ORIGINAL ARTICLE

Roberto Agnesi · Flavio Valentini · Giuseppe Mastrangelo Risk of spontaneous abortion and maternal exposure to organic solvents in the shoe industry

Received: 15 January 1996/Accepted: 24 May 1996

Abstract Object: A high risk of spontaneous abortion was observed in women exposed to organic solvents during pregnancy. Since this risk was not found in the shoe industry, where these solvents are widely used, we carried out a case-control study on the risk of spontaneous abortion in a health district (Veneto, Northern Italy) where about 8,000 people work in shoe manufacturing. Aliphatic hydrocarbons were generally used; their concentrations were repeatedly below the mixture TLVs in the observation period. Methods: Cases (clinically recognized spontaneous abortion, ICD codes 632-634-636) and age-/year-/residence-matched controls (admitted for normal delivery) were traced in the files of the regional hospital discharges register. Data on 108 cases (81% response) and the same number of reference subjects were collected on questionnaires completed by nurses trained in occupational medicine. There were questions on confounding and occupational factors, and an open question to ensure a complete description of work done during pregnancy. An occupational physician, working blind, then coded exposure to organic solvents according to a three-level polytomous variable (no, low, high exposure). Results: The cases/controls not exposed, exposed to low levels and exposed to high levels of organic solvents were 78/88, 12/12, and 18/8 respectively. Adjusted for the confounding factors, the relative risk (RR) of spontaneous abortion for high exposure to organic solvents

G. Mastrangelo ()

Fax: (39) 49 821 66 21 E-mail: op14@ipdunidx.unipid.it during pregnancy was 3.85, with 95% confidence intervals (CI) ranging from 1.24 to 11.9. RR was 1.58 (CI = 0.62-4.06) in women exposed to low solvent concentrations. *Conclusions*: Our results support the hypothesis that spontaneous abortion may be an adverse effect of exposure to high levels of organic aliphatic solvents in women employed in shoe manufacture.

Key words Abortion · Epidemiology · Case-control studies · Solvents · Shoes · Industry

Introduction

An excessive risk of spontaneous abortion has been found in pregnant women employed in several occupations in the course of which organic solvents are widely used: laboratory work [2, 15]; painting [9]; dry cleaning [13, 15]; pharmaceutical industry [22]; plastic and rubber industries [5]; chemical industry [10, 15]; manufacturing industry [18]; and semiconductor factories [20]. Furthermore, detailed studies have shown this risk to be significantly higher in occupations involving exposure to particular solvents; perchloroethylene, trichloroethylene and other aliphatic solvents [26]; aliphatic solvents, particularly in the graphics industry, and toluene, particularly in the shoe-making industry [16]; and finally toluene and xylene in laboratories, research centres and the pharmaceutical industry [22, 23].

In women employed in the shoe and leather industry, where exposure to solvents contained in glues is widespread, other authors did not find an increased risk of spontaneous abortion. Rather, Clarke and Mason [3] reported an increased risk of perinatal mortality, in particular congenital malformations (trisomy 18); and in two further studies McDonald's group [17, 18] confirmed the association with perinatal mortality but not with congenital defects. On the other hand, in a prospective study carried out in a Swedish

R. Agnesi · F. Valentini

Regione Veneto, ULSS 13 Dipartimento di Prevenzione Servizio Prevenzione Igiene e Sicurezza in Ambienti di Lavoro Via. S. Pio X 8, I-30031 Dolo, Italy

Università degli Studi di Padova, Istituto di Medicina del Lavoro Via Facciolati 71, I-35127 Padova (Italy)

Table 1 Threshold limit values(TLV), and averageconcentrations (mg/m^3) ofdifferent solvents found in 31shoe factories in repeatedindustrial hygiene campaigns.(Modified from [25])

