus, we assumed that, for a reported pesticide application on any of these crops, all nonpermanent crops located in the

TABLE 2. Continued

Physicochemical category and pesticide	No. of cases (n = 731)	No. of controls (n = 940)	Conventional logistic regression				Hierarchical logistic regression (multiple pesticide model)†		
			Single-pesticide models		Multiple-pesticide model		OR	95% CI	
			OR	95% CI	OR	95% CI			
Oxydemeton-methyl¶,**	27	22	1.7	1.0, 3.1	3.4	0.8, 14.3	1.2	0.6, 2.5	
Parathion#,**	26	30	1.0	0.6, 1.8	0.8	0.4, 1.8	0.9	0.5, 1.7	
Phosmet**,††	10	12	1.0	0.4, 2.4	0.8	0.3, 2.4	0.8	0.4, 1.9	
Phosphamidon**	4	5	1.0	0.3, 4.1	0.5	0.1, 2.9	1.0	0.5, 2.2	
Phthalimide									
Captan	15	11	2.1	0.9, 4.6	0.9	0.3, 2.9	0.9	0.4, 2.1	
Folpet	7	10	1.1	0.4, 3.0	0.7	0.1, 3.2	0.7	0.3, 1.9	
Pyrethroid/pyrethrin									
Fenvalerate	26	21	1.6	0.9, 3.0	1.2	0.5, 2.9	1.2	0.6, 2.3	
Permethrin	39	34	1.5	0.9, 2.5	1.2	0.5, 2.6	1.1	0.6, 2.1	
Pyrethrins	11	10	1.4	0.6, 3.3	1.0	0.2, 4.0	1.0	0.4, 2.4	
Substituted benzene									
Chlorothalonil	17	18	1.2	0.6, 2.3	0.4	0.2, 1.2	0.6	0.3, 1.5	
Dicloran	13	9	1.6	0.7, 3.8	0.8	0.2, 4.0	0.7	0.2, 1.8	
Triazine/triazole									
Anilazine§	6	5	1.6	0.5, 5.3	0.9	0.1, 5.8	0.9	0.3, 2.4	
Hexazinone	4	7	0.8	0.2, 2.8	0.9	0.2, 4.1	0.9	0.3, 2.5	
Prometryn§	8	9	1.0	0.4, 2.7	0.2	0.02, 1.5	0.7	0.3, 2.1	
Simazine	16	12	1.7	0.8, 3.6	1.5	0.5, 4.2	1.2	0.5, 2.7	
Urea									
Chloroxuron	4	4	1.1	0.3, 4.6	0.8	0.04, 17.8	0.9	0.2, 3.3	
Diuron§	14	17	1.1	0.5, 2.2	0.9	0.3, 2.4	0.9	0.4, 2.3	
Other pesticides									
2,4-D and derivatives	32	29	1.4	0.9, 2.4	1.5	0.8, 2.7	1.2	0.7, 2.0	
Dodemorph acetate	6	8	0.9	0.3, 2.7	0.6	0.2, 2.1	0.9	0.5, 1.7	
Fenbutatin-oxide§	21	10	2.2	1.0, 4.8	1.1	0.4, 3.2	1.0	0.5, 2.0	
Metalaxyl	23	23	1.3	0.7, 2.4	1.1	0.3, 3.3	1.0	0.5, 1.9	
Oxyfluorfen	22	11	2.2	1.1, 4.7	1.7	0.7, 4.6	1.3	0.7, 2.4	
Paraquat dichloride	48	63	1.0	0.7, 1.5	0.7	0.4, 1.1	0.8	0.5, 1.2	
Propargite§,¶	25	32	1.0	0.6, 1.8	0.5	0.3, 1.1	0.8	0.5, 1.4	
Strychnine	11	9	1.8	0.7, 4.5	1.2	0.4, 3.4	1.2	0.6, 2.2	
Triadimefon§,¶,#	16	11	1.9	0.9, 4.3	1.7	0.6, 4.6	1.2	0.6, 2.3	
Trifluralin#	11	16	0.8	0.4, 1.8	0.7	0.3, 1.6	0.8	0.4, 1.6	

\* Each estimate was adjusted for the other pesticides listed, study population, maternal education, ethnicity, periconceptional cigarette smoking, and periconceptional vitamin use. The reference group includes all mothers unexposed to the listed pesticide.

