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Original Article

Spontaneous Abortion in Spouses of Greenhouse Workers Exposed to Pesticides

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ABSTRACT

Objective: The aim of this study was to explore the association between exposure to specific pesticides (including endocrine disrupters) of greenhouse workers and spontaneous abortion in their spouses.

Methods: A group of exposed workers was compared with a non-exposed group. The risk of spontaneous abortion was evaluated using a logistic regression model.

Results: A significantly higher rate of spontaneous abortion was observed among spouses of workers exposed to specific compounds (atrazine, benomyl-carbendazim, carbaryl and DDT) in comparison with spouses of the unexposed group. The logistic regression model confirmed the increased risk of spontaneous abortion (OR=11.8; 95% CI 2.3–59.6).

Conclusions: These findings suggest that occupational exposure to pesticides might have an adverse effect on the partner's reproductive health.

Key words: reproduction, endocrine disrupters, pregnancy loss, epidemiology, paternal exposure

Introduction

There is growing evidence that environmental exposures contribute to reproductive damage (1). The hypothesis of a possible association between exposure to specific pesticides or groups of pesticides and alteration of the male reproductive system is supported by experimental data (2–4). Several pesticides are known to affect endocrine homeostasis in experimental studies *in vivo* through receptor-mediated oestrogenic or antiandrogenic activities, and through enzyme (e.g., aromatase) inhibition (5). Some of these chemicals, for example chlorinated compounds, are of particular concern because they persist in the environment and may bioaccumulate (6, 7). These substances, named endocrine disrupters (ED), may pose a particular risk during the early developmental stages inducing severe reproductive impairment such as genital tract malformations and persistent functional disorders. In adults, exposure to ED may reduce fertility and cause other adverse health effects, even though more evidence is required to support these hypotheses (8). In addition to ED, other pesticides may act through other mechanisms; for example, benzimidazole fungicides inhibit tubulin polymerization, thus exerting a direct cytostatic effect on male germ cells (4).

After the well documented reproductive toxicity of DBCP (9), the issue of the role of pesticides on human reproduction has been explored in several epidemiological studies. Increased conception delay has been observed in couples where the male partners worked in agriculture and were exposed to pesticides (10, 11). A study conducted in Ontario, Canada, among female farmers revealed that the risk of spontaneous abortion was increased in particular by the use of specific active ingredients such as phenoxy-acetic acid herbicides, thiocarbammates and triazines (12). Nevertheless, the available data are insufficient to characterize the risk for female reproductive health induced by agrochemicals affecting sperm quality and integrity.

The aim of this study was to address the question whether the continuous exposure to pesticides in greenhouse workers can be responsible for adverse reproductive effects such as an increased risk of spontaneous abortion in their spouses.

Methods

The present cross-sectional study is based on data obtained

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by interview from the workers of greenhouses in southern Italy during the year 2000.

Eligibility criteria for participation included age 20–55 years and marriage or stable partnership for at least two years. We interviewed 209 married greenhouse workers. Those with no pregnancies (25 subjects) were not included in the analysis. Information was therefore analysed for 184 greenhouse workers.

The workers provided data regarding age, education, occupation, occupational exposure to certain pesticides or pesticide groups, smoking habits and health status, in addition to questions concerning the respondent's spouse, i.e., age, education, occupation, smoking habits and health status, including a complete reproductive history of the spouse. For each pregnancy reported, we collected data on the work and domestic exposures of each partner during the pregnancy and in the months preceding it. The present analysis is based on the first pregnancy of each couple in order to have independent observations.

Exposure to pesticides was ascertained by questions on the use of some active ingredients at the time of the first pregnancy of the workers wives. Using a checklist, we asked greenhouse workers whether they were exposed to at least one of the active ingredients listed. Compounds were selected on the basis of experimental evidence as being either ED or otherwise affecting male and/or female reproduction and included: atrazine, benomyl, carbaryl, carbendazim, dinoseb, lindane, methoxychlor, procymidone, simazine and vinclozolin. The greenhouse workers who, at the time of the first pregnancy, were not using any of the chemicals listed were considered as the reference group. On the basis of the responses, 48 greenhouse workers were considered exposed to pesticides at first pregnancy of their spouses and 136 non-exposed (Table 1).