	TLV	1982–85 Samples = 68	1989 Samples = 30	1992 Samples =10
<i>n</i> -Hexane	176	223	75	76
Cyclohexane	1,030	300	182	95
Ethylacetate	1,440	74	95	43
Methylethylketone	590	67	57	41
Acetone	2,380	-	31	28
Heptane	400	44	27	90
Methylcyclohexane	1,610	65	30	120
Methylcyclopentane	_	77	54	15
2-Methylhexane	_	12	15	13
3-Methylhexane	-	27	16	18
2-Methylpentane	-	86	32	41
3-Methylpentane	-	90	29	35

county, Ahlborg et al. [1] did not find an elevated incidence of spontaneous abortion in women exposed to concentrations of organic solvents below the threshold limit values (TLV) in shoe industries, chemical and microelectronics industries, or hospital laboratories. These conclusions were confirmed in a recent prospective study in Southern Australia, where domestic exposure to solvents and glues was more relevant to spontaneous abortion than occupational exposure, which is very tightly regulated [6].

The conflicting data in the literature led to a further study to evaluate the association between spontaneous abortion and exposure to organic solvents in the shoe industry. We carried out a case-control study in a health district surrounding the city of Dolo (between Padua and Venice in north-eastern Italy), where nearly 400 shoe factories employed about 8,000 workers including many women of child-bearing age. Since the 1970s, when neurotoxicity of n-hexane came to the public's attention, the local Occupational Health Authority of the National Health Service (Servizio Prevenzione Igiene Sicurezza Ambienti di Lavoro, SPISAL) has kept the occupational exposure to organic solvents under control in the shoe factories of the area. The majority of these workplaces have an adequate air exhaust system at the glueing stations. In 1987-1988, solvent mixtures mostly contained ethylacetate, cyclohexane, methylethylketone, and hexane isomers (up to 40% *n*-hexane); at that time, repeated sample investigations showed environmental concentrations of the compounds in the range of 15-70% of the mixture TLVs where glues were used and negligible concentrations in the surrounding environment. The average concentrations in 1989 are shown in Table 1, with the previous and the current values of airborne solvents found in samples of our shoe factories. Although exposure to organic solvents has been prohibited for pregnant women in Italy since 1976 (Act no. 1026/1976), this law was frequently transgressed in the past, whereas it has been widely applied in recent years. For these reasons, we selected the cases of spontaneous abortions and controls from the period 1987–1988.

Subjects and methods

A total of 133 cases of clinically diagnosed spontaneous abortion (IX revision ICD codes 632–634–636) discharged from Dolo Hospital from 1987 to 1988 were found in the Regional Archives of Hospital Discharges. For each case, a control subject (woman discharged from Dolo Hospital following normal childbirth) was selected from the same source, matched to the case for year of birth, city of residence and calendar year of hospital admission. Despite many attempts 6 cases were not traced and another 19 refused to take part when asked to give informed consent prior to the study. The corresponding controls were excluded from the study, which therefore involved 108 cases and 108 controls, a 81% response rate.

Both cases and controls were interviewed using a standard questionnaire. There were questions on marital status (married, single, separated, divorced, at the time of event), gravidity (first, second, third or fourth pregnancy), number of previous spontaneous abortions (0, 1, 2 or more) and education (elementary; junior high; professional or high school; college graduate). Other questions sought information about the period of the pregnancy under study: cigarettes smoked a day (0, 1-10, more than 10); daily consumption of caffeine (0, 1, 2, 3, 4 or more cups of coffee a day) and of alcohol. The content of anhydrous alcohol per drink in different beverages was calculated according to published equivalences [11]. As the mean alcohol consumption was around 10 g/day (no woman drank more than 50 g of alcohol per day), the variable was dichotomized (none versus any). Tea and cola consumption were sporadic. There were two free answer questions; the subjects were asked to specify, if possible with the commercial name, all medications taken during pregnancy. They were also asked to describe their jobs during the pregnancy period under study; the interviewer had the option of asking specific questions about machinery or substances used, the time devoted daily to glueing, the type and dimension of the shoe particular being glued, the dimension of the work environment and presence of a local air exhaust system. Since solvent mixtures were generally used, no attempt was made to specify particular solvent in detail. Subsequently, a dichotomous variable was coded - 0 for women who took no medicines or only those prescribed for threat of abortion and 1 for all other; and a polytomous variable was established -0 (nonexposed subjects), 1 (subjects exposed to low levels) or 2 (subjects exposed to higher levels of organic solvents). Low-level exposure is defined as the presence of 2 out of 3 of the following criteria: glueing time less than 2 h/day, glueing of small surfaces rather than shoe soles, use of glue containers with small openings Table 2 Distribution of cases ofspontaneous abortion andcontrols; odds ratio (OR) forunmatched data

