A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies

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We examined the association between late fetal death due to congenital anomalies (73 cases, 611 controls) and maternal residential proximity to pesticide applications in ten California counties. A statewide database of all applications of restricted pesticides was linked to maternal address to determine daily exposure status. We examined five pesticide chemical classes. The odds ratios from logistic regression models, adjusted for maternal age and county, showed a consistent pattern with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd–8th weeks of pregnancy. For exposure either in the square mile of the maternal residence or in one of the adjacent 8 square miles, odds ratios ranged from 1.4 (95% confidence interval = 0.8–2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95% confidence interval = 1.3–3.9) for halogenated hydrocarbons. Similar odds ratios were observed when a more restrictive definition of nonexposure (not exposed to any of the five pesticide classes during the 3rd–8th weeks of pregnancy) was used. The odds ratios for all pesticide classes increased when exposure occurred within the same square mile of maternal residence. (Epidemiology 2001; 12:148–156)

Keywords: fetal death, pesticides, congenital anomalies, phosphates, pyrethroids, halogenated hydrocarbons, carbamates, endocrine disruptors.

With about 19,000 fetal deaths occurring each year in the United States, the etiology of fetal deaths remains a significant public health issue.[1–3] Few epidemiologic studies have been designed specifically to evaluate the causes of late fetal death (also referred to as stillbirth), although they are often included in studies of birth defects and other adverse birth outcomes. These studies have suggested risk factors for fetal death that include smoking, advanced maternal age, and previous history of fetal death.[4–6]

Experimental studies have suggested that animals exposed to pesticides have a greater risk of adverse reproductive outcomes, including embryonic and fetal death.[7,8] Epidemiologic studies have also found an association between pesticide exposure and stillbirths,[9–12] as well as a variety of congenital anomalies.[13–17]

Toxicology studies have shown that the susceptibility of the fetus to environmental exposures is often dependent on the timing of that exposure with respect to the gestational age of the fetus.[8,18] In humans, the period of organogenesis, about the 3rd–8th week of pregnancy, is the most susceptible time period in which an exposure may have a teratogenic effect on the fetus.[19] For example, thalidomide, a drug that was prescribed to mothers for alleviating morning sickness, was found to cause limb defects. The effects were most severe when exposure took place between the 3rd and 8th weeks of gestation, the period in which the limbs are forming.[19] Although the 3rd–8th-week time window is a critical exposure period for birth defects, few of the epidemiologic studies of pesticide exposure and congenital anomalies have considered timing. Those that have considered timing have limited their definition of exposure to the first trimester of pregnancy.[9,13,17,20–22]

Studies that do not take into account this changing vulnerability with gestational age may not be able to detect an association that exists.[23] In the present study, we used daily pesticide application information to evaluate associations on the basis of the gestational age of the fetus at the time of the exposure. This study is embedded in a larger study of residential proximity to pesticides in relation to fetal death from all causes.[24] This report focuses on the deaths due to congenital anomalies.

Subjects and Methods

SOURCE POPULATION AND DATA EXTRACTION

Cases of fetal death due to congenital anomalies and controls were identified in ten California counties: Madera, Tulare, Kings, Merced, Monterey, Stanislaus, San Joaquin, Riverside, Fresno, and Kern. Counties were

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Editor's note: See related editorial on page 145 of this issue.

TABLE 1. Distribution of Death for 73 Cases

ICD-9 Code	Condition	Number	%
7400	Anencephalus	18	25
7423	Congenital hydrocephalus	2	3
7451	Complete transposition of great vessels	1	1
7469	Unspecified anomaly of heart	2	3
7471	Coarctation of aorta	1	1
7479	Unspecified anomaly of circulatory system	1	1
7485	Agenesis, hypoplasia and dysplasia of lung	9	12
7513	Hirschsprung's disease and other congenital functional disorders of colon	1	1
7519	Unspecified anomaly of digestive system	1	1
7530	Congenital anomalies of urinary system	10	14
7567	Unspecified anomaly of abdominal wall	2	3
7582	Edward's syndrome	1	1
7594	Conjoined twins	1	1
7597	Multiple congenital anomalies as described	21	29
7599	Unspecified congenital anomaly	2	3

ICD-9 = *International Classification of Diseases*, 9th revision.

selected on the basis of the presence of a rural population and high use of pesticides.

