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Parental Agricultural Work and Selected Congenital Malformations

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The authors conducted a case-control study in Comunidad Valenciana, Spain, to assess the relation between occupational exposure to pesticides, mainly as a result of agricultural work, and the prevalence of congenital malformations. A total of 261 cases and 261 controls were selected from those infants born in eight public hospitals during 1993–1994. The cases were those who were diagnosed with selected defects (nervous system, cardiovascular, oral clefts, hypospadias/epispadias, musculoskeletal, and unspecified anomalies) during their first year of life. Information on occupational exposures and potential confounding variables was collected from the parents. For the mothers who were involved in agricultural activities during the month before conception and the first trimester of pregnancy, the adjusted odds ratio was 3.16 (95% confidence interval 1.11–9.01) primarily due to an increased risk for nervous system defects, oral clefts, and multiple anomalies. Paternal agricultural work did not increase the risk, although fathers who reported ever handling pesticides had an adjusted odds ratio of 1.49 (95% confidence interval 0.94–2.35) mainly related to an increased risk for nervous system and musculoskeletal defects. Although the power of this study regarding some associations is limited, the results justify further attention to maternal agricultural work and paternal pesticide exposure. *Am J Epidemiol* 1999;149:64–74.

abnormalities; agriculture; case-control studies; occupational exposure; pesticides

Almost 3 percent of all newborns have a congenital anomaly that requires medical care. One third of these defects are life threatening (1). Therefore, prevention of congenital malformations is a goal worth attaining, and the search for risk factors that have a role in causing this group of defects acquires particular significance. However, until now, only a limited number of factors have been identified as definite human teratogens. Some others are considered likely to cause congenital anomalies, although the evidence is not as conclusive, and, for a large number of other occupational and environmental agents, there is suggestive but not consistent evidence of their teratogenic potential in humans (2, 3).

Birth defects can be a consequence of genetic damage before conception and/or of the direct action of an agent on the embryo or fetus. Both processes can operate as a result of male and/or female exposure at different moments during conception and pregnancy (4). To study the etiology of birth defects, it is optimal to allocate exposure to the relevant time periods according to the postulated pathogenic mechanisms related to paternal and/or maternal exposure (5–7). Maternal exposure to teratogens during organogenesis is the best-known causative pathway for producing birth defects. However, some defects could arise after the critical organogenesis period (7). Several paternal mechanisms have also been proposed: seminal transfer of chemicals, household contamination by substances brought home by the father, and mutagenic or epigenetic damage to paternal germ cells (6). Female germ cell mutagenesis is considered much more unlikely, although there is still a risk during the completion of oogenesis just before ovulation (7). In both parents, later effects (i.e., birth defects due to past exposures) could be related to stem cell damage and to the storage of chemicals in the body (4, 7). Accordingly, for reproductive studies, the use of both chronic and acute exposure models has been recommended (5).

Epidemiologic studies on occupational risks for reproduction (8–17) and specific studies on agricultural workers (18–32) have assessed the association of parental involvement in agricultural work and/or parental exposure to pesticides with congenital defects. Some of these studies evaluated only paternal exposure, some only maternal exposure, and some both. In most of them, exposure was defined by job

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Abbreviations: CI, confidence interval; ICD-9, *International Classification of Diseases*, Ninth Revision; OR, odds ratio.

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title (applicators, farmers, agricultural workers, gardeners, etc). Only some studies present data on occupational exposure to specific pesticides. Most have found nonsignificant increases in the risk. Recent reviews on this subject have concluded that available evidence for an association between maternal and/or paternal pesticide exposure and birth defects is not conclusive (7, 33, 34).

A case-control study intended to provide additional evidence of the association of agricultural work and occupational pesticide exposure with congenital malformations was carried out in Comunidad Valenciana, Spain. In this study, an effort was made to avoid some of the flaws that limited previous research. For example, the source of the information about the cases (hospital discharge records) had been validated previously (35). The study base was defined carefully. Maternal and paternal risks were evaluated, and relevant risk periods for exposure were considered for both parents. The hypothesis that we investigated was that exposures, defined as male and/or female involvement in agricultural work and/or direct handling of pesticides during relevant time periods related to conception and pregnancy, increase the probability of having a child with some selected birth defects.