		Ca	ses	Cont	rols	
Variable		No.	%	No.	%	OR
Solvent exposure	None	78	72.2	88	81.5	1.00
•	Low	12	11.1	12	11.1	1.13
	High	18	16.7	8	7.4	2.54
Gravidity	1	42	38.9	47	43.5	1.00
	2	39	36.1	36	33.3	1.21
	3	17	15.7	21	19.3	0.91
	≥ 4	10	9.3	4	3.7	2.80
Previous	0	87	80.6	97	89.8	1.00
abortions*	1	18	16.7	11	10.2	1.82
	≥ 2	3	2.8	0	0.0	7.80
Coffee (cups/day)*	≥ 0	29	26.9	43	33.3	1.00
	1	24	22.2	18	16.7	1.98
	2	26	24.1	41	38.0	0.94
	3	21	19.4	5	4.6	6.23
	≥ 4	8	7.4	1	0.9	11.9
Cigarettes/day*	0	94	87.0	102	94.4	1.00
	1 - 10	9	8.3	6	5.6	1.63
	≥ 10	5	4.6	0	0.0	11.9
Alcohol	None	82	75.9	83	76.9	1.00
	Any	26	24.1	25	23.1	1.05
Education	Upto age 14	79	73.1	70	64.8	1.00
	Higher level	29	26.9	38	35.2	0.68
Use of medicines	no	96	88.9	95	88.0	1.00
	yes	12	11.1	13	12.0	0.91
Marital status	Married	96	88.9	107	99.1	1.00
	Single*	9	8.3	0	0.0	21.2
	Separated	2	1.9	1	0.9	1.86
	Divorced	1	0.9	0	0.0	3.34

**p* < 0.05

rather than jars or basins. The interviews were carried out in the course of 1989 by two female professional nurses employed by SPISAL; medicine consumption and solvent exposure were scored blindly by an occupational physician employed by SPISAL in 1995, on the basis of the answers on the questionnaire with no knowledge of the case/control status of the subject under study.

The odd ratios (OR), the chi-square test for 2×2 tables (χ^2), and the chi-square test for trend (χ^2_{rend}) for tables $2 \times k$ were calculated using the usual methods for unmatched data. Before calculating both OR and χ^2 , we added 0.5 to all frequencies in 2×2 tables whenever a cell was empty. Furthermore, the relative risk (RR) of spontaneous abortion due to solvent exposure, the 95% confidence intervals (CI), and the *F* test to enter terms were estimated by means of the stepwise multiple conditional logistic regression for matched case-control studies. These estimates were adjusted for the confounding variables: gravidity, previous abortions, level of education, smoking habits, consumption of alcohol, coffee and medicines, and marital status. Statistical analysis was carried out with the BMDP package [4].

Results

Table 2 shows the distribution of cases and controls at every level of the variables considered, and the OR

estimates based on unmatched data. Out of 50 women exposed to organic solvents, 47 worked in shoemaking and three in a purse and leather goods factory. OR (equal to 1.00 in women not exposed to solvents) becomes 1.1 in the exposure to low levels of solvent and increases to 2.54 in the exposure to the higher concentrations; both excesses, however, failed to reach statistical significance. A significantly higher risk of spontaneous abortion was observed in single than in married women ($\chi^2 = 6.84$, P < 0.01); and a significant tendency to an increased risk was found with increasing smoking habits ($\chi^2_{trend} = 4.46$; P < 0.05), coffee consumption ($\chi^2_{trend} = 8.67$; P < 0.01), and number of previous abortions ($\chi^2_{trend} = 4.06$; P < 0.05).

