



Case-Cohort Analysis of Agricultural Pesticide Applications near Maternal Residence and Selected Causes of Fetal Death

Erin M. Bell,¹ Irva Hertz-Picciotto,¹ and James J. Beaumont^{2,3}

The potential association between fetal death and residential proximity to agricultural pesticide applications was examined in 10 California counties for 1984. A case-cohort analysis utilized 319 cases of selected causes of fetal death other than congenital anomalies and 611 noncases. A statewide database of all applications of restricted pesticides was linked to maternal address; residential proximity within 1 mile (1.6 km) provided a surrogate for daily exposure. Pesticides were grouped by chemical class and mechanism of acetylcholinesterase inhibition. Multivariate proportional hazards models using time-dependent exposure variables were fit for each pesticide grouping. Overall, pesticides showed no strong association with fetal death. Slightly elevated risks were observed for women who resided near applications of halogenated hydrocarbons, carbamates, estrogenic pesticides, and carbamate acetylcholinesterase inhibitors during the second trimester, with hazard ratios of 1.3 (95% confidence interval (CI): 1.0, 1.8), 1.3 (95% CI: 1.0, 1.8), 1.4 (95% CI: 0.8, 2.5), and 1.3 (95% CI: 1.0, 1.8), respectively. In a month-by-month analysis, elevated risks were observed when exposure occurred during gestational months 3 and 4 for carbamates and carbamate inhibitors and during months 4 and 5 for halogenated hydrocarbons. Since previous studies have relied on personal recall of exposure, major strengths of this study were the objective source for environmental pesticide exposure assessment and the use of data on the timing of exposure. *Am J Epidemiol* 2001;154:702–10.

environmental exposure; fetal death; pesticides; pregnancy outcome; survival analysis

Fetal death is a significant public health issue, with 19,000 deaths occurring each year in the United States (1). Risk factors include smoking, advanced maternal age, and history of fetal death (2–4). Experimental studies have suggested that animals exposed to some pesticides have a greater risk of fetal death (5, 6). Epidemiologic studies have also found an association between fetal death and occupational (7–11) or environmental (12, 13) exposure to pesticides. Studies have shown that, compared with the general population, persons living near agricultural crops have increased exposure to pesticides due to drift at the time of application (14, 15).

Both epidemiology and toxicology studies have shown that the susceptibility of the fetus is dependent upon the timing of exposures (6, 16). The best understood period of susceptibility in humans is organogenesis, which occurs from the third to the eighth weeks of gestation; birth defects,

whether fatal or not, are most likely to originate in this time window (17). For fetal death due to causes other than congenital anomalies, susceptible time periods are not well characterized. While the organ systems are in place by the end of the eighth week, factors that could influence growth; neural, metabolic, and immunologic development; and, ultimately, survival to birth may have a greater effect during the last two trimesters of gestation (18).

In a previous analysis of fetal death due to congenital anomalies, we found an increased risk among women living near pesticide applications during the period of organogenesis (19). For the 60 percent of women who responded to a self-administered questionnaire, the residential proximity results were not explained by use of pesticides on the job or in the home. A separate analysis of fetal deaths from all causes among questionnaire respondents showed an increased risk among mothers who reported occupational or home use of pesticides (20). Unlike our previous reports, this study evaluated residential proximity to agricultural pesticide applications (not home or occupational use) in relation to fetal death due to causes *other than* congenital anomalies.

MATERIALS AND METHODS

Identification of cases

Ten California agricultural counties served as the source population for cases and noncases: Madera, Tulare, Kings,

Received for publication August 4, 2000, and accepted for publication April 30, 2001.

Abbreviations: CI, confidence interval; HR, hazard ratio; TRS, township, range, section.

¹ Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC.

² Department of Epidemiology and Preventive Medicine, University of California, Davis, CA.

³ Present address: California Environmental Protection Agency, Sacramento, CA.

Reprint requests to Dr. Erin M. Bell, Occupational Epidemiology Branch, National Cancer Institute, 6120 Executive Boulevard, EPS 8111, MSC 7240, Bethesda, MD 20892–7240 (e-mail: belle@mail.nih.gov).

Merced, Monterey, Stanislaus, San Joaquin, Riverside, Fresno, and Kern. Vital statistics records for the study counties were searched to identify all fetal deaths (fetal death certificates) and infant deaths within 24 hours of birth (death certificates) in 1984. A total of 642 deaths were identified; of these, 34 were subsequently excluded due to gestational lengths of 19 weeks or less (by definition, fetal death in California is 20 weeks or more gestation). Using the *International Classification of Diseases*, Ninth Revision (21) code on the death certificates, we excluded congenital anomalies (examined in a previous publication) and other causes not likely to be influenced by environmental exposures (i.e., multiple births and umbilical cord compression), leaving 319 eligible cases, of which 86 were neonatal deaths under 24 hours.