Birth, fetal death, and death certificates were obtained from the California State Vital Statistics Registry. The data abstracted from the certificates included delivery information (day and place of delivery, gender, and plurality), parental information (age, race, ethnicity, occupation, and mother's address), and cause of death and medical data (pregnancy history; date of last menses; month of pregnancy in which prenatal care began; birth weight; and complications of pregnancy, labor, and delivery).[24] Information on additional risk factors was gathered from a self-administered questionnaire, previously described.[23]

Identification of Cases

We searched vital statistics data for the study counties from the California Department of Health Services for 1984 to identify candidates for cases (all fetal deaths and infant deaths within 24 hours of birth) and controls (a sample of live, normal births). A total of 642 cases were identified; of these, 34 were subsequently excluded owing to gestational lengths shorter than 20 weeks (we studied late fetal death after 20 weeks of gestation). For the purposes of this analysis, we focused attention on only those cases due to congenital anomalies, namely *International Classification of Diseases* (9th revision) codes 740.0–759.9 on the death certificates. Seventy-three cases were identified, of which 43 were neonatal deaths within 24 hours of birth. The causes of fetal death were heterogeneous, with 80% of the deaths attributed to four categories: anencephalus (25%), multiple congenital anomalies (29%), anomalies of the lung (12%), and anomalies of the urinary system (14%) (Table 1).

Selection of Controls

Controls were randomly selected from live normal births that occurred in 1984 in the same counties as the cases. Controls were frequency matched by county of maternal residence and maternal age (in 5-year age groups) as recorded on the fetal death certificates (for cases) or birth certificates (for controls). Normal births were defined as livebirths with no congenital malformations recorded on the birth certificate. A total of 611 controls were identified.

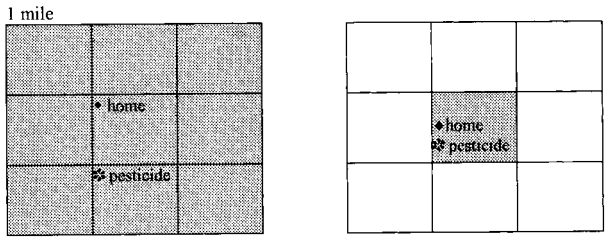

FIGURE 1. Exposure classification based on relation of pesticide application to residence. Left: broad definition of exposure. Right: narrow definition of exposure.

Exposure Ascertainment

The state Pesticide Use Report database for the years 1983–1984 contains information on the application of all restricted-use pesticides, including the specific chemicals used, amount applied, date, and location for each application.[25] Location is identified to the level of township, range, and section (TRS), generally representing 1 square mile. Maternal addresses were obtained from the fetal death, death, and birth certificates. County maps were used to locate the TRS for each maternal address. Pesticide exposure was determined by linking the TRS of the mother's address to the TRS of each pesticide application.

There were two levels of exposure identified for this study population. The Public Land Survey System from the U.S. Geological Survey imposes a grid on the entire United States that divides it into 1-square mile units, each identified by a unique TRS. For the purpose of this study, the TRS of residence and the surrounding eight TRSs were used as geographic markers for residential proximity to pesticide applications (see Figure 1). If the TRS of a pesticide application fell within the same TRS as the mother's residence, or within any of the surrounding eight TRSs, we classified the mother as exposed to that particular pesticide. A narrower classification of exposure limited the definition of exposure to those pesticide applications that fell within the same TRS as the maternal residence.

The date of pesticide application is also recorded in the state Pesticide Use Report database. The date of the mother's last menstrual period (LMP) was abstracted from the birth and death certificates and was used to estimate the days of gestation for each woman, with day 0 equal to the day of conception, defined as the date of LMP plus 14 days. We assigned exposure status for every day of every woman's pregnancy for 327 different pesticides using the dates of each pesticide application within the nine TRSs or the one TRS. For 27 women missing the LMP date, we imputed gestational length using the

hot-deck method[26] with the following sorting variables: case status, birth weight, race, and maternal age.

