ORIGINAL ARTICLE

Maternal and paternal occupational exposure to agricultural work and the risk of anencephaly

M Lacasaña, H Vázquez-Grameix, V H Borja-Aburto, J Blanco-Muñoz, I Romieu, C Aguilar-Garduño, A M García

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See end of article for authors' affiliations

Correspondence to: Dr M Lacasaña, Center for Population Health Research, National Institute of Public Health, Av. Universidad 655, Col. Sta. Maria Ahuacatitlan, CP 62508 Cuernavaca, Morelos, Mexico; mlacasan@correo.insp.mx

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Aims: To evaluate the association between parental occupational exposure to agricultural work and the risk of anencephaly in three Mexican states.

Methods: A paired case control study (1:1) was done based on records of the Epidemiological Surveillance System of Neural Tube Defects in Mexico; 151 cases of anencephaly of more than 20 weeks' gestation were selected between March 2000 and February 2001. Controls were selected from the same maternity services as those of the cases and were born alive without congenital malformations. Information was obtained from both parents by means of a general questionnaire, a food frequency questionnaire, and a specific questionnaire on occupational exposure to pesticides. Exposures were analysed with emphasis on the three months before and one month after the last menstruation periods (acute risk period (ARP)), as well as exposure prior to the abovementioned period (non-acute risk period (NARP)).

Results: The children of mothers who worked in agriculture in the ARP had a greater risk of anencephaly (OR = 4.57, 95% CI 1.05 to 19.96). The risk of fathers having a child with anencephaly was greater in those who applied pesticides irrespective of whether it was done in the ARP or the NARP (OR = 2.50, 95% CI 0.73 to 8.64; and OR = 2.03, 95% CI 0.58 to 7.08, respectively).

Conclusions: These results support the hypothesis of the effect of maternal exposure to agricultural work on anencephaly and suggest that exposure of the father to pesticides in the periconceptional period or prior to this can also increase the risk of having an anencephalic child.

Congenital malformations constitute a public health problem.[1] Malformations of the central nervous system are very frequent, particularly neural tube defects (NTD), of which anencephaly is the most frequent type (37.7%).[2 3]

According to figures of the International Clearinghouse for Birth Defects Monitoring System (ICBD), Mexico in 2002 had the world highest prevalence of anencephaly, with 8.05 cases per 10 000 live births.[4] Even though the NTDs have a wide temporal and geographical variability in Mexico, it has been shown that eight of every ten cases are found in the states of the central zone of the country, mostly in the State of Mexico and Puebla where 40% of all cases of anencephaly are found.[3]

Although there is sufficient evidence of the adverse reproductive effect of some pesticides in experimental research,[5] epidemiological studies are inconclusive. Nevertheless, the results of previous epidemiological studies support the association between agricultural work and/or the exposure to pesticides of the father, of the mother, or both; different adverse reproductive effects have been reported, such as congenital malformations in general,[6 7] cleft lip and palate,[8 9] musculoskeletal defects,[10] limb reduction defects,[11 12] central nervous system defects,[13] spontaneous abortion,[14–17] fetal death due to congenital abnormalities,[18–20] intrauterine growth retardation,[21] and decrease in gestational age.[22] Few studies have evaluated the association between NTDs and exposure to pesticides,[6 8 23 24] and even fewer have studied this association considering anencephaly to be an aetiologically different entity.[25 26] One of the principal limitations of these studies is that the majority considered only maternal or paternal exposure. Nevertheless, in a study of the aetiology of reproductive effects, it is necessary to consider the exposure of both parents during the period of gametogenesis and organogenesis.[27]

The present study evaluated the association between anencephaly and the occupational exposure to agricultural work of both parents during critical periods around conception, in three central states of Mexico.

METHODS

Design and study population

A case control study was conducted, paired (1:1) on maternity clinic, date of birth, and federal entity of the three central states of Mexico: the State of Mexico, Puebla, and Guerrero. Cases of anencephaly were obtained through of the Epidemiological Surveillance System of Neural Tube Defects (ESSNTD) in Mexico. These three central states were chosen because of a generally higher prevalence of anencephaly in the central region states of Mexico; 8 out of 10 cases of NTD are concentrated in the central states of the country, effectively in the State of Mexico and Puebla, where 40% of all identified anencephaly cases in the country have been found,[2] and because the ESSNTD has functioned regularly in these selected states over the last few years.

The study was approved by the Institutional Review Board of the National Institute of Public Health of Mexico. All participants' mothers and fathers were given an informed consent letter, which was signed before participation.

Epidemiological Surveillance System of Neural Tube Defects (ESSNTD) in Mexico

The Epidemiological Surveillance System Register for Neural Tube Defects forms part of the National Epidemiological Surveillance System which compiles information proceeding from all the institutions within the National Health System, such as fetal death certificates and death certificates. Anencephaly is a defect which is incompatible with life, so that every case requires the filling out of a certificate of either

Abbreviations: ARP, acute risk period; ESSNTD, Epidemiological Surveillance System of Neural Tube Defects; NARP, non-acute risk period; NTD, neural tube defect

stillbirth or death by the doctors, and all these certificates must be channelled through the State Health System; for this reason the level of sub-registration is small, for urban areas as well as for rural ones.