† Prespecified residual mean = 0 and variance = 0.35 on the log-odds-ratio scale, with prior second-stage covariates for pesticide physicochemical properties, cholinesterase inhibitors, endocrine disruptors, and developmental toxins.

‡ OR, odds ratio; CI, confidence interval.

§ Developmental toxin listed by the US Environmental Protection Agency.

¶ Developmental toxin listed by the California Environmental Protection Agency.

# Endocrine disruptor.

\*\* Cholinesterase inhibitor.

†† Listed as both an organophosphorus and phthalimide pesticide.

Public Land Survey System section were equally likely sites of application (20).

We used ArcView 3.2 geographic information system software (ESRI, Redlands, California) to link aggregated

pesticide-use report information to the reclassified land-use survey maps. We then overlaid each geocoded residential address by latitude and longitude onto the maps and drew circular buffers of 500- and 1,000-m radii around each

residence. These distances reflect a potential drift range likely to occur during aerial pesticide applications (4, 27). For each buffer, we defined a mother as exposed to a pesticide if any crop type within the buffer was treated with the agent. Because of the low prevalence of residential proximity to any specific pesticide application during the estimated first two calendar months of gestation, we were unable to categorize and rank exposures based on pesticide application rates or the proportion of a buffer area covered by treated crops. We were thus limited to using dichotomous exposure categories for each agent of interest.

### Statistical analysis

We used unconditional logistic regression to estimate the effects of maternal residential proximity to applications of 59 specific pesticides to which at least four cases and four controls were exposed on the risk of NTDs. Each pesticide was evaluated in both single- and multiple-pesticide models. We used polytomous logistic regression to estimate the effects of pesticide exposures on the two main NTD subtypes, anencephaly and spina bifida (28). Each model included indicator variables for study population and for risk factors that could potentially confound the relation between NTDs and pesticide exposures: maternal ethnicity (White, US-born Hispanic, foreign-born Hispanic, other), completed education (college graduate, some college, high school graduate, <high school), periconceptional cigarette smoking (none, 1–19,  $\geq 20$  cigarettes/day), and vitamin use (any vs. none) (9).

We also used hierarchical (multilevel) logistic regression (using a modified version of the SAS-IML program (SAS Institute, Inc., Cary, North Carolina) written by Witte et al. (29)) to reduce the possibility of false-positive results when simultaneously evaluating a large number of pesticides (30, 31). The conventional dichotomous or polytomous first stage of this model incorporates all pesticides of interest and covariates. In the second stage, the pesticide-specific maximum-likelihood coefficients from the first-stage model are regressed on a linear model consisting of 12 dichotomous indicator variables of physicochemical categories containing at least two of the selected pesticides: amides; benzimidazoles; dicarboximides; dithiocarbamates; halogenated organics; methyl carbamates; organophosphorus compounds; phthalimides; pyrethroids or pyrethrins; substituted benzenes, triazines, or triazoles; and ureas. In addition, we added three indicators for cholinesterase inhibitors, endocrine disruptors, and developmental toxins. These second-stage covariates separate the pesticides into groups with similar physicochemical properties assumed to have exchangeable parameters such that the coefficients for pesticides in a physicochemical class are drawn from a common distribution (30). The intercept of the second-stage linear model represents the residual effect of a pesticide not captured by the physicochemical covariates; we assume that the residual has a mean of zero and a prior (second-stage) variance of 0.35 on the log-odds-ratio scale (semi-Bayesian estimation). This variance reflects a 95 percent certainty that the residual odds ratio for each pesticide lies within an  $\exp(2(1.96 \times \sqrt{0.35})) = 10$ -fold range such as 0.5 to 5.

We arbitrarily defined the mean as zero because we did not have enough prior information available to assign a residual effect to any specific pesticide (31).

We estimated risks of all NTDs and common subtypes for exposures to any pesticide in a specific physicochemical class by using dichotomous indicator variables for each of the 12 classes. In addition, we evaluated whether the outcomes were associated with exposures to multiple pesticides. For each mother of all cases and controls, we counted the total number of pesticides she was exposed to out of 59 agents reviewed and created indicator variables for proximity to any pesticides and for numbers (0, 1, 2–5,  $\geq 6$ ) of pesticides (31). We also estimated effects for proximity to any pesticides and for numbers of pesticides classified as multiple endocrine disruptors, cholinesterase inhibitors, and developmental toxins.