Exposure Classification

Because separate analyses of all individual pesticides would be unwieldy, pesticides were categorized into classes on the basis of their chemical structure and biological mechanisms. Five of these categories were chosen for this analysis on the basis of their high use and potential reproductive toxicity suggested by previous animal and epidemiologic studies. These categories were phosphates, carbamates, pyrethroids, halogenated hydrocarbons, and endocrine disruptors. The Hayes and Laws[7] *Handbook of Pesticide Toxicology* was used to identify organophosphates, carbamates, pyrethroids, and halogenated hydrocarbons. The classification of Colborn *et al*[27] was used to identify the pesticides included in the endocrine disruptor category. In addition, a recent article by Sonnenschein and Soto[28] found several pesticides to be estrogenic xenobiotics. Data on two of these estrogenic pesticides were available for analysis (endosulfan and methoxychlor). The individual pesticides within each of these five categories are listed in Table 2. Four definitions of exposure were used for this analysis (Table 3). Exposure definitions A through C examined the importance of gestational age of the fetus at the time of exposure, corresponding to weeks 1–20, 1–13, and 3–8. Exposure definition D evaluated the impact of increasing the restriction for determining nonexposure. This definition classified as nonexposed those without exposure to any of the five pesticide classes of pesticides examined for weeks 3–8.

Statistical Analysis

We used stratified analyses to determine which covariates had potential to be confounders. We assessed the exposure prevalence among controls and the distribution of covariates by case-control status for each of the following: race (white, Hispanic/black, or other), gender of fetus/infant (male or female), trimester prenatal care began (first, second, or third), season of conception (December–February, March–May, June–August, or September–November), and prior fetal loss (yes or no). The distribution of demographic characteristics by case status is described in Table 4. No covariate was materially associated with both exposure and case status. For this reason, the final models included only two covariates: maternal age and county of residence (the matching variables).

We examined stratified odds ratios (ORs) to screen for potential effect modifiers. Inclusion criteria for potential effect modifiers required that stratum-specific ORs differ by 100% or more. On the basis of the results of these stratified analyses, we included no interaction term in the model.

We calculated adjusted ORs and 95% confidence intervals (CIs) using logistic regression for those exposed according to the nine-TRS definition, and again for those exposed in the one-TRS definition, separately for each of the five pesticide classes. Separate analyses for ground and aerial modes of application were also completed for those exposed in the nine TRSs. These analyses were limited to those exposed to the specific pesticide class and mode of interest. For example, in the analysis for subjects exposed to pesticides via ground application, individuals exposed to aerial applications were excluded from the analysis. The unexposed group consisted of those not exposed to the specific pesticide class during the stated time period. The number of individuals exposed within their home TRS was not sufficient for statistical analysis by mode of application.

For those who returned questionnaires (40 cases and 357 controls, 55% of the total cohort), an analysis that adjusted for variables not available from the birth and death certificates was conducted.

Results

Broad Geographic Definition of Exposure

For potential exposure within the nine nearest TRSs of maternal residence, the adjusted ORs (controlling for maternal age and county) and the distributions of exposure prevalence by case status are listed in Table 5. Analyses examining exposure at different time windows during gestation showed a slight, but consistent, trend; ORs increased as the definition of exposure narrowed toward the time of organogenesis (3rd–8th week of gestation). The ORs for exposure during organogenesis ranged from 1.4 (95% CI = 0.8–2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95% CI = 1.3–3.9) for halogenated hydrocarbons. The ORs did not change much when individuals exposed between the 3rd and 8th weeks of gestation were compared with those not exposed to any of the five pesticide classes (exposure definition D) during the same time period. The ORs for this more restrictive definition of nonexposure ranged from a low of 1.4 (95% CI = 0.8–2.5) for phosphates to 2.3 (95% CI = 1.2–4.4) for halogenated hydrocarbons.

Narrow Geographic Definition of Exposure

The adjusted ORs (Table 6) relating fetal death due to congenital anomalies with pesticide application in the same TRS as the residence increased as the time window of exposure decreased toward the period of organogenesis. The ORs for those exposed within the same TRS as the residence during the period of organogenesis ranged from 2.0 (95% CI = 0.8–4.9) for pyrethroids to 3.0 (95% CI = 1.4–6.5) for phosphates. Small numbers prevented the determination of adjusted ORs for those exposed to halogenated hydrocarbons. For the more restrictive definition of nonexposure (not exposed to any of the five classes during weeks 3–8), the ORs ranged from 2.1 (95% CI = 0.8–5.5) for carbamates to 2.9 (95% CI = 1.3–6.6) for phosphates.