Selection of noncases

Noncases consisted of a stratified random sample of normal livebirths occurring in 1984. They were frequency matched to all cases by maternal age (in 5-year age groups) and county of maternal residence recorded on the fetal death or birth certificates to allow for more efficient control of urbanization and potential differences in health care services. Normal births were defined as those with no congenital malformations recorded on the birth certificate. A total of 642 noncases were selected, 31 of which were subsequently excluded due to missing information on the date of last menstrual period.

Data extraction

Fetal death, death, and birth certificates were obtained from the California State Vital Statistics Registry. From these, we abstracted delivery information (date of delivery, gender, and plurality), parental information (age, race, ethnicity, and mother's address), cause of death, and medical data (date of last menses, month prenatal care began, and birth weight). Information on additional risk factors (i.e., smoking status, occupational exposures, alcohol consumption, etc.) was gathered from a self-administered questionnaire returned by 55 percent of the study population, as previously described (22).

Exposure ascertainment

The California Pesticide Use Report database for the years 1983–1984 contains information on applications of all restricted use pesticides, including the chemical used, amount applied, and date and location of each application (23). Location is specific to the level of township, range, and section (TRS). The Public Land Survey System from the US geologic survey imposes a grid on the entire United States that divides it into units of 1 square mile (1.6 km), each identified as a unique TRS. We used county maps to locate the TRS for each maternal address (maternal TRS). Pesticide exposure was determined by linking the maternal TRS to the TRS of each pesticide application.

Two levels of exposure were identified for this study population (figure 1). First, the pregnancy was considered to be exposed to a particular pesticide if the TRS of a pesticide application was the maternal TRS or any of the surrounding eight TRSs ("broad definition"). Second, a "narrow definition" limited exposure to within the maternal TRS.

The date of the mother's last menstrual period 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 last menstrual period plus 14 days. Exposure status was assigned for every day of every woman's pregnancy for 327 different pesticides by using the dates of each pesticide application that fell within the broad or narrow definition. For 27 women who were missing the date of their last menstrual period, gestational length was imputed by using the hot deck method (24) with the sorting variables case status, birth weight, race, and maternal age.

Exposure classification

Since separate analyses of all individual pesticides would be unwieldy, pesticides were categorized into classes based on their chemical structure and biologic action. Five "initial" categories were selected for analysis: phosphates, carbamates, pyrethroids, halogenated hydrocarbons, and endocrine disruptors. These categories were chosen on the basis of their high use and potential adverse effects indicated in animal and epidemiologic studies (5,

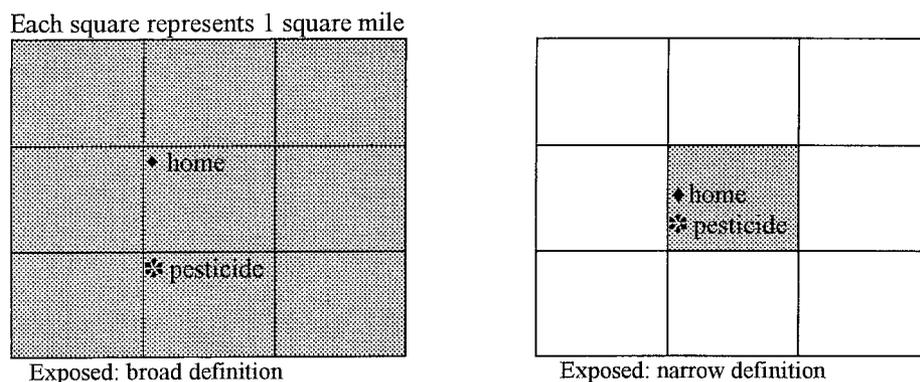


FIGURE 1. Exposure classification based on the proximity of pesticide application to maternal residence for 10 California counties, 1984. With permission of *Epidemiology*, as shown in 2001;12:148–56.

TABLE 1. Pesticides and assigned classes examined in 10 California agricultural counties, 1984

Carbamates/thiocarbamates	
Aldicarb*	
Asulam, sodium salt	
Benomyl	
Carbaryl*	
Chlorpropham	
Cycloate	
Eptam	
Ferbam	
Formetanate hydrochloride*	
3-Iodo-2-propynyl butyl carbamate	
Mancozeb	
Maneb	
Mesuroi*	
Methomyl*	
Oxamyl*	
Phenmediphan	
s-Propyl butyl ethyl thiocarbamate	
Thiobencarb	
Thiram	
Vapam	
Zineb	
Ziram	
Halogenated hydrocarbons	
1,3-Dichloropropene	
Carbon tetrachloride	
Chloropicrin	
Dichloropropanes	
Dicofol	
Endosulfan	
Ethylene dibromide	
Ethylene dichloride	
Methoxychlor	
Methyl bromide	
Pentac	
Polybutene	
Toxaphene	
Phosphates/thiophosphates/phosphonates	
Acephate†	
Aluminum phosphide	
Azinphos-methyl†	
Bensulide	
Carbophenothion†	
Chlorpyrifos†	
Demeton†	
Diammonium phosphate	
Diazinon†	
Dichlorvos‡	
Dicrotophos‡	
Dimethoate†	
Disulfoton†	
Dyfonate	
Ethephon	
Ethion†	
Fenamiphos†	
Fensulfothion†	
Fenthion†	