### RESULTS

The risk of NTDs was increased among foreign-born Latina mothers, mothers with lower educational attainment, and mothers who reported that they were unemployed or did not use vitamins during the periconceptional period (table 1) (9, 17). Overall, 35.2 percent of mothers of cases and 26.8 percent of mothers of controls lived within 1,000 m of any pesticide applications, decreasing to 21.9 percent and 16.3 percent, respectively, within 500 m. Regarding proximity within 500 m, the numbers of exposed cases and controls for 17 of the 59 reviewed pesticides were not sufficient for analysis (i.e.,  $\geq 4$ ). Effect estimates for exposures ascertained within this distance did not appear to differ from those within 1,000 m (results not shown). This paper presents effect estimates for residential proximity within 1,000 m.

Table 2 lists effect estimates from conventional single- and multiple-pesticide and hierarchical multiple-pesticide logistic regression models for 59 pesticides on NTDs. We considered pesticides with an effect estimate of at least moderate size (odds ratio (OR) =  $\geq 1.4$  and lower 95 percent confidence limit:  $\geq 0.9$ ) as possibly associated with NTDs. In single-pesticide logistic models, we observed elevated odds ratios for NTDs for several pesticides, including nappropamide, benomyl, 1,3-dichloropropene, methomyl, chlorpyrifos, dimethoate, disulfoton, naled, and fenbutatin-oxide.

Entering all 59 agents into a conventional multiple-pesticide model, however, resulted in a loss of precision and a general decrease in the magnitude of the effect estimates (table 2). We also observed an attenuation of effect estimates for residential proximity to those pesticides applied in the same time period and in the same area. For example, 69 percent and 65 percent of mothers classified as exposed to applications of captan and fenbutatin-oxide, respectively, were also exposed to benomyl. Thus, parameter estimates for captan and fenbutatin-oxide suggested elevated risks in the single-pesticide models, but these risks were reduced considerably after adjustment for other pesticides, including benomyl. However, in the multiple-pesticide model, effect estimates remained elevated for the benzimidazole fungicide benomyl (OR = 2.3, 95 percent confidence interval (CI): 0.9, 5.6) and the organophosphorous insecticide naled (OR = 2.7, 95 percent CI: 0.9, 8.2).

**TABLE 3. Effect estimates\* for physicochemical categories of agricultural pesticides applied within 1,000 m of maternal residences on NTDs† and subtypes, California, 1987–1991**

Physicochemical category	No. of cases (n = 731)			No. of controls (n = 940)	Any NTDs (dichotomous model)		NTD subtypes (polytomous model)			
	Anencephaly (n = 307)	Spina bifida (n = 390)	Other NTDs (n = 34)		OR†	95% CI†	Anencephaly		Spina bifida	
							OR	95% CI	OR	95% CI
Amides	12	12	0	15	2.2	1.0, 5.3	2.1	0.8, 5.9	3.3	1.2, 9.3
Benzimidazoles	18	19	2	20	2.2	1.1, 4.7	1.8	0.7, 4.7	2.7	1.1, 6.5
Dicarboximides	24	15	2	30	1.1	0.6, 2.1	1.7	0.8, 3.6	0.7	0.3, 1.7
Dithiocarbamates	16	12	3	32	0.7	0.3, 1.5	0.6	0.2, 1.5	0.7	0.2, 1.9
Halogenated organics	38	40	4	92	0.9	0.6, 1.3	0.9	0.6, 1.6	0.8	0.5, 1.4
Methyl carbamates	38	45	4	77	1.5	1.0, 2.3	1.3	0.7, 2.2	1.7	1.0, 2.9
Organophosphorus	73	68	8	142	1.3	0.9, 1.8	1.6	1.0, 2.5	1.1	0.7, 1.6
Phthalimides	12	13	3	30	0.8	0.3, 1.5	0.7	0.3, 1.7	0.7	0.3, 1.6
Pyrethroids/pyrethrins	26	20	2	47	0.9	0.5, 1.6	1.1	0.6, 2.3	0.7	0.3, 1.5
Substituted benzenes	13	11	1	22	0.8	0.3, 1.8	0.9	0.3, 2.5	0.7	0.3, 2.2
Triazines/triazoles	17	13	1	31	0.9	0.4, 1.8	1.3	0.5, 3.2	0.6	0.2, 1.6
Ureas	6	11	1	21	0.9	0.4, 2.1	0.5	0.1, 1.5	1.5	0.5, 4.0

\* Estimated by using conventional logistic regression, adjusting for all other physicochemical categories listed, study population, maternal education, ethnicity, periconceptual cigarette smoking, periconceptual vitamin use, and the uncategorized (other) pesticides listed in table 2. The reference group includes all mothers unexposed to pesticides in the listed category.