The diagnosis of anencephaly was certified by the doctors who filled out the fetal death certificates and death certificates.

Selection of cases and controls

The cases were identified in hospitals and prenatal clinics and reported to the local ESSNTD in each of the three states. All cases were of 20 weeks' gestation or greater (born alive or fetal deaths), and were notified to the local ESSNTD between 1 March 2000 and 28 February 2001; the basic cause of death registered in the death or fetal death certificates was anencephaly (CIE 740.0). Cases of less than 20 weeks' gestation were not included in the study as the registration of abortions (spontaneous or induced) in Mexico is practically non-existent. Other inclusion criteria were that the mothers had to have resided in the corresponding federal entity in the year before the birth and to be contacted during the first three months of the postpartum period.

During the study period, 252 cases of anencephaly were identified in the three states. Fifty seven cases were excluded because of delayed notification (more than three months); we excluded another six cases because their families emigrated, leaving 189 cases whose mothers complied with the inclusion criteria. Of these, 157 mothers (83%) agreed to participate in the study, while 32 (17%) refused; 26 of the mothers did not have a partner, as they did not live with the father of the child for various reasons. As a result, 163 fathers of cases could be contacted, of which 129 (79%) agreed to participate in the study.

For each one of the cases whose mother agreed to participate in the study, a control was defined as the next child born alive in the same maternity clinic where the cases were born, without anencephaly or any other congenital malformation apparent at birth and who complied with the remaining inclusion criteria as the cases. Contact was made with 160 mothers of controls, of which 151 (94%) agreed to participate. Information was only obtained from 110 (79%) fathers of controls, because 11 mothers did not live with the father of the child and 30 fathers of controls refused to participate. It was not possible to find a control that fulfilled the inclusion criteria for six of the participating cases; these cases were not included in the analysis.

With respect to geographical distribution, 14% of cases and controls were from the State of Mexico, 13% from Guerrero, and 73% from Puebla.

Collection of information

The questionnaires were administered by the nursing staff previously trained in each of the participating states. Interviews were conducted in the homes of the cases and the controls. The fathers were interviewed on the same day as the mothers, but separately in order to avoid the possibility that the presence of the partner should condition the person's reply. Though the interviewer knew the case control status of the interviewees before the start of the interviews, he or she did not know the principal hypothesis of the study. The interviewers helped the mothers to define the periconceptional period of interest or the acute risk period (ARP), which was defined as the period from three months prior to the last menstruation to one month after it.

Specific questionnaires for the mother and the father were administered in order to obtain information from both parents on potential confounders, including sociodemographic characteristics (age, marital status, education, and family income), habits (lifetime and periconceptional use of tobacco, alcohol, and illicit drugs), history of illness, use of medication and multivitamins in the periconceptional period, reproductive history of the mother (number of pregnancies, history of stillbirths, spontaneous abortions, premature births, and malformed children), antenatal care in the index pregnancy, family reproductive history, occupational history, and domestic exposure to chemical substances in the periconceptional period.

A standard 85 item food frequency questionnaire was used to assess nutrient intake from diet. This instrument has been validated for use in epidemiological studies with the Mexican population.[28]

A short questionnaire that included summarised information on the principal variables of interest such as age, occupation in the periconceptional period, socioeconomic history (education and monthly family income), and reproductive history was administered to those mothers and fathers that refused to participate in the study.

Exposure assessment

In addition to obtaining information on lifetime occupational activities, with starting and finishing dates of each job, the participants were asked at the end of the general questionnaire if they had at any time been involved in agricultural work, application of pesticides, and/or other activities with potential exposure to these substances (manufacture, formulation, sale, or distribution of pesticides; work in fruit or vegetable warehouses; pest control work or gardening). Additional information on direct handling of pesticides and time periods involved in these activities was obtained from participants that answered positively to the aforementioned questions using a previously validated questionnaire. Accuracy and reliability indices were shown to be high for variables such as crops grown, where the interviewees had been working, time periods for pesticide treatments, and the use of personal protection during treatments (sensitivity 0.81–1.00; kappa index 0.65–0.80).[29]

The variable measuring exposure to agricultural work was analysed in three categories: non-agricultural workers, agricultural workers, and applicators. Additionally, different time periods of exposure were considered, in accordance with the risk periods defined for anencephaly.[30] Although the periconceptional period was considered to be the greatest risk period, many pathogenic mechanisms are not well known and we thought that it would be interesting to evaluate the risk associated with exposure prior to this period.[27]

The relevant exposure periods were defined from the date of the last menstruation of the woman and the same period was assigned to her partner. Two relevant exposure periods were defined: the acute risk period (ARP), which consisted of three months before and one month after the last menstruation; and the non-acute risk period (NARP), when exposure occurred before the ARP. When exposure occurred in both periods, the person was considered to have been exposed during the ARP.