TABLE 1. Continued

Glyphosate	
Malathion†	
Merphos	
Merphos, other	
Methamidophos‡	
Methyl parathion†	
Mevinphos‡	
Monocrotophos‡	
Naled‡	
Oxydemton-methyl‡	
Parathion†	
Parathion, other†	
Phofenofos	
Phorate†	
Phosmet†	
Phosalone†	
Phosphamidon‡	
Phosphamidon, other related‡	
Phosphate esters	
S,S,S-Tributyl phosphorotrithioate	
Sodium tripolyphosphate	
Sulfotep‡	
Sulprophos†	
Temephos‡	
Tetrapotassium pyrophosphate	
Thidathion	
Trichlorphon‡	
Trisodium phosphate	
Zinc phosphide	
Pyrethroids	
Cypermethrin	
Fenvalerate	
Fluthrinat	
Permethrin	
Pyrethrins	
Endocrine disruptors	
Aldicarb	
Amitrole	
Benomyl	
Carbaryl	
Dicofol	
Endosulfan§	
Mancozeb	
Maneb	
Methomyl	
Methoxychlor§	
Metiram-complex	
Metribuzin	
Parathion	
Trifluralin	
Zineb	
Ziram	

* Carbamate acetylcholinesterase inhibitors.

† Indirect acetylcholinesterase inhibitors.

‡ Direct acetylcholinesterase inhibitors.

§ Estrogenically active pesticides.

25). The individual pesticides within each of these five categories are listed in table 1. In addition, a recent article by Sonnenschein and Soto (26) described several pesticides that are estrogenic xenobiotics. We analyzed the two estro-

Table continues

genic pesticides that were in our database (endosulfan and methoxychlor).

Organophosphorus and carbamate insecticides function by inhibiting acetylcholinesterase, an enzyme responsible for breaking down acetylcholine. There are two mechanisms through which the phosphate inhibitors function: indirect and direct. While direct inhibitors act prior to being metabolized, indirect inhibitors must first be metabolized. These two groups were examined separately. Pesticides that inhibit acetylcholinesterase by carbamylation were also examined separately.

Since relevant biologic time periods for exposure were not known, separate analyses were completed for each trimester and each month of gestation. Women were classified as exposed if a pesticide application took place at least once in the gestational period of interest. While this definition potentially lumps women exposed only once with those exposed many times during that time period, multiple pesticide applications occurred during any given gestational month for the vast majority of women classified as exposed.

Statistical analysis

Exposure prevalence among noncases and the distribution of covariates by case status were determined for the following: race, gender of fetus/infant, trimester prenatal care began, season of conception, and prior fetal loss. The covariate-exposure association was examined separately for all pesticide classes and trimesters. No covariates were associated with both exposure and case status (i.e., odds ratios were not less than 0.7 or greater than 1.5), indicating that they did not need to be considered as confounders. The final models included maternal age and county of residence (the two matching variables).

Stratified odds ratios were examined to screen for potential effect modifiers. Our inclusion criterion for meaningful effect modifiers required that stratum-specific odds ratios differ by 100 percent or more. No variable met this criterion.

Given that exposure opportunity (length of observation or, in this case, length of gestation) was also associated with case-status in our data, adjustment was necessary (20, 22, 27). We therefore used survival analysis to control for ges-

TABLE 2. Selected causes of fetal death in 10 California agricultural counties, 1984

ICD-9* code	Name	No.
2792	Combined immunity deficiency	1
2872	Other nonthrombocytopenic purpuras	1
7600	Maternal hypertensive disorders	8
7602	Maternal infections	1
7603	Other chronic maternal circulatory and respiratory diseases	1
7609	Unspecified maternal condition affecting fetus or newborn	3
7611	Premature rupture of membranes	25
7612	Oligohydramnios	1
7613	Polyhydramnios	2
7622	Other and unspecified morphologic and functional abnormalities of placenta	29
7627	Chorioamnionitis	11
7629	Unspecified abnormality of chorion and amnion	2
7649	Fetal growth retardation, unspecified	2
7650	Extreme immaturity	19
7651	Other preterm infants	20
7680	Intrauterine hypoxia and birth asphyxia	40
7684	Fetal distress, unspecified as to time of onset, in liveborn infant	4
7689	Unspecified birth asphyxia in liveborn infant	4
7699	Respiratory distress syndrome	15
7702	Interstitial emphysema and related conditions	2
7704	Primary atelectasis	4
7705	Other and unspecified atelectasis	2
7708	Other respiratory problems after birth	5
7718	Other infection specific to the perinatal period	4
7732	Hemolytic disease due to other and unspecified isoimmunization	3
7753	Neonatal thyrotoxicosis	1
7780	Hydrops fetalis not due to isoimmunization	4
7781	Sclerema neonatorum	1
7798	Other specified conditions originating in the perinatal period	18
7799	Unspecified condition originating in the perinatal period	84
7999	Other unknown or unspecified cause	2
Total		319