MATERIALS AND METHODS

Study base

Cases and controls were selected from those infants born from January 1, 1993, to December 31, 1994 (study period) in eight public hospitals serving the main intensive agricultural zones of Comunidad Valenciana. Only those infants from families residing in the more rural parts of the hospitals' catchment areas (i.e., excluding the towns of Valencia and Castellón) were considered. The study focused on the experience of the base population during previously defined time periods. Two risk periods for exposure, defined according to known or suspected mechanisms for congenital malformations, were used for analysis. One was the "acute risk period" (for the father, exposure during the 3 months before conception and/or the first trimester of pregnancy; for the mother, exposure 1 month before conception and/or during the first trimester of pregnancy). The other was the "nonacute risk period" (for both parents, exposures occurring before the acute risk period and/or during the second trimester of pregnancy).

Definition of cases

For our study, we considered the following malformations or groups of defects that have a relatively high prevalence at birth and, in previous epidemiologic research, have been related to pesticide exposure: nervous system defects (*International Classification of Diseases,* Ninth Revision (ICD-9) codes 740.0–742.9), cardiovascular defects (ICD-9 codes 745.0–747.9), oral clefts (ICD-9 codes 749.0–749.2), hypospadias/epispadias (ICD-9 code 752.6), and musculoskeletal defects (ICD-9 codes 754.0–756.9). Multiple and unspecified defects (ICD-9 codes 759.7–759.9) were included as well. As a result of a previous validation study of the quality of hospital discharge records as a source of information on congenital malformations, which compared data in the records with data in the clinical history (35), we decided to exclude from our study those cases with ICD-9 code 746.9 (unspecified anomalies of the heart, a code frequently used to refer to "heart murmur under surveillance" or "innocent heart murmur") or 754.3 (congenital dislocation of the hip) without any other code for the selected defects. This decision was made because the hospitals had very different practices regarding the use of these codes and whether they were included in the clinical history and the discharge records. Children with chromosomal syndromes were not included.

The goal was to identify all cases born with one of the chosen malformations during the study period in any of the selected hospitals. Malformations were identified by using hospital discharge records. For practical reasons, it was decided to limit the study to cases for whom diagnoses were made up to age 1 year and to select only those cases born and diagnosed during the study period.

Definition of controls

The case-control ratio was 1:1. A control was defined as the infant born closest to the date of birth of the case, in the same hospital, and with no diagnosis of congenital malformations during the study period. No more restrictions related to health problems at birth were considered for controls.

Interviews

The parents of the selected cases and controls were located and interviewed by telephone. Face-to-face interviews were also conducted with those parents whose telephone numbers were not available from hospital records. The interviews were highly structured, and a different questionnaire was used for the fathers and the mothers. All interviews were carried out by four trained interviewers. As interviews were completed, one of the authors (E. O.) reviewed the questionnaires and provided the interviewers with feedback on mistakes and omissions, which were subsequently corrected.

Once they had contacted the parents of the selected infants, the interviewers presented the study as research into children's health problems and related factors. Before the interview began, the interviewer did not know whether the subject was a case or a control. However, the answers to some of the questions on the questionnaire might have revealed this information. The interviewers were also unaware of the principal hypothesis under study, although the questionnaire clearly emphasized agricultural work.

By using the questionnaire, interviewers collected information on potential confounding variables for both parents (age, cigarette smoking, alcohol consumption and drug use, medical history, health of the immediate family, socioeconomic data, and, for the mother, reproductive history including problems during the index pregnancy) and on activities with a potential for exposure to pesticides.

Exposure assessment

A final set of questions asked whether interviewees had ever been involved in agricultural work, pesticide application, and/or any other activity with a potential for exposure to pesticides. For those who answered yes, the interviewers collected additional information regarding direct handling of pesticides and the time period of involvement in the activity.