Exposure by Mode of Application (Ground vs Aerial): Broad Definition of Exposure

Again, most of the ORs increased as timing of exposure decreased to the critical 3rd–8th-week time window

TABLE 2. Pesticides and Assigned Classes

Carbamates/Thiocarbamates
- Aldicarb
- Asulam, sodium salt
- Benomyl
- Carbaryl
- Chlorpropham
- Cycloate
- Eptam
- Ferbam
- Formetanate hydrochloride
- 3-Iodo-2-propynyl butyl carbamate
- Mancozeb
- Maneb
- Mesurol
- Methomyl
- Oxamyl
- Phenmediphan
- S-Propyl
- Butylethylthiocarbamate
- Thiobencarb
- Thiram
- Vapam
- Zineb
- Ziram

Halogenated Hydrocarbons
- 1,3-Dichloropropene
- Carbon tetrachloride
- Chloropicrin
- Dichloropropanes
- Dicofol
- Endosulfan
- Ethylene dibromide
- Ethylene dichloride
- Methoxychlor
- Methyl bromide
- Pentac-R
- Polybutene
- Toxaphene

Phosphates/Thiophosphates/Phosphonates
- Acephate
- Aluminum phosphide
- Azinphos-methyl
- Bensulide
- Carbophenothion Chlorpyrifos
- Dichlorvos
- Demeton
- Diammonium phosphate
- Diazinon
- Dicrotophos
- Dimethoate
- Disulfoton
- Dyfonate
- Ethephon
- Ethion
- Fenamiphos
- Fensulfothion
- Fenthion
- Glyphosphate
- Malathion
- Merphos
- Merphos, other
- Methamidophos
- Methyl parathion
- Mevinphos
- Monocrotophos
- Naled
- Oxydemton-methyl
- Parathion
- Parathion, other
- Phofenofos
- Phorate
- Phosmet
- Phosolone
- Phosphamidon
- Phosphamidon, other related phosphateesters
- S,S,S-Tributyl
- Phosphorotrithioate
- Sodium tripolyphosphate
- Sulfotep
- Sulprophos
- Temephos
- Tetrapotassium pyrophosphate
- Thidathion
- Trichlorophon
- Trisodium phosphate
- Zinc phosphide

Pyrethroids
- Auitrol 200-R
- Cypermethrin
- Difenzoquat
- Dodemorph
- Fenvalerate
- Flucthrinate
- Mepiquat chloride
- Morpholine
- Norflurazon
- Paraquat dichloride
- Permethrin
- Piperonyl butoxide
- Technical
- Pyrazon
- Pyrazon, other related
- Pyrethrins
- Strychnine

Endocrine Disruptors
- Aldicarb
- Amitrole
- Benomyl
- Carbaryl
- Dicofol
- Endosulfan*
- Mancozeb
- Maneb
- Methomyl
- Methoxychlor*
- Metiram-Complex
- Metribuzin
- Parathion
- Trifluralin
- Zineb
- Ziram

* Estrogenically active pesticides.

(data not shown). Analyses of pesticides applied during organogenesis solely by the ground application method in the nine-TRS area (Table 7) resulted in ORs ranging from 1.5 (95% CI = 0.8–2.7) for endocrine disruptors to 2.1 (95% CI = 0.9–4.7) for pyrethroids and halogenated hydrocarbons. The number of individuals exposed to pesticides applied solely by aerial methods, even within the nine-TRS area, was small, particularly for halogenated hydrocarbons. The general pattern of ORs was not different from that for ground application.

Multiple Exposure Classes

Table 8 shows the number of controls and cases exposed to multiple pesticide classes in the nine-TRS area (all modes of application). Cases were more likely to have been exposed to three or more of the five pesticide classes, whereas controls were more likely to have not been exposed at all. The adjusted OR for those exposed to three or more pesticide classes was 2.6 (95% CI = 1.3–5.3), whereas those exposed to one or two pesticide classes showed no association, with an OR of 1.1 (95% CI = 0.6–2.1). In addition, whereas the majority of cases due to anencephaly and lung anomalies were unexposed, cases with urinary system anomalies and multiple congenital anomalies were more likely to have been exposed to at least three different pesticide classes.