† NTDs, neural tube defects; OR, odds ratio; CI, confidence interval.

The hierarchical multiple-pesticide model drew the effect estimate for each pesticide within a physicochemical or toxicologic category toward the mean of all agents in the category (table 2), and only benomyl (OR = 2.0, 95 percent CI: 0.9, 4.3) was still associated with NTDs, whereas the estimate for naled was reduced (OR = 1.4, 95 percent CI: 0.7, 2.7). The effect estimate from this model for methomyl (OR = 1.4, 95 percent CI: 0.9, 2.2), a methyl carbamate insecticide, also suggested an association, which was slightly more precise than the estimate from the conventional model (OR = 1.4, 95 percent CI: 0.8, 2.5).

Using a polytomous conventional multiple-pesticide model to estimate the effects of 44 pesticides (with sufficient numbers of exposed NTD subtype cases and controls) on anencephaly and spina bifida (results not shown), we obtained similar subtype point estimates for benomyl, which suggested no difference between subtypes. We also observed subtype associations between anencephaly and naled, glyphosate, and oxydemeton-methyl and between spina bifida and chlorpyrifos, disulfoton, and napropamide. When we used a hierarchical polytomous model (results not shown), only chlorpyrifos (OR = 1.5, 95 percent CI: 0.9, 2.7) remained associated with spina bifida and naled (OR = 2.0, 95 percent CI: 0.9, 4.3) with anencephaly.

NTD and subtype effect estimates for pesticides by physicochemical category are listed in table 3. Residential proximity to applications of amide, benzimidazole, methyl carbamate, and organophosphorus compounds appeared to be associated with NTDs. For NTD subtypes, we observed increases in anencephaly associated with organophosphorus pesticides and spina bifida with amides, benzimidazoles, and methyl carbamates.

Table 4 lists effect estimates for maternal residential proximity to any or multiple specific pesticides, as well as for endocrine disruptors, cholinesterase inhibitors, and developmental toxins. We observed elevated NTD and subtype odds ratios for proximity to applications of any specific pesticides, any endocrine disruptors, and cholinesterase inhibitors. Compared with those for no exposure to any pesticides, the risk increases for NTDs or subtypes with exposure to 2–5 or  $\geq 6$  specific pesticides were greater than the increase associated with exposure to applications of one specific pesticide. Estimated effects for proximity to multiple endocrine disruptors or developmental toxins were stronger than those for exposure to a single agent in either of these categories. When we compared NTD subtypes, we observed that effect estimates for cholinesterase inhibitors appeared to be consistently stronger for anencephaly than for spina bifida.

## DISCUSSION

Increases in NTD risk were associated with maternal residence within 1,000 m of agricultural applications of benomyl, methyl carbamate or organophosphorus pesticides, or pesticides listed as endocrine disruptors, cholinesterase inhibitors, or developmental toxins. In addition, we observed associations for specific pesticides with anencephaly and spina bifida. Our estimates of effect did not change or were very similar when we examined proximity to pesticide applications within 500 m of a maternal residence. Our finding that residential proximity to agricultural applications of benomyl was associated with NTDs is supported by evidence of

**TABLE 4. Effect estimates\* for the number of agricultural pesticides† applied within 1,000 m of maternal residences on NTDs‡ and subtypes,§ California, 1987–1991**