* ICD-9, *International Classification of Diseases*, Ninth Revision.

tational age and to simultaneously analyze time-dependent exposures. Since the data were originally collected in a "standard" case-control design based on cumulative risk of death at 1 day after birth, survival analysis (i.e., estimation of failure probabilities over time) required knowledge of the sampling probabilities. These sampling probabilities were $p = 1$ for cases and $p = 0.01$ for noncases (67,915 livebirths occurred in the 10 counties in 1984). To convert standard (cumulative incidence) case-control data to a case-cohort design, the controls and a random sample of cases (selected with the same probability as the controls) can be combined to create a subcohort that is a random sample of the original cohort from which the cases arose. In this study, random sampling of controls was conducted within strata of maternal age and county. At the time of failure, the case is compared with a risk set composed of those from the subcohort still eligible to be a case, i.e., those who have not yet delivered. Adjusted hazard ratios (HRs) and 95 percent confidence intervals were calculated by using multivariate proportional hazards models, with time-dependent exposures for each trimester and each month of gestation.

Analyses were completed separately for the broad and narrow exposure definitions. Because of the small number of persons exposed to only one pesticide class, the analyses for each pesticide class were completed without adjusting for or removing those exposed to other pesticide classes.

RESULTS

The causes of fetal death were heterogeneous (table 2), with the largest group being "unspecified condition originating in the perinatal period." The distribution of demographic characteristics is presented in table 3. Cases sought prenatal care later than did noncases and were more likely to have had a previous fetal death and to have conceived between December and February. Of those exposed, more than 60 percent were exposed to three or more of the five pesticide classes during any given trimester of gestation (data not shown).

For the broad definition of exposure, the adjusted HRs and the distribution of exposure prevalence for each trimester by case status are shown in tables 4 and 5. Overall, the pesticide classes examined were not strongly associated with fetal death, regardless of trimester of exposure. Weakly elevated risks were observed for applications of halogenated hydrocarbons, carbamates, estrogenic pesticides, and carbamate acetylcholinesterase inhibitors during the second trimester, with HRs of 1.3 (95 percent confidence interval (CI): 1.0, 1.8), 1.3 (95 percent CI: 1.0, 1.8), 1.4 (95 percent CI: 0.8, 2.5), and 1.3 (95 percent CI: 1.0, 1.8), respectively. Similar results were observed for exposures within the maternal TRS, i.e., using the narrow definition, as well as for causes originating in the perinatal period (*International Classification of Diseases*, Ninth Revision, codes 7649–7799, excluding those not likely to be due to environmental exposures) (data not shown). Further analysis by specific causes (i.e., complications of the placenta) was not feasible due to the small number of exposed cases.

The adjusted HRs for each gestational month for pesticide classes with elevated risks in the trimester analysis are

presented in table 6. A modestly increased risk of fetal death was observed when exposure to halogenated hydrocarbons occurred during the fourth and fifth months of gestation, with HRs of 1.4 (95 percent CI: 1.0, 2.0) for both months. Results were similar for exposure to carbamates and carbamate inhibitors during the third and fourth months of gestation.

For those who returned questionnaires (166 cases and 342 controls (55 percent of the study population)), results were not confounded by maternal smoking, alcohol use, income, education, or pesticide use in the home or on the job (variables not available from the birth and death certificates).

DISCUSSION

Overall, no strong associations were observed for residential proximity to applications of pesticides and the causes of fetal death examined. Risks were elevated 30–40

TABLE 3. Fetal deaths in 10 California counties, 1984: distribution and percent of maternal characteristics by case status

	Cases		Noncases	
	No.	%	No.	%
Total	319		611	
Counties*				
Fresno	61	17	102	16
Kern	46	16	97	15
Kings, Madera, Merced	34	11	65	11
Monterey	31	9	58	9
Riverside	67	22	135	22
San Joaquin	31	9	55	9
Stanislaus	29	9	53	8
Tulare	20	7	46	8
Maternal age (years)*				
18–24	142	45	272	45
25–29	94	29	172	28
30–34	54	17	110	18
≥35	29	9	57	9
Maternal race				
White	154	48	320	52
Hispanic	106	33	203	34
Other	58	18	88	15
Missing	1	<1	0	0
Gender				
Male	172	54	311	51
Female	147	46	300	49
Month prenatal care began				
1st trimester	192	60	452	74
2nd trimester	58	18	123	20
3rd trimester, none	35	11	28	5
Missing	34	11	8	1
Season of conception				
December–February	105	33	158	26
March–May	66	21	151	25
June–August	63	20	146	24
September–November	85	27	156	25
Prior fetal loss				
Yes	90	28	108	18
No	229	72	503	82

* Matching factor.