Questionnaire Data

The results for those who returned questionnaires, after adjustment for maternal smoking, alcohol use, and occupational exposure to pesticides, showed that the association between maternal residential pesticide exposure and fetal death due to congenital anomalies was not confounded by these factors for this subset of subjects. Nevertheless, with the exception of exposure to phosphates, the ORs (adjusted for county and age) for those who returned questionnaires were higher than for those with no questionnaire data. To explore further the potential for selection bias, we examined the distribution of demographic characteristics by questionnaire-return status. Those who returned questionnaires were more likely to be white, to be older, and to have sought prenatal care during the first trimester (data not shown).

Discussion

In ten agricultural counties of California, proximity to commercial pesticide applications was associated with

TABLE 3. Definitions of Exposure, Nonexposure, and Restrictiveness of Definition

Definition	Exposure Period*	Nonexposure Period†	Restrictiveness‡
A	<20 weeks gestation	<20 weeks gestation	Least restrictive
B	First trimester (1–13 weeks)	First trimester (1–13 weeks)	Moderately restrictive
C	3–8 weeks gestation	3–8 weeks gestation	Most restrictive
D	3–8 weeks gestation	3–8 weeks gestation	

* Exposure = exposure to specified pesticide class for all definitions.
† Definitions A–C: Nonexposure = not exposed to specific pesticide class. Definition D: Nonexposure = not exposed to any of the five pesticide classes.
‡ With respect to exposure.

an elevated risk of fetal death due to congenital anomalies. Furthermore, a consistent pattern was found with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd–8th weeks of pregnancy. This pattern held for all five pesticide classifications, with ORs ranging from 1.4 to 2.2 for those exposed within a 9-square mile area of their residence (Table 5). Narrowing the area of potential exposure to the same TRS as the mother's residence produced a stronger association between pesticide exposure and fetal death due to congenital anomalies as compared with the associations observed for the nine-TRS exposure definition (Table 6). In addition, for both the nine- and one-TRS analyses, the ORs did not change when the definition of nonexposure was restricted to those not exposed to any of the five pesticide classes during the 3rd–8th weeks of pregnancy. Restricting the definition of nonexposure did decrease precision owing to the fact that fewer people were included in the analysis, given that those removed from the nonexposure group were excluded entirely.

Although epidemiologic studies of pesticides have not looked at exposure during the 3rd–8th weeks of pregnancy in relation to birth defects or fetal death due to congenital anomalies, several have examined these outcomes in relation to exposure by trimester. Elevated ORs for birth defects in livebirths in those with occupational exposure to pesticides during the first trimester of pregnancy were observed by Garcia *et al*,[17] Nurminen *et al*,[29]and Zhang *et al*[21] with ORs of 1.8 (95% CI = 0.3–10.5), 1.4 (95% CI = 0.9–2.0), and 3.2 (95% CI = 1.1–9.0), respectively. Increased ORs for congenital anomalies were also observed for women reporting household use of pesticides and living within 0.25 miles of an agricultural crop at any time during the month before conception and the first trimester of pregnancy.[13]

Pastore *et al*[11] evaluated self-reported occupational and home pesticide exposure for the cases and controls in the present study with completed questionnaires. Occupational pesticide exposure during the first 2 months of pregnancy was associated with stillbirths due to congenital abnormalities (OR = 2.4, 95% CI = 1.0–5.9) and during the first two trimesters for all causes of fetal death (OR = 1.3–1.4, 95% CI = 1.0–1.7). Home pesticide exposure was positively associated with fetal death due to congenital anomalies (OR = 1.7, 95% CI = 1.0–2.9). Neither occupational nor home pesticide use, however, explained the association we observed with agricultural pesticide applications.

Pesticides were applied using ground and aerial modes of application. Ground application can include injection of the pesticides directly into the soil, as well as spraying of the pesticide onto the fields from tractor-drawn rigs.