Statistical analysis

The association between maternal exposure to pesticides and the risk of anencephaly was evaluated using conditional logistic regression. Paternal exposure was evaluated by non-conditional logistic regression as there were 69 women without a participating partner. In this case, given that the matching was broken, the potential confounding effect of the matching variables was evaluated (place of delivery, date of birth of the child, and federal entity).

Likewise, non-conditional logistic regression was used to evaluate the association between anencephaly and the joint exposure of both parents, comparing couples that had not worked in agriculture with couples in which at least one partner worked in agriculture and couples in which both

Table 1 Crude odds ratios for anencephaly according to some maternal characteristics

Variable	Cases (n = 151)		Controls (n = 151)			
	n	%	n	%	OR	(95% CI)
Age						
<18 years	10	6.62	16	10.60	1	
18–34 years	124	82.12	126	83.44	1.61	0.66–3.87
35–44 years	17	11.26	9	5.96	3.24	0.99–10.65
Educational level (years of school)						
High school and greater (≥12)	9	6.00	22	14.97	1	
Secondary and preparatory incomplete (9–11)	23	15.33	37	25.17	1.69	0.59–4.80
Primary and secondary incomplete (6–8)	47	31.33	46	31.29	3.46	1.25–9.53
No education/primary incomplete (<6)	71	47.33	42	28.57	5.92	2.09–16.72
Income level (US$1 = 10 pesos)						
>2500 pesos a month	16	10.96	22	14.86	1	
1000–2500 pesos a month	55	37.67	75	50.68	1.27	0.51–3.15
<1000 pesos a month	75	51.37	51	34.46	2.95	1.29–7.74
Familial reproductive history						
Reproductive problems*	14	8.92	9	5.96	1.62	0.67–3.92
Congenital malformations	9	5.73	2	1.32	4.50	0.97–20.83
Number of pregnancies						
1–2	78	51.66	109	72.19	1	
3–4	39	25.83	27	17.88	1.93	1.07–3.49
>4	34	22.52	15	9.93	3.84	1.72–8.59
Fever in the ARP	22	14.01	13	8.61	1.90	0.88–4.09
Adverse reproductive antecedents with previous children†						
Multiparous without antecedents	52	35.14	55	38.19	1	
Multiparous with antecedents	46	31.08	20	13.89	2.12	1.12–4.02
Primiparous	50	33.78	69	47.92	0.71	0.40–1.27
Lifetime alcohol use	50	32.48	62	41.61	0.59	0.34–1.05
Use of alcohol at the beginning of pregnancy	13	25.49	15	25.00	1.00	0.20–4.95
Lifetime use of tobacco	23	14.65	32	21.33	0.59	0.30–1.17
Use of tobacco at the beginning of pregnancy	6	26.09	9	28.13	0.66	0.24–1.87
Use of wood to cook during the ARP	87	57.62	60	39.74	3.25	1.70–6.21
Exposure to domestic pesticides during the ARP	37	24.50	35	23.18	1.08	0.63–1.86
Exposure to domestic solvents during the ARP	11	7.69	12	7.95	0.92	0.40–2.08
Occupation in the ARP‡						
Professionals	5	3.31	9	5.96	0.5	0.15–1.66
Industry	14	9.93	11	8.09	1.43	0.54–3.75
Agriculture	13	10.08	8	6.02	3.67	1.02–13.14
Services	6	4.03	7	4.83	0.86	0.29–2.55
Other occupations§	15	9.93	25	16.56	0.55	0.27–1.10
Occupation in the NARP‡						
Professionals	1	0.66	4	2.65	0.25	0.03–2.24
Industry	10	7.30	13	9.42	0.67	0.24–1.87
Agriculture	27	18.88	17	11.97	1.78	0.79–4.02
Services	2	1.38	6	4.17	0.33	0.07–1.65
Other occupations§	11	7.28	14	9.27	0.75	0.32–1.78
Folate intake						
≥400 μg/day	48	32.00	59	40.41	1	
<400 μg/day	102	68.00	87	59.59	1.58	0.94–2.63

*Includes problems with having children, repeated abortions, and infertility.
†Includes stillbirths, premature births, malformations, and abortions.
‡Reference level includes total women except the occupation considered in each case.
§Does not include women who are housewives or students.
ARP (acute risk period): three months before and one month after the last menstruation; NARP (non-acute risk period): exposure prior to the ARP.

partners worked in agriculture. In order to evaluate the exposure of men and women, three statistical models were constructed considering the exposure periods of interest: during the lifetime, during the ARP, or during the NARP.

All variables associated with outcome in the bivariate models were selected in order to evaluate the presence of confounding. Multivariate models were constructed, adding each of the variables previously selected, including those that changed the OR (odds ratio) by 10% or more.