TABLE 4. Fetal deaths in 10 California counties, 1984: adjusted* hazard ratios for fetal deaths and potential exposure within the maternal or eight surrounding townships, ranges, and sections during each trimester for each of six pesticide classes (n = 319)

Trimester†	Phosphates				Pyrethroids				Halogenated hydrocarbons			
	No. of noncases	No. of cases	Adjusted HR‡	95% CI‡	No. of noncases	No. of cases	Adjusted HR	95% CI	No. of noncases	No. of cases	Adjusted HR	95% CI
First												
No	353	183			555	290			415	210		
Yes	258	136	1.0	0.7, 1.3	56	29	1.0	0.6, 1.6	196	109	1.1	0.8, 1.0
Second												
No	336	183			543	68			421	208		
Yes	275	136	0.9	0.7, 1.3	68	32	0.9	0.6, 1.5	190	111	1.3	1.0, 1.8
Third												
No	357	256			552	300			434	279		
Yes	254	63	0.8	0.5, 1.2	19	19	1.4	0.7, 2.8	177	40	0.8	0.5, 1.2
Trimester†	Carbamates				Endocrine disruptors				Estrogenic pesticides			
	No. of noncases	No. of cases	Adjusted HR	95% CI	No. of noncases	No. of cases	Adjusted HR	95% CI	No. of noncases	No. of cases	Adjusted HR	95% CI
First												
No	390	193			348	174			570	295		
Yes	221	126	1.2	0.9, 1.6	264	145	1.1	0.8, 1.5	41	24	1.0	0.6, 1.8
Second												
No	382	182			348	173			581	297		
Yes	229	137	1.3	1.0, 1.8	264	146	1.2	0.9, 1.6	30	22	1.4	0.8, 2.5
Third												
No	403	262			371	252			587	307		
Yes	208	57	1.1	0.7, 1.6	241	67	1.0	0.6, 1.5	24	12	1.9	0.7, 4.9

* All models are adjusted for age and county.

† First trimester: 1–13 weeks' gestation; second trimester: 14–27 weeks' gestation; third trimester: ≥28 weeks' gestation.

‡ HR, hazard ratio; CI, confidence interval.

percent above background for several pesticide classes when exposure occurred in the second trimester. Increasing residential proximity (narrow definition of exposure) did not increase the magnitude of observed associations. The HRs for pesticide exposure by month of gestation varied over the course of the pregnancy. In most cases, the increased HRs for exposures during the first or second trimester were not the result of increased HRs for all months in the trimester.

For example, while slightly elevated HRs were observed for exposure to carbamates during the first and second trimesters of gestation, the monthly analysis showed that risk increased only for exposure during the third and fourth months of gestation.

Several epidemiologic studies have found occupational and environmental exposures to pesticides to be associated with fetal death, with estimated risk ratios ranging from 1.4

TABLE 5. Fetal deaths in 10 California counties, 1984: adjusted* hazard ratios for fetal deaths and potential exposure within the maternal or eight surrounding townships, ranges, and sections during each trimester for acetylcholinesterase inhibitors (n = 319)

Trimester†	Direct inhibitors				Indirect inhibitors				Carbamate inhibitors			
	No. of noncases	No. of cases	Adjusted HR‡	95% CI‡	No. of noncases	No. of cases	Adjusted HR	95% CI	No. of noncases	No. of cases	Adjusted HR	95% CI
First												
No	563	288			426	225			442	223		
Yes	48	31	1.2	0.7, 2.1	185	94	0.9	0.7, 1.3	169	96	1.2	0.9, 1.6
Second												
No	553	289			423	220			428	206		
Yes	58	30	1.0	0.6, 1.7	188	99	1.0	0.8, 1.4	183	113	1.3	1.0, 1.8
Third												
No	556	305			436	278			442	274		
Yes	55	14	0.9	0.4, 2.0	175	41	0.8	0.5, 1.2	169	45	1.0	0.6, 1.6

* All models are adjusted for age and county.

† First trimester: 1–13 weeks' gestation; second trimester: 14–27 weeks' gestation; third trimester: ≥28 weeks' gestation.

‡ HR, hazard ratio; CI, confidence interval.