TABLE 4. Distribution (%) of Maternal Characteristics by Case Status (Total N = 684)

	Cases (N = 73)		Controls (N = 611)			
	No.	%	No.	%	OR	95% CI
Counties						
Fresno	16	22	102	17	NR*	
Kern	3	4	97	16		
Kings, Madera, Merced	6	8	65	11		
Monterey	8	11	58	9		
Riverside	19	26	135	22		
San Joaquin	8	11	55	9		
Stanislaus	7	10	53	9		
Tulare	6	8	46	8		
Maternal race						
White†	31	42	320	52	1.0	
Hispanic	33	45	203	34	1.7	1.0–2.9
Other	9	12	88	14	1.1	0.5–2.3
Maternal age						
18–24	34	47	272	45	NR*	
25–29	21	29	172	28		
30–34	12	17	110	18		
>35	5	7	57	9		
Missing	1	<1				
Gender of fetus/infant						
Female†	40	55	300	49	1.0	
Male	33	45	311	51	0.8	0.5–1.8
Prenatal care months						
1–3†	40	55	452	74	1.0	
4–6	16	22	123	20	1.5	0.8–2.7
7–9, none	7	10	28	5	2.5	1.0–6.0
Missing	10	14	8	1		
Season of conception						
December–February†	26	36	158	26	1.0	
March–May	15	21	151	25	0.6	0.3–1.3
June–August	16	22	146	24	0.7	0.3–1.3
September–November	16	22	156	26	0.6	0.3–1.3
Prior fetal loss						
No†	58	79	503	82	1.0	
Yes	15	21	108	18	1.2	0.6–1.7

* NR = not reported, matching factor.

TABLE 5. Adjusted* Odds Ratios and 95% CIs for Potential Exposure within the Same Location or Eight Surrounding Township, Range, and Sections for Four Exposure Definitions

	Phosphates				Pyrethroids				Halogenated Hydrocarbons				Carbamates				Endocrine Disruptors				Estrogenically Active			
Exposure Definitions†	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI
A																								
No	320	37			343	42			378	37			331	38			307	33			565	64		
Yes	291	36	1.0	0.6–1.7	268	31	0.8	0.5–1.5	233	36	1.7	0.9–2.9	280	35	1.0	0.6–1.6	304	40	1.4	0.7–1.9	46	9	1.7	0.8–3.8
B																								
No	358	39			390	46			417	40			392	46			350	40			571	68		
Yes	253	34	1.2	0.7–2.1	221	27	1.1	0.6–1.9	194	33	2.0	1.6–3.5	219	27	1.0	0.6–1.7	261	33	1.1	0.7–1.8	40	5		NR
C																								
No	408	42			471	47			497	49			467	51			427	45			585	69		
Yes	203	31	1.4	0.8–2.4	140	26	1.9	1.1–3.2	114	24	2.2	1.3–3.9	144	22	1.4	0.8–2.4	184	28	1.4	0.8–2.4	26	4		NR
D																								
No	329	33			329	33			329	33			329	33			329	33			329	25		
Yes	203	31	1.4	0.8–2.5	140	26	1.9	1.0–3.6	114	24	2.3	1.2–4.4	144	22	1.7	0.9–3.2	184	28	1.5	0.9–2.7	26	4		NR

* NR = not reported because there were fewer than five exposed cases. All models adjusted for maternal age and county.
† Exposed to specified class *vs* not exposed to same class during exposure A (the first 20 weeks of gestation), exposure B [the first 13 weeks of gestation (first trimester)], exposure C (the 3rd–8th-week period of gestation), or exposure D (exposed to specified class between 3 and 8 weeks of gestation *vs* not exposed to any of the five classes during the 3rd–8th-week period of gestation).