Saturated models were constructed with the selected variables; those variables that did not contribute to the model were excluded from the final model. The variables associated with the outcome and therefore selected for inclusion in the multivariate models for women were: age, family income, education, history of congenital malformations in first degree relatives, number of pregnancies, fever in the ARP, adverse reproductive outcomes in previous pregnancies, (stillbirths, prematurity, spontaneous abortions, congenital abnormalities), folate intake, energy, use of tobacco and alcohol, exposure to domestic pesticides and solvents during the ARP, and habitual use of wood fires to cook. The decision to include wood fires was taken because during the combustion process of wood and carbon, a large quantity of aromatic hydrocarbons is emitted, including dioxins which have teratogenic, carcinogenic, and mutagenic effects. In Mexico, people in rural areas quite frequently use wood for cooking, so that this may represent a potential confounding factor concerning associations with agricultural work.

The potential confounders evaluated in the multivariate models for men, using the same criteria were: age, education, history of congenital malformations in first degree relatives, folate intake of the mother, energy of the mother, exposure of the mother to agricultural work during the ARP, and exposure to domestic pesticides and solvents during the ARP.

In order to evaluate the precision of the estimates, 95% confidence intervals were calculated. The analysis was done using the statistical package Stata 7.[31]

RESULTS

All mothers of cases who refused to participate, answered the short questionnaire; of the fathers who refused to participate, only 26% answered the short questionnaire. Owing to the fact that only nine fathers answered the questionnaire for non-participants, a comparative analysis between participants and non-participants was made, only taking into account the mothers of the cases. No relevant differences were found between the participant and the non-participant cases, in terms of the principal variables of interest (age, educational level, family income, maternal reproductive history, paternal and maternal occupation); results have been published previously.[32]

No relevant differences were found between the participant and non-participants cases in terms of the principal variables of interest (age, educational level, family income, maternal reproductive history, paternal and maternal occupation) (data not shown).

There were only nine pairs of parents in the control group that did not accept the invitation to participate in the study; three of them answered the questionnaire for non-participants. In view of this, we did not consider it necessary to analyse the differences between the controls that participated in the study and those that did not participate, in terms of the aforementioned variables.

The distribution of some characteristics of the mothers and fathers and their association with anencephaly are shown in tables 1 and 2. In addition to agricultural work, industrial work during the ARP by the father and the mother demonstrated an increased risk of anencephaly, but with wide confidence intervals: crude OR = 1.43 (95% CI 0.54 to 3.75) for women and 1.37 (95% CI 0.66 to 2.83) for men.

Daily folate intake less than 400 μg/day of the mother was associated with a increase of the risk of anencephaly: crude OR = 1.58 (95% CI 0.94 to 2.63). It is worth mentioning that 13 women reported the use of multivitamins containing folic acid during pregnancy, but only three (two cases and one control) took them in the acute risk period.

Of the 67 women involved in agricultural work (table 3), only nine (six mothers of cases and three of controls) reported having applied pesticides at some time in their lives, but none of them did it during the ARP. As a result, female applicators of pesticides were not differentiated from other women in the construction of the final models.

Table 3 presents the results obtained from the bivariate and multivariate models for the exposure to agricultural activities of the fathers and the mothers as well as the joint exposure of both parents. The mothers involved in agricultural work during the ARP experienced a higher risk of having anencephalic children: adjusted OR = 4.58 (95% CI 1.05 to 19.96). The risk of having an anencephalic child was higher, although non-significant, in those fathers that applied pesticides independently of whether it was done in the ARP or the NARP: adjusted OR = 2.50 (95% CI 0.73 to 8.64) and adjusted OR = 2.03 (95% CI 0.58 to 7.08), respectively. Nevertheless, when the parents were involved in agricultural activities but had never applied pesticides, no increase in the risk of having a child with anencephaly compared to the reference group was observed.

By means of a variable that combined exposure to agricultural work of both parents, couples that were never involved in any agricultural activity were compared with couples in which one of the partners was involved in agricultural work and couples in which both partners were involved. A higher risk was observed in couples in which both partners were involved in agricultural work during the NARP (crude OR = 4.82; 95% CI 1.00 to 23.29). Owing to the small number of subjects in this category it was not possible to adjust the model for confounders.

Pesticides most used by the parents of the cases in decreasing order of frequency were: permethrin (pyrethroid), methamidophos (organophosphate), methyl parathion (organophosphate), atrazine (triazine), 2,4-dichlorophenoxyacetic acid (chlorinated phenoxy), chlorpyrifos (organophosphate), mancozeb (dithiocarbamate), picloram (pyridine), dimethoate (organophosphate), and carbofuran (carbamate); pesticides most used by the parents of the controls in decreasing order of frequency were: methyl parathion (organophosphate), methamidophos (organophosphate), 2,4-dichlorophenoxyacetic acid (chlorinated phenoxy), chlorpyrifos (organophosphate), and monocrotophos (organophosphate).

DISCUSSION

The results of this study support the hypothesis of the effect of maternal exposure to agricultural work on neural tube closure and suggest that exposure to pesticides of the father in the periconceptional period or prior to this can also increase the risk of having an anencephalic child. In the case of mothers, exposure during the periconceptional period constitutes the most important risk period such that women involved in agricultural work during the ARP have a fourfold increased risk of having an anencephalic child with respect to women not exposed to agricultural activities. Women involved in agricultural work before the ARP demonstrated a much smaller increase.