TABLE 6. Fetal deaths in 10 California counties, 1984: adjusted* hazard ratios for fetal deaths and potential exposure within the maternal or eight surrounding townships, ranges, and sections to six pesticide classes examined† during the 10 4-week periods of gestation (n = 319)

Gestation months‡	Halogenated hydrocarbons		Carbamates		Endocrine disruptors		Estrogenic		Direct inhibitors		Carbamate inhibitor	
	Adjusted HR§	95% CI§	Adjusted HR	95% CI	Adjusted HR	95% CI	Adjusted HR	95% CI	Adjusted HR	95% CI	Adjusted HR	95% CI
1	1.1	0.8, 1.6	1.0	0.7, 1.5	0.9	0.7, 1.3	1.2	0.6, 2.6	1.2	0.6, 2.3	1.1	0.7, 1.6
2	1.1	0.8, 1.6	1.1	0.8, 1.6	1.2	0.9, 1.7	0.9	0.5, 1.9	1.5	0.8, 3.0	1.1	0.8, 1.6
3	1.2	0.8, 1.7	1.4	1.0, 1.9	1.2	0.9, 1.6	1.6	0.7, 3.5	1.3	0.7, 2.6	1.4	1.0, 2.1
4	1.4	1.0, 2.0	1.4	0.9, 1.9	1.3	1.0, 1.8	1.1	0.4, 2.6	1.1	0.5, 2.0	1.4	1.0, 1.9
5	1.4	1.0, 2.0	1.1	0.8, 1.5	1.2	0.9, 1.7	1.5	0.6, 3.7	0.9	0.4, 1.9	1.1	0.7, 1.5
6	0.8	0.5, 1.2	1.2	0.8, 1.7	1.0	0.7, 1.4	0.9	0.4, 2.4	0.9	0.5, 1.8	1.2	0.8, 1.8
7	1.2	0.7, 1.9	1.0	0.7, 1.6	1.0	0.7, 1.4	1.4	0.5, 3.9	0.7	0.3, 1.6	1.0	0.6, 1.6
8	0.8	0.5, 1.4	1.2	0.7, 2.0	1.1	0.6, 1.7	1.7	0.5, 5.6	0.5	0.2, 1.4	1.0	0.6, 1.8
9	0.6	0.3, 1.2	0.6	0.3, 1.1	1.0	0.7, 1.3	2.1	0.5, 9.3	0.7	0.2, 2.1	0.7	0.3, 1.4
10	0.6	0.2, 1.6	0.6	0.3, 1.5	0.7	0.3, 1.4	NR§		NR		0.7	0.9, 1.6

* All models are adjusted for age and county.

† Phosphates, pyrethroids, and indirect acetylcholinesterase inhibitors not presented.

‡ Month 1: 1–4 weeks; month 2: 5–8 weeks; month 3: 9–12 weeks; month 4: 13–16 weeks; month 5: 17–20 weeks; month 6: 21–24 weeks; month 7: 25–28 weeks; month 8: 29–32 weeks; month 9: 33–36 weeks; month 10: ≥37 weeks.

§ HR, hazard ratio; CI, confidence interval; NR, not reported because there were fewer than five cases.

to 3.6 (7, 9–11, 13). Specific pesticide classes were not examined in any of these studies, and the exposure assessments were based on broad job classifications or self-reported use of pesticides. For the same study population, Pastore et al. (20) evaluated self-reported occupational and home pesticide use for all cases and controls with completed questionnaires. Occupational pesticide exposure during the first two trimesters of gestation was associated with all causes of fetal death (HR = 1.4, 95 percent CI: 1.0, 1.7) and fetal death due to complications of the placenta, cord, and membranes (HR = 1.6, 95 percent CI: 1.1, 2.3). In our evaluation of residential proximity to applications of agricultural pesticides, however, adjustment for self-reported use of pesticides on the job or in the home among those who returned the questionnaires did not alter the findings.

We found that subjects were more likely to be exposed to multiple pesticide classes than to one specific pesticide class, making it difficult to examine the impact of exposure to only one pesticide class. In a previous analysis of these data, an association with fetal death due to congenital anomalies was observed for exposure to three or more pesticide classes during the period of organogenesis. A much weaker association was observed for those exposed to one to two classes (19). In both the trimester and the monthly analyses presented here, we observed increased HRs for carbamates, direct and carbamate acetylcholinesterase inhibitors, and halogenated hydrocarbons. Several animal studies have shown specific pesticides within these classes to be fetotoxic, but at exposure levels not likely for humans. Thus, plausible mechanisms involving maternal, fetal, or placental units have yet to be identified. Human case studies have shown that pesticides can cross the placenta and accumulate in fetal organs (28, 29). In addition, decreased levels of placental acetylcholinesterase have been observed in pesticide-exposed rodents (30, 31). However, the effect of decreased acetylcholinesterase on the fetus is not well understood.

In many respects, exposure assessment was a strength in this study. Exposure was determined from state-maintained

computer databases covering all applications of the pesticide classes examined here. Hence, its ascertainment was independent of birth outcome, a distinct advantage over case-control studies that rely on self-reports and therefore are subject to differential recall by case status. Exposure assessment was also improved over previous studies in that information was specific for each day of pregnancy. The daily exposure data enabled trimester- and month-specific analyses and control of differential exposure opportunity between cases and controls. The availability of data on specific pesticides and proximity of the pesticide application to maternal residence as defined in the TRS allowed us to refine the exposure definition further.