TABLE 6. Adjusted* Odds Ratios for Potential Exposure within the Same TRS* for Four Exposure Definitions

	Phosphates				Pyrethroids				Halogenated Hydrocarbons				Carbamates				Endocrine Disruptors			
Exposure Definitions†	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI
A																				
No	546	59			538	61			567	64			545	67			527	62		
Yes	65	14	2.0	1.0–4.0	73	12	1.4	0.7–2.7	44	9	1.7	0.8–3.6	66	6	2.0	0.8–5.2	84	11	1.1	0.5–2.3
B																				
No	561	61			563	64			588	67			570	63			557	63		
Yes	50	12	2.3	1.1–4.7	48	9	1.5	0.7–3.3	23	6	2.0	0.75–5.1	41	6	2.1	0.8–5.7	54	10	1.6	0.8–3.5
C																				
No	574	62			584	66			599	70			585	51			576	65		
Yes	37	11	3.0	1.4–6.5	27	7	2.0	0.8–4.9	12	3		NR	26	6	2.3	0.9–6.4	35	8	2.1	0.9–4.8
D																				
No	535	58			535	58			535	58			535	63			535	58		
Yes	37	11	2.9	1.3–6.4	27	7	2.2	0.9–5.3	12	3		NR	26	10	2.3	0.9–6.4	35	8	2.3	0.9–5.3

TRS = location identified at the level of township, range, and section. NR, not reported because there were fewer than five exposed cases.
* All models adjusted for maternal age and county.
† Exposed to specified class *vs* not exposed to same class during exposure A (the first 20 weeks of gestation), exposure B [the first 13 weeks of gestation (first trimester)], exposure C (the 3rd–8th-week period of gestation), and exposure D (exposure to specified class between 3 and 8 weeks of gestation *vs* not exposed to any of the five classes during the 3rd–8th-week period of gestation).

TABLE 7. Adjusted* Odds Ratios for Potential Exposure to Ground and Aerial Applications within Nine TRSs for Those Exposed to the Specified Pesticide Class between the 3rd and 8th Weeks of Pregnancy by Mode of Application

Exposure Definition†	Phosphates				Pyrethroids				Halogenated Hydrocarbons				Carbamates				Endocrine Disruptors			
	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI	Control	Case	Adjusted OR	95% CI
Ground application																				
Exposure D																				
No	380	26			380	26			380	26			380	26			380	26		
Yes	128	25	1.5	0.8–2.8	115	22	2.1	0.9–4.7	96	16	2.1	0.9–4.1	104	18	1.6	0.8–3.0	143	24	1.5	0.8–2.7
Aerial application																				
Exposure D																				
No	462	40			462	40			465	40			462	40			462	40		
Yes	105	15	1.4	0.7–2.8	53	11	2.4	0.7–8.5	12	4		NR	84	13	1.6	0.8–3.4	90	13	1.4	0.7–3.0

TRS = location identified at the level of township, range, and section. NR = not reported because there were fewer than five exposed cases.
* All models adjusted for maternal age and county.
† Exposed to specified class between 3rd and 8th weeks of gestation compared with not exposed to any of the five classes between the 3rd and 8th weeks of gestation.

Aerial application is the spraying of pesticides from airplanes as they fly over the crops. Although both methods may result in pesticide drift, potentially exposing nearby residents, we wanted to determine whether mode of application influenced the association between pesticide exposure and fetal death due to congenital anomalies. In this analysis, the association did not differ greatly between the two modes of applications. We lacked data on meteorology or other factors, however, that might have influenced the extent of drift, and addresses were not geocoded to exact locations within the TRS.

A major strength of this study is the fact that exposure assessment was not based on recall. Exposure was determined from state-maintained computer databases covering all commercial applications of a large number of pesticides. Hence, its ascertainment was independent of birth outcome, which is a distinct advantage over most case-control studies of birth outcomes and environmental exposures. Exposure assessment is also improved in that exposure information was collected for each day of pregnancy. The daily exposure measurements provided the opportunity to evaluate the association with fetal death by examining pesticide application during the critical biological period of relevance, organogenesis. The availability of data on the mode of pesticide application and the proximity of the pesticide application to the maternal residence allowed us to refine further the exposure definition.