Table 3 shows the RRs of spontaneous abortion obtained by the conditional logistic regression for matched data. In this model, cigarette, coffee and alcohol consumption, gravidity and number of previous abortion were considered as interval variables; education and use of medicines, coded as 0/1 variables according to the definition shown in Table 2, and marital status (married vs unmarried) and solvent exposure **Table 3** Stepwise logistical regression for matched case-control studies: risk factors of spontaneous abortion (*Terms*), *F* to enter terms and degrees of freedom (*df*), relative risk (*RR*) and 95% confidence intervals (*CI*)

Terms		F to enter	df	df	RR	CI
Coffee intake (cups/day)	Step 1 Step 2	8.14*	1	107	1.37	1.08-1.73*
Number of previous abortions Coffee intake (cups/day)	Step 2 Step 3	4.54*	1	106	2.19 1.36	1.00-4.82* 1.07-1.73*
Solvent exposure: low versus none high versus none Number of previous abortions Coffee intake (cups/day)	Step 5	3.37*	2	104	1.58 3.85 2.50 1.36	0.62–4.06 1.24–11.9* 1.05–1.73* 1.06–1.76*

* P < 0.05

were categorical variables. Positive trends were observed with coffee intake and number of previous abortion. The risk of spontaneous abortion is 2.50 or 1.36 times the number of previous abortions, or cups of coffee drunk during pregnancy, respectively. A significant influence is also shown by solvent exposure, which was a polytomous variable with three levels (0, 1, 2 res)pectively for no, low-level and high-level organic solvent exposure). Compared with nonexposed women, those with a high exposure to organic solvents have a significant increase in index abortion, with the confounding factors of coffee intake and previous abortion held constant. The cumulative risk of spontaneous abortion is equal to the product of the single risk shown in Table 3. Therefore, the risk is estimated to be around 10 (3.85×2.50) in mothers exposed to a high level of organic solvents and at same time with a history of one spontaneous abortion; it rises further if they drank coffee during the index pregnancy. The occupational exposure to organic solvents shows a tendency to an increased risk of spontaneous abortion even at lower concentrations; the corresponding RR (1.6), however, with CI including unity (0.6-4.1), is also compatible with coincidence. In Table 2, the risk of fetal death was high in single women; but when they were pooled with the separated or divorced women in a dichotomous variable, marital status was no longer significant when this risk was adjusted for coffee intake, previous abortion, and solvent exposure (Table 3). The program did not enter the cigarette consumption and other non-significant variables in the regression equation.

Table 4 shows the distribution of cases and references according to whether they worked in shoemaking or not. The OR of abortion, 1.67, is not statistically significant ($\chi^2 = 2.53$; P = 0.11).

Table 5 shows another model of stepwise logistic regression, in which all variables of Table 3 except solvent exposure are used. The occupational risk indicator is the dichotomous variable "occupation in shoe manufacture". The RRs for coffee intake and history of

 Table 4 Distribution of cases and controls according to maternal occupation (shoemakers vs other occupations)

	Cases	Controls	Total
Shoemaker Other occupations	31 77	21 87	52 164
Total	108	108	216

abortion have the same numerical values as in the first two steps of Table 3. In the third step the occupational variable entered in equation, as its "F to enter" value is slightly above the level of significance (P = 0.0453). When the corresponding RR was adjusted for coffee consumption and previous abortion, the 95% CI of RR, ranging from 0.99 to 4.12, suggested only a borderline significance of the association ($P \sim 0.03$) between maternal exposure in shoe manufacture and spontaneous abortion.