Although it is infrequent that women are involved in the application or direct handling of pesticides, their exposure to these substances in agricultural work could be due to its presence in the fields or to the storage of pesticides or applicator equipment in their homes.[5 33]

Toxicological studies have demonstrated that fetal susceptibility to environmental exposures depends on the time period of the exposure with respect to the gestational age of the fetus. The period of organogenesis is the most susceptible period in which an exposure may have a teratogenic effect.[27] The neural tube, under normal circumstances, closes during the first four weeks of gestation. When for some reason this does not occur, various types of NTD may occur, such as spina bifida, when closure of the caudal neuropore is affected, or anencephaly, when the lesion affects the closure of the cephalic neuropore.[3]

There are few epidemiological studies on pesticide exposure and congenital malformations that have evaluated critical periods of exposure. This aspect is important because if the period of maximum fetal vulnerability is not taken into account, it is possible to miss an association that exists.[7] Nevertheless, this teratogenic damage can also be caused if the mother is exposed during the period prior to pregnancy, which can permit the accumulation of toxins in body tissues which are then liberated into the bloodstream during the period of greatest cellular differentiation, thus producing retarded fetal damage as a consequence of these exposures. On the other hand, during the first month of pregnancy the presence of pesticides in the seminal fluid of the father, derived from the liberation of toxins accumulated in the organism, is able to contaminate the mother during sexual relations and cause damage to the fetus.[27]

In 1984, Hearey *et al*[25] reported an association between agricultural work of fathers and the prevalence of spina bifida and anencephaly only when the fathers were smokers. The study, however, lacked statistical power. Brender and Suárez,[26] in 1990, reported a weak association between cases of anencephaly located in Texas between 1981 and 1986 and paternal exposure to pesticides (OR = 1.28; 95% CI 0.8 to 2.1), but they did not define critical periods of exposure in the analysis.

Shaw *et al*, in 1999,[24] reported a higher risk of NTD among women exposed to carbamates and organophosphates during

Table 2 Crude odds ratios for anencephaly according to some paternal characteristics

Variables	Cases (n = 129)		Controls (n = 110)			
	n	%	n	%	OR	95% CI
Age >40 years	7	5.43	4	3.64	1.52	0.43–5.34
Educational level (years of school)						
High school and greater (≥12)	13	10.08	19	17.27	1	
Secondary and preparatory incomplete (9–11)	23	17.83	36	32.73	0.93	0.39–2.25
Primary and secondary incomplete (6–8)	45	34.88	26	23.64	2.53	1.08–5.95
No education/primary incomplete (<6)	48	37.21	29	26.36	2.42	1.04–5.62
Lifetime use of alcohol	105	81.40	89	80.91	1.12	0.57–2.21
Use of alcohol at the beginning of pregnancy	59	45.74	51	46.36	0.99	0.56–1.77
Lifetime use of tobacco	93	72.09	73	66.36	1.31	0.75–2.29
Use of tobacco at the beginning of pregnancy	54	41.86	41	37.27	1.35	0.73–2.51
Familial reproductive history						
Reproductive problems*	6	4.65	7	6.36	0.72	0.24–2.20
Congenital malformations	5	3.88	1	0.91	4.39	0.51–38.20
Exposure to domestic pesticides during the ARP	32	24.81	20	18.18	1.08	0.63–1.86
Exposure to domestic solvents during the ARP	15	11.63	23	20.91	0.50	0.25–1.02
Occupation in the ARP†						
Professionals	2	1.55	5	4.55	0.32	0.06–1.67
Industry	22	17.05	14	12.73	1.37	0.66–2.83
Agriculture	38	29.46	23	20.91	1.81	0.97–3.38
Services	20	15.50	19	17.27	0.91	0.46–1.81
Construction	20	15.50	17	15.45	1.09	0.54–2.21
Other occupations	63	48.84	66	62.86	0.69	0.40–1.18
Occupation in the NARP†						
Professionals	0	0.00	2	1.82	–	–
Industry	4	3.10	5	4.55	0.69	0.18–2.67
Agriculture	29	22.48	20	18.18	1.59	0.82–3.10
Services	3	2.33	1	0.91	2.59	0.26–25.36
Construction	4	3.10	2	1.82	1.85	0.33–10.39
Other occupations	16	12.40	11	10.00	1.05	0.44–2.51

*Includes problems with having children, repeated abortions, and infertility.
†Reference level includes the total of men except the occupation considered in each case. The sum of the cases and controls can be greater than the total as there were persons who had different jobs in the period considered.
ARP (acute risk period): three months before and one month after the last menstruation; NARP (non-acute risk period): exposure prior to the ARP.

the first trimester of pregnancy compared to those exposed to the same pesticides during the three months prior to conception (OR = 2.1, 95% CI 0.51 to 7.6; and OR = 1.6, 95% CI 0.71 to 3.7, respectively). Also in 1999, Shaw *et al*[8] observed a greater risk of NTD for mothers that applied pesticides themselves at home during the periconceptional period (defined as one month prior to conception and three months after) (OR = 2.9; 95% CI 1.3 to 7.6) or when a professional applied the pesticides (OR = 2.5; 95% CI 0.9 to 6.9). Nevertheless, they did not find an association with occupational exposure of the father to pesticides during the three months before and after conception.