Despite these strengths, several limitations pertaining to exposure assessment are still present. A surrogate of exposure, the TRS of pesticide applications in relation to maternal address at time of delivery or fetal death, was used as the determining factor for exposure classification. The smallest unit of the TRS system is 1 square mile. Hence, the exact distance of the pesticide application from the home (for example, a few feet or >1 mile) could not be determined. In addition, exposure assessment data such as daily activity patterns, home monitoring, and biological samples were not available. Our exposure classification method did not guarantee that a mother was, in fact, exposed, because wind and weather conditions, hour of application, and the location of the mother at the time of the application are all factors that would determine actual exposure. Mothers who worked away from the home (and were not exposed to pesticides at work) would potentially have fewer hours in the day to be exposed compared with mothers at home. In addition, because maternal residence at the time of delivery was used as the marker for determination of exposure, misclassification of exposure could result for those mothers who moved during pregnancy. Residential history was available for those mothers who returned questionnaires. For those who moved, the TRS for the address reported by the mother on the questionnaire was used to determine exposure. Residential history was not available for mothers who did not return questionnaires, and therefore it is possible that some misclassification of exposure may have occurred for those women.

TABLE 8. Number and Percentages of All Cases and Controls by Number of Different Pesticide Classes of Potential Exposure

					Specific Case Groups							
	Controls		All Cases		Anencephaly		Lung Anomalies		Urinary System Anomalies		Multiple Congenital Anomalies	
Exposure Status	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Not exposed	329	54	33	45	12	67	5	56	2	20	6	29
1–2 classes	125	21	12	17	1	6	2	22	3	30	5	24
3–5 classes	157	26	28	38	6	28	2	22	5	50	10	48

Exposure to a given pesticide class was defined as residential proximity to pesticide applications equal to or greater than the median number of pounds applied during the 3rd–8th weeks of gestation for this study population.

In addition, only five of the many pesticide classes that were applied in the ten counties in 1984 were examined. Although these five classes have shown fetotoxic effects in animal studies, it is possible that other pesticide classes that were not evaluated may have an association with fetal death due to congenital anomalies. Given the likelihood that women were exposed to multiple pesticide classes, other pesticides could have confounded these results.

A further issue is the possibility that, although all applications of restricted pesticides are required to be reported to the state database in California, some applications or dates or areas of application may have been recorded inaccurately. In addition, it is possible that some pesticides that were not declared restricted in 1983–1984 could potentially cause adverse health effects on the fetus; these pesticides were not captured in the state pesticide database for those years. Nevertheless, given that many pesticides must go through an extensive battery of toxicologic tests before approval for use, the number of potentially harmful pesticides not on the 1983–1984 list is expected to be small.

An additional limitation is the potential for biased results due to the inability to adjust for occupational pesticide exposure, maternal alcohol consumption, and maternal smoking in the analysis of all subjects. These factors were determined not to be confounders in the subset who returned questionnaires. Different ORs were observed, however, for those with and without questionnaire data. For example, for those exposed to halogenated hydrocarbons during the period of organogenesis, the OR among those without questionnaire data was 1.8 compared with 2.8 for those who returned questionnaires. Because those with and without questionnaire data differed on race, age, and trimester in which prenatal care began, it is also possible that they would differ on factors such as smoking status, alcohol consumption, and occupational pesticide exposure. If these factors are functioning as confounders among the group without questionnaire data, and the confounding is strong enough to impact the risk estimate for the entire cohort, then our inability to adjust for these factors may have led to the observation of slightly biased ORs.

It is important to note that this study was designed to examine fetal death and not congenital anomalies. Because the congenital anomalies examined in this analysis are a unique group, that is, they were fatal, the results may not be generalizable to congenital anomalies among all births, particularly among those infants who survive. Nevertheless, our case group did include neonatal deaths in the first 24 hours, which constituted 59% of these cases. As previously discussed, studies that have examined congenital anomalies among livebirths have also found increased associations with pesticide exposures.

Given that most teratogens are associated with specific anomalies or syndromes, it seems unlikely that pesticides could be related to all congenital anomalies. In our data, the pattern suggested a higher percentage of fetuses with urinary and multiple congenital anomalies exposed to multiple pesticides as compared with those with other anomalies; however, numbers were far too small to make firm conclusions.

Overall, the results of this study show an increased association between fetal death due to congenital anomalies and several classes of pesticides when exposure occurs during the 3rd–8th weeks of pregnancy. The risk was highest for those individuals living within the same square mile as the pesticide application. The plausibility that these associations are causal is enhanced by our use of objective measures of exposure and by the increasing magnitude in the ORs when exposure was limited to relevant biological time periods. Because of the strong correlation among pesticide classes, we were unable to identify which specific pesticide classes are the most likely lethal teratogens.

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