Discussion

As recently reported, epidemiological reproductive studies present several methodological weaknesses: misclassification of outcome and exposure, small sample size, selection and confounding bias [14]. The response rate in this study, more than 80%, was fairly similar to that in most other studies [14]. Data on outcome were collected from hospital records, which are considered a reliable data source [14]. Few investigations have used objective data in the exposure assessment [14]. In our study information on solvent exposure is based on interviews of cases and controls carried out by occupational health care personnel; exposure assessment, moreover, was blindly established by an industrial hygienist; the nurses who conducted interviews were no longer working at SPISAL at the time. Lastly, potential confounding factors were controlled by either matching in the design of the study or use of **Table 5** Stepwise logistical regression for matched case-control studies: risk factors of spontaneous abortion (*Terms*), *F* to enter terms and degrees of freedom (*df*), relative risk (*RR*) and 95% confidence intervals (*CI*)

Terms		F to enter	df	df	RR	CI
	Step 1					
Coffee intake (cups/day)	-	8.14*	1	107	1.37	1.08-1.73*
	Step 2					
Number of previous abortions	1	4.54*	1	106	2.19	1.00 - 4.82*
Coffee intake (cups/day)					1.36	1.07-1.73*
	Step 3					
Occupations as shoemaker	-	4.10*	1	105	2.02	0.99-4.12*
Number of previous abortions					2.40	1.09-5.28*
Coffee intake (cups/day)					1.36	1.07 - 1.76*

* P < 0.05

multivariate methods in the statistical analysis. Unfortunately, there were no items in the questionnaire on hobbies or home exposure to solvents, which have recently been reported to entail a risk of spontaneous abortion [6]. In our study, however, there would be a confounding bias only in the unlikely event that domestic exposure was associated with occupational exposure to high levels of solvents. The marital status was included among the confounding variables following an early report by Murphy et al. in 1984 [19]. Similar findings were subsequently reported in Greece [24], Japan, Cuba and Sweden [7]. The excess fetal death cannot be explained by maternal age, parity, or even the fact that unmarried mothers often smoke more than the rest of the population [8, 12]. A Norwegian birth registry analysis showed that unmarried status conferred a major disadvantage even in Scandinavia, where the unmarried status was relatively common and thought not to imply social disadvantage [21]. In our hospital-based case-control study, unmarried status could not be considered an indicator of induced illegal abortion, because the latter has a different ICD code.

In our study a high level of organic solvents was the risk factor for spontaneous abortion (Table 3). The finding agrees with the findings of many authors. Windham et al. [26] collected indirect measurements of the intensity of exposure from women exposed to solvents: reporting of odours, skin contact or symptoms (versus none), presence of ventilation; wearing of masks or respirators during solvent handling. For most solvents, the ORs for spontaneous abortion were higher among women reporting a more intense exposure. Lindbohm [16] distinguished three exposure levels: high, low and none, assessed on the basis of the reported frequency of the use of solvents and the available information on typical levels of exposure in that particular job. In this study, the OR of spontaneous abortion for high exposure to aliphatic hydrocarbons during pregnancy was 3.9, the same risk estimate as observed in our women working in shoe manufacture (Table 3), who were mainly exposed to ethylacetate, methylethylketone, hexane isomers, and cyclohexane. Taskinen et al. [22] classified the exposure as rare if the chemical

was handled on 1 or 2 days a week and frequent if the chemical was handled at least 3 days a week. The risks of spontaneous abortion were elevated in women who were more frequently exposed to toluene, xylene, or formalin, and in women with high overall solvent exposure (formalin excluded). In all the above studies, women exposed to low levels of solvents have a risk of spontaneous abortion not significantly different than that of women who have never been exposed. These findings suggest, on the one hand, a "cumulative exposure" model, according to which repair of or compensation for damage may be possible with lower exposure levels, whereas higher probabilities of adverse outcomes result from the accumulation of irreversible changes. On the other, they also suggest a threshold effect, even though assessment of exposure is always based on anamnestic information. Thorough hygiene studies in our shoe factories demonstrated that organic solvent exposures, and also most of the "high" exposures, were below the TLVs at the time of epidemiological study. Therefore, the threshold dose may be lower than the current TLVs, which are established on the basis of acute irritating effects. Since 1988, the TLVs for hexane isomers, *n*-hexane, cyclohexane, ethylacetate and methylethylketone have not been changed.