Relevant exposure periods were computed for the mothers and fathers according to the dates of conception and pregnancy of the child selected for the study. As defined previously, two main exposure periods were considered: the acute risk period and the nonacute risk period. If exposure occurred during both periods, that person was considered exposed during the acute risk period.

Analysis

The analysis by agricultural activities compared interviewees who had ever been involved in agriculture and/or in pesticide application with interviewees who had never been involved in any activity with a potential for exposure. The analysis by direct handling of pesticides compared those who reported ever having handled pesticides with those who had never handled pesticides. An additional analysis was also conducted for direct handling of pesticides as compared with never being involved in any activity with a potential for exposure.

Univariate and multivariate conditional logistic regression models were built for both indicators of exposure (involvement in agricultural work and/or pesticide application and direct handling of pesticides). Potential confounding variables to be included in the multivariate models were chosen by following three steps: 1) selecting variables that had a significant association ($p \leq 0.10$) with the outcome in univariate models, 2) building saturated models with indicators of exposure and all of the covariates selected in step 1, and 3) excluding from the final models those covariates that did not contribute significantly to the model (likelihood ratio test $p > 0.10$). Most of the analysis in this paper is presented separately for mothers and fathers, although some models were built that included variables of exposure for both parents simultaneously. Data analysis was conducted by using Stata statistical software (36).

RESULTS

A total of 336 cases and 355 controls were initially selected for the study. The parents of 292 of the cases (87 percent) and 284 of the controls (80 percent) were located. The father and/or the mother of 31 cases (11 per 100 located families) and 23 controls (8 per 100 located families) refused to be interviewed (difference not statistically significant, $p = 0.30$). Therefore, a total of 261 cases and 261 controls were finally included in the study. For the cases, 232 parents (89 percent) were interviewed by telephone and 29 (11 percent) in face-to-face interviews; for the controls, this distribution was 229 (88 percent) and 32 (12 percent), respectively.

The distributions of potential confounding variables for the mothers and fathers of the selected cases and controls are shown in tables 1 and 2. Maternal variables selected for inclusion in multivariate models were a previous history of difficulty in becoming pregnant; a previous history of spontaneous abortion; a multiple pregnancy (in the index pregnancy); drug use and heavy smoking during the first trimester of the index pregnancy; educational level; and a job as a manager, professional, or clerk during the acute risk period. Selected paternal variables were age >40 years; educational level; and a job as a manager, professional, clerk, or industrial/production worker during the acute risk period. Family income was chosen as well. When a model was built using only these potential confounding variables, a previous history of difficulty in becoming pregnant; maternal and paternal occupations as managers, professionals, or clerks; paternal educational level; and family income lost their significant association with congenital malformations. Hence, these variables were not considered further in multivariate analysis with exposure variables.

The distribution of mothers and fathers according to their involvement in activities with a potential for exposure, reported direct handling of pesticides, and

TABLE 1. Distribution of potential confounding variables for the mothers of selected cases and controls born in eight public hospitals in Comunidad Valenciana, Spain, 1993–1994

Variable	Cases (*n* = 261)		Controls (*n* = 261)	
	No.	%*	No.	%*
Age >35 years	25	10	17	7
Reproductive history				
Difficulty in becoming pregnant	33	13	16	6
Previous spontaneous abortion	56	22	34	13
Previous stillbirth	3	1	2	1
Close family pattern				
Direct kinship with husband†	2	1	4	2
Reproductive problems (family)	44	17	42	16
Health problems‡	26	10	25	10
Congenital malformations (family)	8	3	9	3
Hereditary problems§	70	27	59	23
Index pregnancy history				
Birth order				
First	134	51	137	52
Second	94	36	92	35
Third or more	33	13	32	12
Twins	20	8	6	2
Diseases¶,#	41	16	44	17
Drug use¶	112	43	79	30
Smoking ≥15 cigarettes/day¶,**	20	12	5	3
Drinking ≥6.1 cc of pure alcohol/day¶,**	4	2	4	2
Socioeconomic data				
Education (no. of years of school)††				
Less than primary level (<5)	24	9	12	5
Primary level (5–8)	178	68	182	70
Secondary level (11–13)	37	14	53	20
University (14–19)	22	9	14	5
Working before the birth of the child	183	70	172	66
Job during ARP‡‡				
Manager/professional/clerk	28	11	41	16
Shop/trades/catering worker	42	16	35	13
Health worker	9	3	8	3
Driver/transportation worker	2	1	0	0
Construction/maintenance worker	0	0	0	0
Industrial/production worker	35	13	32	12
Other services worker	3	1	4	2