Despite these strengths, several limitations pertaining to exposure assessment were still present. The smallest unit of the TRS system is 1 square mile. Hence, the exact distance of the pesticide application from the home (e.g., a few feet or >1 mile) could not be determined. In addition, daily activity patterns, home monitoring, and biologic samples were not available. Wind and weather conditions, the hour of application, and the location of the mother at the time of the application are all factors that would determine actual exposure. For example, mothers who worked away from the home (and who were not exposed to pesticides at work) would have a lower likelihood of being exposed compared with mothers at home. More generally, whether absorption occurs by inhalation, dermal absorption, contamination of ground water, or ingestion of residues on foodstuffs or by a combination of these remains unclear.

In addition, since maternal residence at the time of delivery was used, misclassification of exposure could occur for those mothers who moved during pregnancy. Residential history was available for those mothers who returned questionnaires. For those who reported having moved, the updated address reported by the mother was used to determine exposure for the corresponding days of pregnancy. For mothers who did not return questionnaires, incorrect addresses may have been used to assign exposure. Exposure

misclassification would be greater for the early months of pregnancy because women would have had more time to change residences. Since there is a greater probability of a bias toward rather than away from the null, our specific findings for the third to fifth months of pregnancy may be underestimates of the true effect. Estimated associations for later months may have also been underestimated, but probably less so.

Another limitation was the lack of questionnaire data on potential confounders for almost 45 percent of the study participants. Twenty-five percent were not locatable (i.e., they had moved, and no forwarding addresses were obtainable), and 20 percent did not respond to the questionnaire. These two groups did not differ from respondents by case status. Both groups, however, were more likely to be younger, to be Hispanic, and to have sought prenatal care later in pregnancy compared with those who returned questionnaires. Smoking status, alcohol consumption, income, education, and occupational and home use of pesticides did not confound the observed associations among the questionnaire group, but our inability to adjust for these factors in the entire study cohort may have led to some bias in the HRs.

Fetal deaths may have been underascertained and/or misclassified regarding their causes. In California, fetal deaths at 28 weeks or more gestation are more likely to be reported than are those that occur between 20 and 27 weeks gestation (32). If environmental exposure to pesticides influences the risk differentially for the earlier versus the later fetal deaths, our results may not represent effects for earlier events. In addition, uncertainties in assigning cause make it difficult to assemble etiologically homogenous groups, particularly since causes are frequently unspecified (as was true for 25 percent of our cases). We attempted to address the problem of heterogeneity by excluding those fetal deaths not likely to be caused by environmental exposures; however, our cases were still very heterogeneous.

Finally, because fetal death rates are higher for young and old maternal ages, the matching by maternal age resulted in a study population age distribution that was not representative of the full cohort (all pregnant women who delivered after 20 weeks in 1984 in the study counties). If maternal age is an effect modifier, our summary effect estimates could differ slightly from those in the total cohort. The likelihood that matching on county constituted overmatching with respect to exposure is extremely low, given that the controls were not matched on date of birth (pesticide applications occur in specific seasons) and that the counties are large (1,426–6,017 square miles) (33).

In summary, in 10 agricultural counties of California, residential proximity to application of several pesticide classes during pregnancy was, on the whole, not associated with fetal death. Slightly elevated risks were observed for women who lived near applications of halogenated hydrocarbons in the fourth and fifth months of gestation and of carbamates and carbamate inhibitors in the third and fourth months of gestation. However, despite our attempts to improve exposure assessment and case definition, the increased HRs observed in this study were small ($HR < 1.5$); thus, we are not able to rule out the possibility that our observations were

the result of some unmeasured factor. Nevertheless, the consistency of the time period for these associations suggests that months 3–5 of gestation may be a period of fetal vulnerability to some pesticide exposures.

ACKNOWLEDGMENTS

This work was partially supported by grant ES03767 from the National Institutes of Environmental Health Sciences.

The authors acknowledge Dr. Andrew Olshan, Dr. David Savitz, and Dr. Ernest Hodgson for their comments on earlier versions of this paper and Lawrence Park for his computer programming assistance. The authors also thank Dr. Steven Samuels, James Singleton, and Susan Lutzenhiser for their contributions to study design, data collection, and database development.