For purposes of comparability, the study most similar to ours in terms of the evaluation of the exposure and definition of the relevant exposure periods is that of García *et al* in 1999.[6] In a hospital based case-control study in Comunidad Valenciana, Spain, an increase in the risk of having children with congenital abnormalities was observed in women involved in agricultural work in the month prior conception and the first trimester of pregnancy (adjusted OR = 3.16; 95% CI 1.11 to 9.01). With respect to paternal exposure, a greater risk was observed when the fathers directly handled pesticides, although the difference in paternal risk for the ARP or NARP was not significant.

These results are compatible with the hypothesis that environmental or occupational exposure to pesticides may cause alterations in the male gametes prior to conception, as well as embryonic damage or feto-placental complex. The latter may be due to exposure of the mother during the period of organogenesis or due to the presence of pesticides in the seminal fluid of the father from liberation of the toxin accumulated in the organism.

In relation to the toxic effect on the male gametes, some studies suggest that pesticides, especially the organophosphates (methyl parathion and mathamidophos), interfere with migration during meiosis owing to their transferral to DNA, causing alterations in the gametes.[34 35] By this mechanism, paternal exposure to this type of compound may increase the frequency of congenital malformations and spontaneous abortions as it has been found that at least 35% of the latter are aneuploid, while the frequency of this anomaly in live births is 4 per 1000.[35]

Another mechanism that has not been frequently studied could be the involvement of the female germinal cells, although mutagenesis of the female germinal cells is a less likely mechanism. There is however, a risk during the period of oogenesis just before ovulation.[27] In an experimental study in female hamsters, the fungicide carbendazim demonstrated antimitotic activity that interfered with the maturation of the oocyte, causing a decrease in fertility and early losses in pregnancy.[36]

Very few studies exist which have evaluated the association between exposure to specific pesticides and NTDs or other adverse reproductive outcomes. Nordby *et al*, in 2005,[37] observed a moderate association between mancozeb exposure and neural tube defects. Greenlee *et al*, in 2004,[38] observed that in vitro exposure of embryos pre-implanted from murine into various specific pesticides and mixtures of these, demonstrated the fact that pesticides (dicamba, pendimethalin, 2,4-D, atrazine, chlorotalonil, mancozeb diquat, metolachlor, ammonium nitrate, chlorpyrifos, and terbufos) increased the percentage of apoptosis, and that atrazine (herbicide), chlorpyrifos, and terbufos all reduced the development of embryos on the path towards becoming blastocytes, demonstrating that these pesticides when used in agriculture cause damage in the development of re-implanted embryos. Many of the pesticides mentioned had been used by the parents of anencephaly cases: chlorpyrifos, methyl parathion, methamidophos, mancozeb, 2,4-D, atrazine; parents of the controls had been exposed to methyl

Table 3 Crude and adjusted odds ratios for anencephaly according to agricultural work and the application of pesticides by the parents

	Cases		Controls		Crude		Adjusted*	
Variable	**n**	**%**	**n**	**%**	**OR**	**95% CI**	**OR**	**95% CI**
Mothers								
Sometime in life								
Non-agricultural workers	110	72.85	125	82.78	1		1	
Agricultural workers	41	27.15	26	17.22	2.15	1.12–4.16	1.47	0.79–4.93
Acute risk period (ARP)								
Non-agricultural workers	110	89.43	125	93.98	1		1	
Agricultural workers	13	10.57	8	6.02	3.67	1.02–13.14	4.57	1.05–19.96
Non-acute risk period (NARP)								
Non-agricultural workers	110	80.29	125	88.03	1		1	
Agricultural workers	27	19.71	17	11.97	1.78	0.79–4.02	1.65	0.43–6.39
Fathers								
Sometime in life								
Non-agricultural workers	62	48.06	67	60.91	1		1	
Agricultural workers	34	26.36	29	26.36	1.27	0.69–2.32	0.66	0.30–1.48
Applicators	33	25.58	14	12.73	2.55	1.24–5.20	2.17	0.86–5.49
Acute risk period (ARP)								
Non-agricultural workers	62	64.58	67	75.28	1		1	
Agricultural workers	20	20.93	13	14.61	1.66	0.76–3.62	0.79	0.257-2.36
Applicators	14	14.58	9	10.11	1.68	0.68–4.16	2.50	0.73–8.64
Non-acute risk period (NARP)								
Non-agricultural workers	62	65.26	67	76.14	1		1	
Agricultural workers	14	14.74	16	18.18	0.95	0.43–2.10	0.61	0.21–1.74
Applicators	19	20.00	5	5.68	4.11	1.45–11.66	2.03	0.58–7.08
Both parents								
Sometime in life								
Neither is an agricultural worker	56	43.41	60	54.55	1		1	
One is an agricultural worker and/or applicator	49	37.98	38	34.55	1.38	0.79–2.42	1.02	0.54–1.94
Both are agricultural workers and/or applicators	24	18.60	12	10.91	2.14	0.98–4.69	1.46	0.60–3.52
Acute risk period (ARP)†								
Neither is an agricultural worker	56	58.33	60	70.59	1			
One is an agricultural worker and/or applicator	36	37.50	22	25.88	1.75	0.92–3.34		–
Both are agricultural workers	4	4.17	3	3.53	1.43	0.31–6.67		–
Non-acute risk period (NARP)†								
Neither is an agricultural worker	56	62.92	60	70.59	1			
One is an agricultural worker and/or applicator	24	26.97	23	27.06	1.12	0.57–2.20		–
Both are agricultural workers	9	10.11	2	2.35	4.82	1.00–23.29		–