* All percentages were rounded.
† The mother's husband was also her cousin.
‡ In other children in the family.
§ Answers were grouped in four broad categories: cancer, cardiovascular diseases, diabetes, and other.
¶ During the first trimester.
# Very high fever, diabetes, thyroid diseases, cancer, epilepsy, German measles, syphilis, any other infectious disease, and any other serious disease (requiring special medical care).
** The limits for heavy smoking and heavy drinking were based on the value for the 75th percentile of mothers of controls.
†† The highest level of education successfully completed by the interviewee and the minimum number of years of schooling necessary to complete each level.
‡‡ ARP, acute risk period for the mother; 1 month before conception through the first trimester of pregnancy.

time periods of exposure is shown in table 3. To enable analysis of maternal exposure according to the activities listed in this table, "pesticide application" was combined with "agricultural work" for later analysis because of the small number of women pesticide applicators. Only three fathers and no mothers were exclusively exposed during the second trimester of pregnancy. Therefore, exposure during the nonacute risk

TABLE 2. Distribution of potential confounding variables for the fathers of selected cases and controls born in eight public hospitals in Comunidad Valenciana, Spain, 1993–1994

Variable	Cases (n = 261) No.	Cases (n = 261) %*	Controls (n = 261) No.	Controls (n = 261) %*
Age >40 years	25	10	10	4
Smoking ≥20 cigarettes/day†,‡	100	66	94	61
Drinking ≥36 cc of pure alcohol/day†,‡	80	36	58	26
Serious diseases	38	15	32	12
Drug use§	44	17	41	16
Close family pattern				
Reproductive problems (family)	29	11	21	8
Health problems¶	24	9	16	6
Congenital malformations (family)	7	3	4	2
Hereditary problems#	53	20	47	18
Socioeconomic data				
Education (no. of years of school)**				
Less than primary level (<5)	25	10	23	9
Primary level (5–8)	188	72	169	65
Secondary level (11–13)	35	13	52	20
University (14–19)	13	5	17	6
Working before the birth of the child	258	99	255	98
Job during ARP††				
Manager/professional/clerk	24	9	41	16
Shop/trades/catering worker	38	15	41	16
Health worker	1	0	2	1
Driver/transportation worker	21	8	24	9
Construction/maintenance worker	40	15	51	20
Industrial/production worker	89	34	68	26
Other services worker	10	4	9	3
Monthly family income‡‡ (pesetas§§)				
<100,000	63	24	52	20
100,000–149,999	115	44	103	39
150,000–199,999	36	14	60	23
≥200,000	33	13	32	13
No response	14	5	14	5

* All percentages were rounded.
† Before the birth of the child.
‡ The limits for heavy smoking and heavy drinking were based on the value for the 75th percentile of fathers of controls.
§ Other than usual drugs for minor pains or symptoms during the 3 years before the birth of the child.
¶ In other children in the family.
# Answers were grouped in four broad categories: cancer, cardiovascular diseases, diabetes, and other.
** The highest level of education successfully completed by the interviewee and the minimum number of years of schooling necessary to complete each level.
†† ARP, acute risk period for the father; 3 months before conception through the first trimester of pregnancy.
‡‡ Total for both mother and father.
§§ Approximately 1 US dollar = 152 pesetas (September 1998).

period refers mainly to the time before the acute risk period.

The results of univariate and multivariate analysis for exposure according to activities are shown in table 4. We found a statistically significant association for maternal involvement in agricultural work during the acute risk period (adjusted odds ratio (OR) = 3.16, 95 percent confidence interval (CI) 1.11–9.01). However, the data did not show an increased risk for paternal involvement in agricultural worker or pesticide application.