Exposure	Any NTDs					NTD subtypes (polytomous model)				
	NTD cases (n = 731)		Controls (n = 940)		OR‡	95% CI‡	Anencephaly		Spina bifida	
	No.	%	No.	%			OR	95% CI	OR	95% CI
<b>Any pesticides</b>										
No exposure	474	64.8	688	73.2	1.0		1.0		1.0	
Any exposure¶	257	35.2	252	26.8	1.5	1.2, 1.9	1.5	1.1, 2.0	1.5	1.2, 2.0
No. of pesticides¶										
1	69	9.4	82	8.7	1.2	0.9, 1.7	0.8	0.5, 1.4	1.5	1.0, 2.3
2–5	127	17.4	109	11.6	1.7	1.3, 2.3	1.9	1.3, 2.8	1.7	1.2, 2.4
≥6	61	8.3	61	6.5	1.4	1.0, 2.1	1.6	1.0, 2.6	1.1	0.7, 1.9
<b>Any endocrine disruptors</b>										
No exposure	564	77.2	788	83.8	1.0		1.0		1.0	
Any exposure¶	167	22.9	152	16.2	1.5	1.2, 2.0	1.7	1.2, 2.4	1.4	1.0, 1.9
No. of pesticides¶										
1	84	11.5	81	8.6	1.4	1.0, 2.0	1.4	0.9, 2.2	1.5	1.0, 2.2
≥2	83	11.4	71	7.6	1.6	1.1, 2.2	2.0	1.3, 3.0	1.2	0.8, 1.9
<b>Any cholinesterase inhibitors</b>										
No exposure	563	77.0	780	83.0	1.0		1.0		1.0	
Any exposure¶	168	23.0	160	17.0	1.5	1.1, 1.9	1.7	1.2, 2.3	1.3	0.9, 1.8
No. of pesticides¶										
1	69	9.4	57	6.1	1.7	1.1, 2.4	1.9	1.2, 3.1	1.6	1.0, 2.5
≥2	99	13.5	103	10.9	1.3	1.0, 1.8	1.6	1.1, 2.3	1.1	0.8, 1.6
<b>Any developmental toxins#</b>										
No exposure	598	81.8	791	84.1	1.0		1.0		1.0	
Any exposure¶	133	18.2	149	15.9	1.1	0.9, 1.5	1.3	0.9, 1.8	1.1	0.8, 1.5
No. of pesticides¶										
1	70	9.6	95	10.1	0.9	0.7, 1.3	0.8	0.5, 1.3	1.0	0.7, 1.5
≥2	63	8.6	54	5.7	1.5	1.0, 2.2	2.0	1.2, 3.1	1.1	0.7, 1.9

\* Estimated by using conventional logistic regression, adjusting for study population, maternal education, ethnicity, periconceptual cigarette smoking, and periconceptual vitamin use.

† Of the 59 pesticides listed in table 2.

‡ NTDs, neural tube defects; OR, odds ratio; CI, confidence interval.

§ Some percentages do not total 100 because of rounding.

¶ Compared with the reference group of all mothers unexposed to pesticides in the specified category.

# Listed by the US or California Environmental Protection Agency.

teratogenic effects observed in laboratory animal studies. Increases in malformations, including NTDs, have been reported in the fetuses of rats and mice treated with high doses of benomyl (32, 33). Benzimidazoles impede fungal mitosis by inhibiting microtubule formation and tubulin polymerization. This same inhibitory process may also adversely affect neuroepithelial cell development. A recent study showed inhibited neural tissue differentiation in frog embryos treated with benomyl solution (34). Benomyl production was voluntarily suspended in the United States in 2001 (35).

There is limited evidence of teratogenic effects from animal studies for other specific pesticides that appeared to be associated with NTD subtypes (36). Methyl carbamate and

most organophosphorus pesticides are known cholinesterase inhibitors. Anticholinesterase activity has been observed to affect fetal neuronal differentiation after the neurulation period in laboratory animals and thus delay neurodevelopment (37). Chlorpyrifos, an insecticide commonly used in US households (until 2001) and agriculture, has been associated with inhibition of mitosis of neuroepithelial cells during neurulation in some animal studies (37), but evidence of teratogenicity has been inconsistent (38).

Our results for residential proximity to applications of any of 59 or multiple pesticides suggest that pesticide mixtures or agents applied in combination may increase the risk of NTDs. We used hierarchical logistic regression to



adjust for multiple comparisons and correlated pesticide exposures, but our second-stage model grouped pesticides into only broad categories according to general physicochemical and toxicologic properties because little or no information is available about teratogenicity in humans. The low exposure prevalences for any specific pesticide prevented us from evaluating potential synergistic effects between specific agents beyond such broad grouping. Although there is generally limited data from laboratory animal studies of teratogenic effects from pesticide mixtures (39), a recent study of low-dose mixtures of agricultural pesticides observed an increase in apoptosis and a decrease in blastocyst development in treated preimplantation mouse embryos (40).