REFERENCES

1. United States Department of Health and Human Services. Infant mortality: 1950–1995. Washington, DC: United States Department of Health and Human Services, 1997.
2. Fretts RC, Schmittdiel J, McLean FH, et al. Increased maternal age and the risk of fetal death. *N Engl J Med* 1995;333:953–7.
3. Magann FF, Winchester MI, Carter DP, et al. Factors adversely affecting pregnancy outcome in the military. *Am J Perinatol* 1995;12:462–6.
4. Walles B, Tyden T, Herbst A, et al. Maternal health care program and markers for late fetal death. *Acta Obstet Gynecol Scand* 1994;73:773–8.
5. Hayes J, Laws E. Handbook of pesticide toxicology. San Diego, CA: Academic Press, Inc, 1991.
6. Klassen C. Cassarett and Doull's toxicology: the basic science of poisons. New York, NY: McGraw-Hill, Inc, 1996.
7. Savitz DA, Whelan EA, Kleckner RC. Self-reported exposure to pesticides and radiation related to pregnancy outcome—results from National Natality and Fetal Mortality Surveys. *Public Health Rep* 1989;104:473–7.
8. Thomas D, Petitti D, Goldhaber M, et al. Reproductive outcomes in relation to malathion spraying in the San Francisco Bay area, 1981–1982. *Epidemiology* 1992;3:32–9.
9. Goulet L, Theriault G. Stillbirth and chemical exposure of pregnant workers. *Scand J Work Environ Health* 1991;17:25–31.
10. McDonald AD, McDonald JC, Armstrong B, et al. Fetal deaths and work in pregnancy. *Br J Ind Med* 1988;45:148–57.
11. Vaughn TL, Daling JR, Starzyk PM. Fetal death and maternal occupation: an analysis of birth records in the State of Washington. *J Occup Med* 1984;26:676–8.
12. White FM, Cohen FG, Sherman G, et al. Chemicals, birth defects and stillbirths in New Brunswick: associations with agricultural activity. *CMAJ* 1988;138:117–24.
13. Taha TE, Gray RH. Agricultural pesticide exposure and perinatal mortality in central Sudan. *Bull World Health Organ* 1993;71:317–21.
14. Fenske RA, Lu C, Simcox NJ, et al. Strategies for assessing children's organophosphorus pesticide exposures in agricultural communities. *J Expo Anal Environ Epidemiol* 2000;10:662–71.
15. Simcox NJ, Fenske RA, Wolz S, et al. Pesticides in household dust and soil: exposure pathways for children of agricultural families. *Environ Health Perspect* 1995;103:1126–34.

16. Kurzel RB, Cetrulo CL. Chemical teratogenesis and reproductive failure. *Obstet Gynecol Surv* 1985;40:397-424.
17. Sadler TW. *Langman's medical embryology*. Baltimore, MD: Williams & Wilkins, 1995.
18. Dencker L, Eriksson P. Susceptibility in utero and upon neonatal exposure. *Food Addit Contam* 1998;15(suppl):37-43.
19. Bell EM, Hertz-Picciotto I, Beaumont J. A case-control study of pesticides and fetal death due to congenital anomalies. *Epidemiology* 2001;12:148-56.
20. Pastore LM, Hertz-Picciotto I, Beaumont JJ. Risk of stillbirth from occupational and residential exposures. *Occup Environ Med* 1997;54:511-18.
21. World Health Organization. *International classification of diseases. Manual of the international statistical classification of disease, injuries, and causes of death. Ninth Revision*. Geneva, Switzerland: World Health Organization, 1977:1.
22. Hertz-Picciotto I, Pastore L, Beaumont H. Timing and patterns of exposures during pregnancy and their implications for study methods. *Am J Epidemiol* 1996;143:597-607.
23. Department of Pesticide Regulation, Information Systems Branch, California Environmental Protection Agency. *Pesticide use reporting: an overview of California's unique full reporting system*. Sacramento, CA: Department of Pesticide Regulation, 1995.
24. Reilly M. Data analysis using hot deck multiple imputation. *Statistician* 1993;42:307-13.
25. Colburn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 1993;101:378-84.
26. Sonnenschein C, Soto AM. An updated review of environmental estrogen and androgen mimics and antagonists. *J Steroid Biochem Mol Biol* 1998;65:143-50.
27. Poole C. Exposure opportunity in case-control studies. *Am J Epidemiol* 1986;123:352-8.
28. Autrup H. Transplacental transfer of genotoxins and transplacental carcinogenesis. *Environ Health Perspect* 1993;101:33-8.
29. Saxena MC, Siddiqui MKJ, Bhargava AK, et al. Placental transfer of pesticides in humans. *Arch Toxicol* 1981;48:127-34.
30. Astroff AB, Young AD. The relationship between maternal and fetal effects following maternal organophosphate exposure during gestation in the rat. *Toxicol Ind Health* 1998;14:869-89.
31. Guven K, Deveci E, Akba O, et al. The accumulation and histological effects of organometallic fungicides Propineb and Maneb in the kidneys of fetus and female rats during pregnancy. *Toxicol Lett* 1998;99:91-8.
32. Goldhaber M. Fetal death ratios in a prospective study compared to state fetal death certificate reporting. *Am J Public Health* 1989;79:1268-70.
33. Department of Water Resources. Assistance Division of Planning and Local Assistance. *Sacramento Area Council of Governments. 1990 census geographic areas reference list, Part A*. Sacramento, CA: Department of Water Resources, 2001.