Table 5 presents the results of univariate and multivariate analysis according to reported direct handling of pesticides. As shown in table 3, only 13 mothers (nine mothers of cases and four mothers of controls) reported ever having handled pesticides. Of these, only two mothers of cases and one mother of a control handled pesticides during the acute risk period. These small numbers are reflected in the wide confidence intervals. Regarding paternal exposure, there was almost no difference between the crude and adjusted

TABLE 3. Time periods of exposure to activities and pesticides among mothers and fathers of selected cases and controls born in eight public hospitals in Comunidad Valenciana, Spain, 1993–1994

	Cases (*n* = 261)*				Controls (*n* = 261)			
	Mothers		Fathers		Mothers		Fathers	
	Acute risk period†,‡	Nonacute risk period§,‡	Acute risk period	Nonacute risk period	Acute risk period	Nonacute risk period	Acute risk period	Nonacute risk period
Activity								
Pesticide application	0	3	38	26	1	1	31	25
Agricultural work	15	72	26	66	7	80	23	78
Other¶	11	33	5	6	11	26	3	3
Never involved	127		90		134		93	
Pesticide handling								
Yes	2	7	47	36	1	3	33	28
Never	251		173		257		195	

* All selected malformations combined: nervous system (*n* = 37), cardiovascular (*n* = 117), oral clefts (*n* = 18), hypospadias/epispadias (*n* = 18), musculoskeletal (*n* = 79), unspecified anomalies (*n* = 14); there are cases with defects pertaining to more than one group of malformations.

† 1 month before conception through the first trimester of pregnancy for the mother and 3 months before conception through the first trimester of pregnancy for the father.

‡ Some interviewees did not remember the exact time period of exposure and were therefore excluded.

§ Before the period covered by the acute risk period and during the second trimester of pregnancy (only three fathers were exclusively exposed during the second trimester of pregnancy).

¶ Includes involvement in other activities with a potential for pesticide exposure (e.g., gardening, wood treatment, pesticide marketing, forestry, white collar work in pesticide plants, fruit and/or vegetable processing, and sanitation).

odds ratios for reported exposure during either the acute risk period or the nonacute risk period. However, a slight increase in risk was observed for fathers who directly handled pesticides.

When exposure related to direct handling of pesticides was analyzed by using as the unexposed reference group those interviewees who had never been involved in any activity with a potential for exposure (including mainly agricultural activities without direct handling of pesticides), the adjusted odds ratio for direct maternal handling of pesticides was 3.35 (95 percent CI 0.80–14.07). Maternal adjusted odds ratios for the nonacute and acute risk periods were 3.82 (95 percent CI 0.67–21.83) and 2.47 (95 percent CI 0.20–30.84), respectively. When this reference group was used for the fathers, the adjusted odds ratios were 1.40 (95 percent CI 0.82–2.37) for ever handling pesticides and 1.33 (95 percent CI 0.69–2.56) and 1.48 (95 percent CI 0.75–2.90) for exposure during the nonacute and acute risk periods, respectively.

Some additional analysis was carried out by including variables of exposure for the mothers and fathers in the same models. The results were not substantially different from those observed in the previous analysis. A combined index of exposure was created by comparing couples in which neither parent was ever involved in any activity with a potential for exposure (n = 120) with couples in which only one parent was ever involved in any such activity (n = 264, adjusted OR = 1.28, 95 percent CI 0.77–2.14) and with couples in which both parents were ever involved (n = 138, adjusted OR = 1.30, 95 percent CI 0.71–2.37). A similar approach was applied to the handling of pesticides; couples in which neither parent had ever handled pesticides (n = 368) were compared with couples in which only one parent had ever handled pesticides (n = 147, adjusted OR = 1.42, 95 percent CI 0.90–2.25) and with couples in which both parents had ever handled pesticides (n = 7, adjusted OR = 5.94, 95 percent CI 0.59–59.55).