The risk of spontaneous abortion was evaluated by a logistic regression model controlling for the potential confounding due to partners' age, smoking habits, educational level, the spouse's occupation in agriculture, and the time that elapsed between the first pregnancy and the interview.

Results

Out of the list of ten chemicals, the use of five was reported by these workers. In particular, in the 48 exposed workers 30 reported exposure to benomyl and 6 to the closely related carbendazim. Among them we observed 5 spontaneous

Table 1 Socio-demographic characteristics of the greenhouse workers and their spouses by exposure

Variables at the time of first pregnancy	Exposed		Non-exposed		X^2 test
	n	%	n	%	P
Total pregnancies	48		136		
Abortions	7	14.6	6	4.4	0.02
Male partner's age (years)					
<30	31	70.5	86	66.1	
≥30	13	29.5	44	33.9	n.s.
Spouse's age (years)					
<20	4	9.5	10	7.9	
20–30	36	85.7	98	77.2	
≥30	2	4.8	19	15.0	n.s.
Male partner's smoking habit					
No	29	60.4	80	58.8	
Yes	19	39.6	56	41.2	n.s.
Spouse's smoking habit					
No	45	97.3	114	83.8	
Yes	3	6.3	22	16.2	n.s.
Male partner's educational level					
Primary	30	62.5	113	83.1	
Secondary	18	37.5	23	16.9	0.003
Spouse's educational level					
Primary	30	62.5	96	70.6	
Secondary	18	37.5	40	29.4	n.s.
Spouse's work					
Housekeeper	31	66.0	75	55.6	
Work in agriculture	8	17.0	27	20.0	
Other work	8	17.0	33	24.0	n.s.
Time interval between interview and pregnancy date in years					
<3	10	22.7	30	23.0	
3–6	8	18.2	24	18.5	
>6	26	59.1	76	58.5	n.s.

Table 2 Crude and adjusted ORs of spontaneous abortion among the 1st pregnancies of the greenhouse workers' spouses

Predictor	Crude OR	(95% CI)	Adjusted OR*	(95% CI)
Exposure				
non-exposed	1		1	
exposed	3.7	1.2–11.7	11.8	2.3–59.6
Male partner's age (years)				
<30	1		1	
≥30	1.5	0.5–5.0	2.6	0.3–21.7
Spouse's age (years)				
<20	0.8	0.09–6.6	0.3	0.01–7.1
20–30	1		1	
≥30	1.2	0.1–10.5	0.7	0.06–8.1
Male partner's smoking habit				
No	1		1	
Yes	2.5	0.8–7.9	2.9	0.6–15.2
Spouse's smoking habit				
No	1		1	
Yes	2.0	0.5–8.0	9.2	1.2–68.7
Male partner's educational level				
Primary	1.6	0.3–7.6	16.0	0.7–380.6
Secondary	1		1	
Spouse's educational level				
Primary	2.7	0.6–12.5	2.8	0.3–23.5
Secondary	1		1	
Spouse's work				
Housekeeper	1		1	
Work in agriculture	0.8	0.2–4.4	0.2	0.01–4.1
Other work	2.2	0.6–8.1	3.5	0.6–20.5
Time interval between interview and pregnancy date in years				
<3	1		1	
3–6	1.2	0.1–20.9	3.7	0.1–99.8
>6	4.3	0.5–34.2	6.2	0.5–78.5

*Adjusted for all variables.

abortions (13.9%). Thirty-one workers reported exposure to carbaryl; of these 4 experienced spontaneous abortions (12.9%). DDT was reported by 11 workers with 3 cases of abortion (27.3%). Two workers exposed to atrazine reported no abortion. In all there were 7 cases of spontaneous abortion among the exposed (14.6%), and 6 cases among the non-exposed (4.4%).

Table 1 shows the other characteristics of interest of the two groups. The two groups were different with respect to the abortion rate and to the male partner's educational level and the differences are significant. No significant differences were observed with respect to the other characteristics of the two groups. The time that elapsed between the interview and the first pregnancy was also similar in the two groups. However, since all these may be confounding factors for spontaneous abortion, all the above variables were entered in the logistic regression for estimating the adjusted odds ratios.

Table 2 shows the crude and the adjusted odds ratio obtained by the logistic regression model. The crude OR for spontaneous abortion was 3.7 (95% CI 1.2–11.7), while the OR adjusted for age, smoking habit and education of both partners and for spouse's type of work and time between the pregnancy outcome and the interview, was 11.8 (95% CI 2.3–59.6). The only other risk factor associated with spontaneous abortion was the smoking of the mother (adjusted OR 9.2, 95% CI 1.2–68.7).