*Multivariate model adjusted for:
Mothers: age of the mother, socioeconomic level (family income and education of the mother), adverse reproductive history with previous children, folate intake of the mother, and energy of the mother.
Fathers: age of the father, socioeconomic level (family income and education of the mother), adverse reproductive history with previous children, exposure of the mother to agricultural work during the ARP, work activity of both parents in industry, folate intake of the mother, and energy of the mother.
Both parents: age of the mother, socioeconomic level (family income and education of the mother), adverse reproductive history with previous children, work activity of both parents in industry, folate intake of the mother, and energy of the mother.
†The models were not adjusted due to the low values of the cells. The sum of the cases and controls does not necessarily equal the total due to missing values.
ARP (acute risk period): three months before and one month after the last menstruation; NARP (non-acute risk period): exposure prior to the ARP.

parathion, methamidophos, 2,4-D, and chlorpyrifos. However, studies with greater sample size are necessary to analyse the affects of specific pesticides and mixtures and to identify those pesticides which present the greatest risk to the exposed population.

One of the strengths of this study is that the outcome studied is one type of congenital malformation; the majority of previous studies have evaluated neural tube defects as a group. Although the aetiology of NTDs is still not well understood, available evidence suggests that NTDs may be aetio-pathologically heterogeneous.[39 40] The evidence for heterogeneity is derived from the fact that the neural tube closure encompasses two distinct pathogenic processes—neurulation and canalisation—with the likelihood that abnormalities in these two processes would have different aetiologies.[41] On the other hand, although there has been a general tendency for the prevalence of NTDs to diminish, there has been no similar decline among the other subgroups of NTDs, suggesting that these were aetiologically different.[42] Nevertheless, there is no evidence from experimental studies performed on animals permitting us to deduce how the possible differential mechanisms define specific pesticide activity as causes of anencephaly and other NTD subgroups. Neither do studies carried out on humans, owing to their epidemiological design, permit us to clarify the mechanisms resulting from activity caused by exposure to pesticides. However, this gap in knowledge does not permit us to eliminate the possibility of the existence of differential mechanisms concerning the activity of pesticides, specific to the various kinds of NTD.

Another strength of this study is the evaluation of critical periods of exposure as well as potential confounders in the father and the mother, which has been one of the principal limitations in previous studies that have evaluated environmental and occupational exposures as risk factors for neural tube defects.

Despite these strengths, some limitations of the study should be acknowledged, such as the small sample size, resulting in a reduction of the power of the study and wide confidence intervals owing to the sparseness of data referring to some of the categories analysed, especially concerning the evaluation of paternal exposure to pesticides. However, it is necessary to undertake an analysis, making multiple comparisons, taking into account the fact that the impact of

mechanisms in pesticides which can cause congenital malformations depends on the periods of exposure.

We consider that the possibility that we might have made an error of classification is very low, owing firstly to the fact that we used a previously validated questionnaire in order to evaluate retrospective exposure to pesticides. This ensured certain high levels of precision and reproducibility, for example concerning the crops which the workers had been cultivating during the last two years prior to the interview, the periods of pesticide treatment, and the use of personal protection during the application of pesticides (sensitivity 0.81–1.00 and kappa index 0.65–0.80).

On the other hand, although one of the principal challenges of environmental and occupational epidemiology is the evaluation of specific active compounds, we should not forget that agricultural work involves other risks associated with infectious agents, physical stress, excessive heat, and exposure to other chemicals that are used in agriculture such as fertilisers and chemical components other than active principles found in pesticides.

In this study the cases were selected from births or stillbirths with a diagnosis of anencephaly that were notified to the ESSNTD. The controls were not selected from the civil registry of birth certificates owing to the fact that in Mexico, especially in rural areas, some parents delay the registration of the births of their children, which may introduce selection bias as the possibility of capturing parents who work in agriculture as controls would be decreased. Additionally, the selection of controls from the same medical care unit where the case was born was not related to the odds of selecting a parent involved in agricultural activities. The proportion of children born at home or in medical units was similar for parents involved or not in agricultural activities.