Although we were able to use data from a population-based birth defect registry and detailed pesticide-use reports and land-use surveys to assess residential proximity to specific pesticides, our study had limitations due to potential exposure misclassification. Residential address histories were self-reported by mothers after the date of delivery and may be subject to some recall error, especially for mothers in the 1987–1988 study population whose residential histories were obtained almost 4 years after the date of delivery. Errors in residential-address geocoding may have occurred as a result of geocoding procedures that approximate locations by interpolating within a range of address numbers (41). This interpolation method is the default geocoding procedure commonly used in geographic information system software and may result in errors in rural areas where actual residences may lie hundreds of meters away from the street curb location where the address is geocoded and may even be obstructed from the street by a crop field, vineyard, or orchard (27, 41). Thus, for a residence where the true distance to crops is close to or just beyond 1,000 m, geocoding error may lead to misclassification of residential proximity. In addition, to capture the embryonic neurulation period, we assessed exposures for a 2-month period starting with the estimated calendar month of conception. We may have misclassified mothers as exposed to pesticides that were in fact applied prior to conception or after the end of neurulation. However, pesticides may persist in the environment after application, and thus mothers may have been exposed to residues from agents applied before the month of conception.

Because countywide land-use surveys are conducted every 7–10 years, changes in land use occurring between survey years near maternal residences may have led to misclassification of residential proximity (20). These changes may have occurred as a result of urban or suburban development expanding into rural areas or changes in the agricultural production of crop types (e.g., changing an orchard to a vegetable crop field). By combining all nonpermanent crops such as cotton, tomatoes, and wheat into a single class, the linkages between the pesticide-use reports and land-use surveys for these crops were not as specific as those for orchards and vineyards. It is also possible that some individual pesticide-use report records may have been filed with inaccurate locations, dates, or chemical or crop names, and pesticide applications may have been underreported. We expect exposure misclassification resulting from these errors to be non-

differential between cases and controls, thus leading to a possible attenuation of true effects.

Our exposure metric assumes that agents applied within a 1,000-m radius can drift to the residence at the center and ignores pesticides applied outside this arbitrary distance. This simple approach ignores several factors that affect the fate and drift potential of pesticides in the environment, such as wind speed and direction at the time of application, method of application and equipment used, and solvents and adjuvants that may affect the persistence of the active ingredient in the pesticide (42). However, a recent study observed a moderate association between pesticide-use reports of agricultural applications of chlorpyrifos and diazinon and outdoor air concentrations of these pesticides monitored 3 miles (4.8 km) from the Public Land Survey System section of application 2–4 days after the application date (43). The low prevalence of residential proximity to applications of specific agents during the narrow early gestational time window prevented us from estimating effects for rarely applied pesticides and comparing estimates for proximity to high- and low-intensity pesticide applications. In addition, we were concerned that exposures to the agents evaluated in this study may have also occurred through other routes such as occupational or household use. However, in the 1987–1988 study population, an industrial hygienist designated only three mothers of NTDs case and 18 mothers of controls as likely to have had occupational exposure to pesticides. For active ingredients such as chlorpyrifos, diazinon, and carbaryl that were registered for both agricultural and household use during the study period, mothers exposed because of household use but not agricultural applications may have been misclassified as unexposed to these pesticides. Mothers in the 1987–1988 study population were asked whether they used pesticides in the household, but 40 percent of the mothers who reported any use were unable to provide product names (9).

Despite these limitations regarding exposure assessment, our results suggest that residential proximity to agricultural applications of benomyl and other pesticides may have contributed to the occurrence of NTDs in California. The observed association between NTDs and proximity to applications of benomyl is supported by limited evidence of teratogenicity from laboratory animal studies and its listing as a developmental toxin in the United States. In addition, our results imply that mothers exposed to combinations of two or more pesticides may be at increased risk of delivering an NTD-affected infant. These findings suggest future directions for evaluating teratogenic effects resulting from exposure to these agents at low doses in the environment.

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