Discussion

Environmental factors may adversely affect human reproduction through either direct tissue toxicity or endocrine-mediated mechanisms (13). However, sufficient evidence is not available to assess the possible effects on female reproduction mediated through the exposure of the male partner (14).

Toxicological studies in rodents showed that the five compounds for which exposure has been reported in the present study may affect reproductive function through different mechanisms. The chloro-S-triazine herbicides, such as atrazine, may disrupt the hypothalamic-pituitary-gonadal axis, thus altering endocrine homeostasis in both genders (15). DDT, in addition to being a persistent and bioaccumulating pollutant, is an estrogen agonist, while DDE, a major metabolite, is an antiandrogen (7). Both compounds may affect male reproduction; moreover increased levels of DDE in serum were associ-

ated with increased risk of miscarriage in a recent case-control study of Chinese women working in the textile industry (16).

Other compounds may directly affect spermatogenesis such as the benzimidazole fungicides benomyl and carbendazim; these chemicals impair cellular division and also induce postimplantation loss through embryonic damage (4, 17). Carbaryl is another testicular toxicant which both reduces sperm production and increases the rate of abnormal sperm, although the mechanisms have yet to be clarified (2).

Experimental findings lend support to the biological plausibility that male exposure to specific contaminants, including pesticides, increase the risk of miscarriage. In fact, embryonic death may be induced by paternal exposure to compounds affecting genomic expression and/or protein synthesis, e.g., lead (18); moreover, hormonally active compounds may also impair the integrity of the male germ cell (3, 19).

The present observations that wives of greenhouse workers showed an increased risk for spontaneous abortion is consistent with previous findings. Indian male cotton field workers applying organochlorine and organophosphorous pesticides without protective equipment had a significant increased risk in spontaneous abortion and stillbirths of their wives (20). In Spain, male greenhouse workers with daily exposure to a multitude of pesticides (including most of those recorded in our study) showed an elevated risk of spontaneous abortion (RR=3.14, 95% CI 1.25, 7.88) of their wives (21). In the Ontario Farm Family Health Study, an increased risk of miscarriage was associated with the use of thiocarbammates (RR=1.9, 95% CI 1.1, 3.3), carbaryl (RR=1.9, 95% CI 1.1, 3.1) and the generic use of herbicides (RR=1.4, 95% CI 1.0, 2.0) and insecticides (RR=1.6, 95% CI 1.1, 2.4) (22). A more detailed analysis of the timing of paternal exposure showed that male preconception exposure to phenoxyherbicides was particularly associated with early (<12 weeks) spontaneous abortion (RR=2.5, 95% CI 1.0, 6.4). Since the majority of early abortions also have gross chromosomal anomalies, the authors suggested that exposure to phenoxyherbicides may be responsible for a genetic effect on paternal gametes, rather than a direct effect on the embryo (23).

The size of the present study does not permit more detailed analysis of the timing of the exposure or of the role of single product used in association with the risk of spontaneous abortion. Overall the unadjusted odds ratio observed among the greenhouse workers was 3.7 (95% CI 1.2, 11.7) and the adjusted 11.8 (95% CI 2.3, 59.6). The high odds ratio may be attributable in part to the low abortion rate among the non-exposed workers (4.4% versus 14.6% in the exposed). Considering, however, that the abortion rate of first pregnancies in the general population in Italy has been estimated to be 6.7% (24), the rate observed among the exposed greenhouse workers appears comparatively high.

The present findings, however, must be considered with caution, especially because of the small sample size which reduces the precision of the estimated risk. Moreover, we cannot exclude that recall bias might have contributed to the high OR. Finally, the reported exposure to DDT is somewhat surprising considering that this product was banned several decades ago. This might prompt the attention to the possible persistent use of outlawed, toxic chemicals in some agricultural settings.

In conclusion, the present results offer additional evidence in support of the hypothesis of an association between reproductive problems and male occupational exposure to ED agrochemicals. Nevertheless, additional epidemiological studies are needed to further characterize the risks; in particular, ED deserve attention because of their complex mechanism affecting human reproduction and their potentially long-lasting impact on reproductive health.