Another limitation of our study is that 25% of the mothers of identified cases were not interviewed, largely because of delays in case notification. Given that the opportunity for notification in Mexico is more precarious in communities of low socioeconomic level where the major activity is agriculture work, which might introduce selection bias in this study, this would tend to underestimate the true association between agricultural work and anencephaly, owing to the fact that delays in certification and notification are more prone to occur in rural areas.

The mothers of the cases that refused to participate in the study, 34 and 32 respectively, did not differ from the participants in terms of the exposure of interest; we therefore believe that the possibility of selection bias due to non-participation is minimal. The main reason for the parents refusing to participate in the study was the fact that, besides responding to the questionnaire, participating parents were asked to give a blood sample. This, added to the fact that the average time taken for the questionnaire to be applied was approximately one hour, led some parents to refuse to participate in the study.

Only products of conception with a gestational age of more than 20 weeks were included in the study; spontaneous and induced abortions were excluded. This prenatal selection could have affected the estimation of the association if the probability of reaching 20 weeks of gestational age was related to the exposure of interest and anencephaly. We could not evaluate whether exposure to pesticides increases the risk of early loss in the affected fetuses because of the study design. However, information concerning elective termination of pregnancy, in the case of congenital malformations in Mexico, is practically non-existent. Neither do we have access to data concerning the frequency of cases of neural tube defects, which are identified prior to birth. In the case of anencephaly, an upwards trend has been shown to exist in Mexico between 1980 and 1989; rates remained constant from 1989 to 1997.[2] We might assume that elective termination of pregnancy, owing to these kinds of problems, has had no impact on its prevalence; however neither should we forget that the examination and identification of these defects has increased and this trend may be a reflection of this. However, if it is the case that some frequency for voluntary termination of pregnancy exists, we should consider that in the Mexican context this frequency is small and is almost exclusively confined to women of high socioeconomic level who have access to this option; taking this factor into account may therefore mean we have overestimated these associations.

The possibility exists that cases and controls respond differently to a questionnaire as a result of the presence or absence of the event being studied, causing a differential classification.[43] In this study the parents of the cases did not

Main messages

- Although there is sufficient evidence concerning the teratogenic effect of some pesticides in experimental research, results from epidemiological studies are inconclusive.
- Results of this study suggest an increase in the magnitude of the association when the exposure to agricultural work occurs during the periconceptional period, notably in the case of maternal exposure.
- A greater risk was observed when the intensity of exposure to pesticides was increased among the fathers (applying versus non-applying agricultural workers). This was independent of whether this took place in the periconceptional period (three months before and one month after the last menstruation) or before this.
- These results are compatible with the hypothesis that occupational exposure to pesticides may cause alterations in the male gametes prior to conception, as well as embryonic damage or damage to the fetal-placental complex.
- Some of the pesticides previously reported as having possible adverse reproductive effects were used by the fathers of the anencephaly cases: chlorpyrifos, methyl parathion, methamidophos, mancozeb, 2,4-D, atrazine.

Policy implications

- Women involved in agricultural work, or who are living with men who work in agriculture, should be protected from direct and indirect pesticide exposure, especially during the periconceptional period if they are planning to have a child.
- There is a need for further studies that permit the evaluation of specific active compounds and mixtures, in order to identify substances which have highly toxic effects on reproductive health, as much as to take concrete action for the prevention of these effects on exposed workers.
- In the future, efforts should be oriented towards promoting activities which provide information and training among male and female agricultural workers and their families, as well as promoting a preventive culture and developing codes of behaviour.

associate the exposure to pesticides in agricultural work with having a child with anencephaly: when they were asked about any health effects that could be associated with exposure they only indicated nausea and headaches; they never associated it with any adverse reproductive event. In this respect there were no differences observed between the cases and controls.

Finally, the interviewers may have introduced differential information bias into the study by knowing the status of the cases and controls. Nevertheless, they were unaware of the hypothesis of the study, and were carefully trained to apply the questionnaire in the same manner to the cases and controls.

The results of this study suggest a possible causal association between exposure to agricultural work and anencephaly for various reasons; there is consistency with the results of other studies in terms of an increase in the magnitude of the association when the exposure occurs during the biologically important period, notably in the case of maternal exposure. A greater risk was observed when the intensity of exposure to pesticides was increased in the fathers (applicators versus non-applicator agricultural workers). In Mexico, pesticide applicators typically do not wear safety equipment; in this study, only two workers used appropriate safety equipment. There is a need for studies to evaluate specific active compounds in order to identify substances that have highly toxic effects on reproductive health, and to prevent these effects in exposed workers.

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Authors' affiliations

M Lacasaña, H Vázquez-Grameix, J Blanco-Muñoz, I Romieu, C Aguilar-Garduño, Center for Population Health Research, National Institute of Public Health, Cuernavaca, Mexico
M Lacasaña, Andalusian School of Public Health, Granada, Spain
V H Borja-Aburto, Mexican Institute of Social Security, Mexico DF, Mexico
C Aguilar-Garduño, Public Health Department, History of Science and Gynaecology, Miguel Hernandez University, Alicante, Spain
A M García, Department of Preventive Medicine and Public Health, University of Valencia, Spain

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