A descriptive analysis by groups of congenital malformations was performed for maternal involvement in agricultural work and for direct paternal handling of pesticides. The distribution of discordant pairs for groups of congenital malformations according to these variables is shown in table 6. For maternal involvement in agricultural work, the number of discordant pairs that included those mothers of cases who had ever been exposed versus discordant pairs that included those mothers of cases who were unexposed was higher for every type of defect but was the highest for nervous system defects, oral clefts, and multiple anomalies. When we considered maternal exposure during the acute risk period, multiple anomalies had the strongest association, although the small numbers did not enable a proper analysis at this level. Regarding direct paternal handling of pesticides, nervous system defects and musculoskeletal defects had the highest

TABLE 4. Results of univariate and multivariate analysis for reported involvement in agricultural work and/or pesticide application and all selected malformations, Comunidad Valenciana, Spain, 1993–1994

Exposure variable	Wald test*	Odds ratio†	95% confidence interval†
Mothers			
Univariate model			
Agricultural work (ever)‡	0.744	1.07	0.72–1.58
Multivariate model§			
Agricultural work (ever)‡	0.430	1.20	0.76–1.90
Multivariate model§			
Agricultural work‡			
During nonacute risk period¶	0.823	1.06	0.65–1.70
During acute risk period#	0.031	3.16	1.11–9.01
Fathers			
Univariate model			
Agricultural work (ever)	0.805	0.95	0.62–1.45
Pesticide application (ever)	0.649	1.11	0.70–1.76
Multivariate model§			
Agricultural work (ever)	0.913	1.03	0.63–1.67
Pesticide application (ever)	0.840	1.06	0.62–1.81
Multivariate model§			
Agricultural work			
During nonacute risk period	0.618	0.87	0.51–1.50
During acute risk period	0.281	1.50	0.72–3.13
Pesticide application			
During nonacute risk period	0.666	1.17	0.57–2.39
During acute risk period	0.924	1.04	0.51–2.09

* Two-tailed *p* values from Wald test for exposure variables in the models.

† The numbers of exposed parents of cases and controls in each category are given in table 3; odds ratios and 95% confidence intervals were obtained from conditional logistic regression models.

‡ Includes applicators and agricultural workers.

§ Adjusting by maternal and paternal confounders: spontaneous abortion (mother), twins (index pregnancy), drug use during pregnancy (mother), heavy smoking during pregnancy (mother), education (mother), industrial work (father), and age >40 years (father).

¶ Before the period covered by the acute risk period and during the second trimester of pregnancy (only three fathers were exclusively exposed during the second trimester of pregnancy).

\# 1 month before conception through the first trimester of pregnancy for the mother and 3 months before conception through the first trimester of pregnancy for the father.

number of discordant pairs that included the exposed fathers of cases.

DISCUSSION

The power of this study to detect a significant odds ratio of ≥3.00 for maternal involvement in agricultural work during the acute risk period and all selected congenital malformations was a relatively low 0.33 (significance level, 0.05) (37). For fathers, there was a similar power to detect a significant odds ratio of ≥1.50 between handling pesticides during the acute risk period and all selected congenital malformations. For fathers who had ever handled pesticides, this same power was 0.55. The analysis by maternal handling of pesticides and by groups of congenital malformations was very limited by small numbers. In this study, significant associations were observed for maternal involvement in agricultural activities during the acute risk period. The risk for reported paternal handling of pesticides was increased as well, although the increase was not statistically significant.

Only those infants who were born alive and were admitted to the hospital were included in this study. The limitations of using this approach for surveillance of human teratogens have been discussed thoroughly elsewhere (38, 39). An association between a risk factor and a birth defect can be observed if that factor decreases the probability of early loss of defective conceptuses, substantially increases the probability of early loss of conceptuses without the defect, or is an actual determinant of the abnormality. Studies based on births cannot differentiate between these various explanations. In this study, the effect of prenatal selec-

TABLE 5. Results of univariate and multivariate analysis for reported direct handling of pesticides and all selected malformations, Comunidad Valenciana, Spain, 1993–1994