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Reference

(1) Petrelli G., Mantovani A. Environmental risk factors and male fertility and reproduction. Contraception 2002; 65: 297–300.
(2) Pant N, Srivastava SC, Prasad AK, Shankar R, Srivastava SP. Effects of carbaryl on the rat's male reproductive system. Vet Hum Toxicol 1995; 37: 421–425.
(3) Traina ME, Rescia M, Urbani E, Mantovani A, Macrì C, Ricciardi C, Stazi AV, Fazzi P, Cordelli E, Eleuteri P, Leter G, Spano M. Long-lasting effects of lindane on mouse spermatogenesis induced by in utero exposure. Reprod. Toxicol. 2003; 17: 25–35.
(4) World Health Organization. Carbendazim. Environmental Health Criteria, 149. Geneva: WHO, 1993.
(5) Mantovani A. Hazard identification and risk assessment of endocrine disrupting chemicals with regard to developmental effects. Toxicology 2002; 181-182: 367–370.
(6) Mantovani A., Stazi, AV, Maranghi, F, Macri' C, Ricciardi C. Problems in testing and risk assessment of endocrine disrupting chemicals with regard to developmental toxicology. Chemosphere 1999; 39: 1293–1300.
(7) Turusov V, Rakitsky V, Tomatis L. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ. Health Perspect. 2002; 110: 125–128.
(8) Joffe M. Are problems with male reproductive health caused by endocrine disruption? Occup. Environ. Med. 2001; 58(4): 281–287
(9) Whorton MD, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. Lancet 1977; 2: 1259–1261.
(10) De Cock J, Westveer K, Heederik D, De Velde E, van Kooij R. Time to pregnancy and occupational expsoure to pesticides in fruit growers in The Netherlands. Occup. Environ. Med. 1994; 51(10): 693–699.
(11) Petrelli G., Figà-Talamanca I. Reduction in fertility in male greenhouse workers exposed to pesticides. Eur. J. Epidemiol.

2001; 17: 675–677.
(12) Arbuckle TE, Lin Z, Mery LS. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ. Health Perspect. 2001; 109(8): 851–857.
(13) Hoyer PB. Reproductive toxicology: current and future directions. Biochem. Pharmacol. 2001; 62(12): 1557–1564.
(14) Hales BF, Robaire B. Paternal exposure to drugs and environmental chemicals: effects on progeny outcome. J. Androl. 2001; 22(6): 927–936.
(15) Stoker TE, Laws SC, Guidici DL, Cooper RL. The effect of atrazine on puberty in male wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. Toxicol. Sci. 2000; 58(1): 50–59.
(16) Korrick SA, Chen C, Damokosh AI, Ni J, Liu X, Cho SI, Altshul L, Ryan L, Xu X. Association of DDT with spontaneous abortion: a case-control study. An. Epidemiol. 2001; 11(7): 491–496.
(17) Hess RA. Effects of environmental toxicants on the efferent ducts, epididymis and fertility. J. Reprod. Fertil. Suppl 1998; 53: 247–259.
(18) Gandley R, Anderson L, Silbergeld EK. Lead: male-mediated effects on reproduction and development in the rat. Environ. Res. 1999; 80(4): 355–363.
(19) Anderson D, Dobrzynska MM, Basaran N. Effect of various genotoxins and reproductive toxins in human lymphocytes and sperm in the Comet assay. Teratog. Carcinog. Mutagen. 1997; 17(1): 29–43.
(20) Rupa DS, Reddy PP, Reddi OS. Reproductive performance in population exposed to pesticides in cotton fields in India. Environ. Res. 1991; 55(2): 123–128.
(21) Parròn T, Hernandez AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. Hum. Exp. Toxicol. 1996; 15: 957–963.
(22) Savitz D, Arbukle T, Kaczor D, Curtis KM. Male pesticide exposure and pregnancy outcome. Am. J. Epidemiol. 1997; 146: 1025–1036.
(23) Arbuckle TE, Savitz DA, Mery LS, Curtis KM. Exposure to phenoxy Herbicides and the risk of spontaneous abortion. Epidemiology 1999; 10: 752–760.
(24) Osborn JF, Cattaruzza MS, Spinelli A. Risk of spontaneous abortion in Italy, 1978-1995, and the effect of maternal age, gravidity, marital status, and education. Am. J. Epidemiol. 2000; 151: 98–105.