Exposure variable	Wald test*	Odds ratio†	95% confidence interval†
Mothers			
Univariate model			
Direct handling of pesticides (ever)	0.121	2.50	0.78–7.97
Multivariate model‡			
Direct handling of pesticides (ever)	0.166	2.66	0.67–10.64
Multivariate model‡			
Direct handling of pesticides			
During nonacute risk period§	0.235	2.88	0.50–16.41
During acute risk period¶	0.622	1.87	0.16–22.26
Fathers			
Univariate model			
Direct handling of pesticides (ever)	0.042	1.50	1.02–2.22
Multivariate model‡			
Direct handling of pesticides (ever)	0.087	1.49	0.94–2.35
Multivariate model‡			
Direct handling of pesticides			
During nonacute risk period	0.134	1.62	0.86–3.05
During acute risk period	0.195	1.48	0.82–2.68

* Two-tailed *p* values from Wald test for exposure variables in the models.

† The numbers of exposed parents of cases and controls in each category are presented in table 3; odds ratios and 95% confidence intervals were obtained from conditional logistic regression models.

‡ Adjusting by maternal and paternal confounders: spontaneous abortion (mother), twins (index pregnancy), drug use during pregnancy (mother), heavy smoking during pregnancy (mother), education (mother), industrial work (father), and age >40 years (father).

§ Before the period covered by the acute risk period and during the second trimester of pregnancy (only three fathers were exclusively exposed during the second trimester of pregnancy).

¶ 1 month before conception through the first trimester of pregnancy for the mother and 3 months before conception through the first trimester of pregnancy for the father.

TABLE 6. Distribution of discordant pairs* by major groups of congenital malformations, Comunidad Valenciana, Spain, 1993–1994

	Malformation†													
	Nervous system defects (*n* = 37)		Cardiovascular defects (*n* = 117)		Cleft lip and/or palate (*n* = 18)		Hypospadias/ epispadias (*n* = 18)		Musculoskeletal defects (*n* = 79)		Unspecified anomalies (*n* = 14)		Multiple anomalies‡ (*n* = 35)	
	+/–	–/+	+/–	–/+	+/–	–/+	+/–	–/+	+/–	–/+	+/–	–/+	+/–	–/+
Mothers														
Agricultural work (ever)	9	5	20	18	5	1	4	2	12	11	3	2	11	3
Agricultural work (during acute risk period)§	2	1	4	2	2	0	1	0	1	1	0	0	4	1
Fathers														
Pesticide handling (ever)	12	1	26	22	3	3	5	4	19	13	1	2	4	11
Pesticide handling (during acute risk period)§	7	1	13	16	2	0	2	2	11	6	1	0	3	8

* +/–, exposed parent of case and unexposed parent of control; –/+, unexposed parent of case and exposed parent of control.

† *International Classification of Diseases*, Ninth Revision codes: nervous system defects, 740.0–742.9; cardiovascular defects, 745.0–747.9 (excluding 746.9); cleft lip and/or palate, 749.0–749.2; hypospadias/epispadias, 752.6; musculoskeletal defects, 754.0–756.9 (excluding 754.3); unspecified anomalies, 759.7–759.9.

‡ More than one defect from different groups of malformations.

§ 1 month before conception through the first trimester of pregnancy for the mother and 3 months before conception through the first trimester of pregnancy for the father.

tion could have affected the risk estimates if the probability of being born alive was related to exposure status and was different for cases and controls in the base population.

In hospital-based case-control studies, differential referral patterns can be a source of selection bias if they are related to the exposure (37, 40). Every inhabitant requiring medical assistance in Spain is referred to a specific public hospital, depending on the area of residence (the catchment area for that hospital). The base population for this study was defined by deliveries of infants in selected public hospitals in agricultural areas. Cases and controls were chosen from the same hospitals; hence, they were representative of the same population experience (41). A differential referral is possible if cases at private hospitals are prenatally diagnosed with congenital malformations and are delivered at the hospitals selected for the study, as some of these hospitals are reference centers that have the medical resources and highly qualified staff necessary for complicated deliveries and births. However, because controls include other complicated births as well, controls would follow the same pathway from private to public hospitals, and it is reasonable to expect some compensation for this potential "differential referral" effect.

Using hospital records as a source for congenital malformations and other relevant information is not problem free. The information recorded and the record-keeping methods may vary among physicians and among hospitals (42, 43). The optimum time for admission (and subsequent treatment) of cases can vary because of different medical opinions and is not standardized clearly. Underascertainment of defects can especially affect the recording of minor malformations, but the reporting of major defects can be deficient as well (44). Problems may also arise when two or more defects are present, generally resulting in undernotification of coexisting defects (45). However, any such underascertainment is not likely to be related to exposure status in this study; therefore, the risk estimates should not be biased (46, 47).

Recall bias for exposure or related to other variables of interest is a well-known problem in case-control studies of pregnancy outcomes (48). Recall bias could mainly affect factors suspected to be related to the outcome (49). However, we can reasonably assume that agricultural work would not frequently be perceived as a risk factor for congenital defects among this study population. Regarding direct handling of pesticides, interviewees' perception of the risk is more likely. Additionally, differential bias is more frequent as general inaccuracies in recall increase for both cases and controls (50). Indeed, highly structured questionnaires and skilled interviewers are considered to protect against differential recall of exposure (51), and these points were carefully addressed in our research. A short time period between the interview and the period of interest is also considered to decrease the probability of nondifferential recall (50, 51). For this study, the acute risk period extended to a maximum of 4 years before the interview.

We observed that maternal involvement in agricultural activities during the acute risk period was associated with a threefold increase in the risk of having a child with at least one of the defects selected for study (adjusted OR = 3.16, 95 percent CI 1.11–9.01). There was no increase in risk for mothers who were involved in agricultural activities during the nonacute risk period. The increased risk remained almost unaffected when controlling by paternal involvement in agricultural activities (adjusted OR = 2.84). Pesticide exposure is still likely in agricultural work without directly handling pesticides. Entering a field after it has been treated with pesticides can be a major source of exposure, as residues are present on foliage and soil for some time after spraying (52). Although the level of exposure is usually lower during reentry than during other activities involving direct handling of pesticides, reentry is a much more frequent activity and usually involves many more hours (53). On the other hand, other factors related to agricultural activities, such as infections and physical stress, and other nonpesticide chemicals, such as fertilizers, can potentially act as teratogens. Exposure to these factors was not assessed in this study.

Previous epidemiologic research has yielded inconsistent results on the association of maternal involvement in agricultural activities and/or occupational exposure to pesticides with birth defects. Significant associations have been observed with all congenital malformations (13, 25, 26), developmental defects (14), and orofacial clefts (30). An association between maternal exposure to pesticides and neural tube defects also has been described (32, 54). Tikkanen et al. (15, 16) have studied occupational risk factors for cardiovascular defects and have not found a relation with maternal agricultural work, although they had a small number of mothers who were exposed.

Male-mediated teratogenesis has been postulated repeatedly, although conclusive evidence for humans is not yet available (4, 5, 7, 55, 56). Several studies have assessed paternal agricultural work and/or occupational exposure to pesticides as risk factors for congenital malformations in the offspring. Significant associations were observed for all congenital malformations in a study of applicators in cotton fields in India (27) and a study of floriculture workers in Colombia (25, 26). In our study, a small increase in the risk for direct paternal handling of pesticides was observed, although the adjusted odds ratio was not significant. Classification by time period of exposure had little effect on the magnitude of the risk.

The results from this study add to existing evidence of the association between maternal agricultural work and congenital malformations. The increased risk appears to be related to several distinct groups of defects, although a larger study is necessary to adequately assess the magnitude of the risks for specific malformations. If pesticides are responsible for the observed association, the increased risk may be related to relatively low levels of maternal exposure (that derived from agricultural work without clear, direct handling of pesticides), and this detail should be taken into account. For the fathers, our data suggest that simple involvement in agricultural work does not increase the risk, especially given the absence of an association with the more highly exposed group of pesticide applicators during the acute risk period. However, a small increase was related to direct paternal handling of pesticides, although the adjusted odds ratio was not statistically significant. Further studies are needed to determine which specific pesticides, if any, are involved in the increased risk of birth defects.

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