In our study, employment of expectant mothers in the shoe industry, as against exposure to high levels of organic solvent, did not have any marked effect on the pregnancy outcome (Tables 4, 5). Similarly, Clarke and Mason [3] and McDonald [17]and McDonald and McDonald et al. [18], who used occupation in shoe/leather industry as their surrogate risk factor, also did not find an increased risk of spontaneous abortion in these workers. Furthermore, Ahlborg et al. [1] and Ford et al. [6], who used a dichotomous variable to assess maternal exposure to organic solvents, stated that such exposure was not associated with an increased risk of spontaneous abortion. As occurrence of irreversible changes is dependent upon level of exposure, the split of a variable into two categories (exposed vs not exposed) may have led to the inclusion of women with minimal solvent exposure, diluting the risk of abortion in the two latter studies.

References

- 1. Ahlborg G, Hogstedt C, Bodin L, Barany S (1989) Pregnancy outcome among working women. Scand J Work Environ Health 15:227-233
- Axelsson G, Lutz C, Rylander R (1984) Exposure to solvents and outcome of pregnancy in university laboratory employees. Brit J Ind Med 41:305–312
- 3. Clarke M, Mason ES (1985) Leatherwork: a possible hazard to reproduction. Brit Med J 290:1235–1237
- Dixon WJ (1992) BMDP Statistical Software Manual. University Press of California, Los Angeles
- Figà-Talamanca I (1984) Spontaneous abortions among female industrial workers. Int Arch Occup Environ Health 545: 163–171
- Ford JH, MacCormac L, Hiller J (1984) PALS (pregnancy and lifestyle study): association between occupational and environmental exposure to chemicals and reproductive outcome. Mutation Research 313:153–164
- 7. Golding J (1990) The third report of the WHO study of social and biological effects on perinatal mortality. University of Bristol, Bristol
- Golding J, Henriques J, Thomas P (1987) Unmarried at delivery, II. Perinatal morbidity and mortality. Early Hum Dev, 14:217–225
- Heidham LZ (1984) Spontaneous abortion among dental assistants, factory workers, painters and gardening workers: a followup study. J Epidemiol Community Health 38:149–155
- Hemminki K, Franssila E, Vainio H (1980) Spontaneous abortions among female chemical workers in Finland. Int Arch Occup Environ Health 45:123–126
- International Agency for Research on Cancer (1988) Alcohol drinking. (IARC monographs on the evaluation of carcinogenic risks to humans, vol 44) IARC, Lyon
- 12. Kiely M (1991) Reproductive and perinatal epidemiology. CRC Press, Boca Raton, Fl
- Kyyronen P, Taskinen H, Lindbohom ML, Hemminiki K, Heinonen OP (1989) Spontaneous abortions and congenital malformations among women exposed to tetrachloroethylene in dry cleaning. J Epidemiol Community Health 43:346–351

- Lindbohom ML (1995) Effects of parental exposure to solvents on pregnancy outcome. JOEM 37:908–914
- Lindbohom ML, Hemminki K, Kyyronen P (1984) Parental occupational exposure and spontaneous abortion in Finland. Am J Epidemiol 120:370–378
- Lindbohom ML, Taskinene H, Salimén M, Hemminki K (1990) Spontaneous abortions among women exposed to organic solvents. Am J Ind Med 17:449–463
- McDonald AD, McDonald JC (1986) Outcome of pregnancy in leatherworkers. BMJ J 292:979–981
- McDonald AD, McDonald JC, Armstrong B, Cherry NM, Coté R, Lavoie J, Nolin AD, Robert D (1988) Fetal death and work in pregnancy. Br J Ind Med 45:148–157
- Murphy JF, Dauncey M, Newcombe R, Garcia J, Elbourne D (1984) Employment in pregnancy: prevalence, maternal characteristics, perinatal outcome. Lancet I: 1163–1166
- Pastides H, Calabrese EJ, Hosmer DW, Harris DR (1988) Spontaneous abortion and general illness symptoms among semiconductor manufacturers. J Occup Med 30:543–551
- 21. Skjaerven R, Irgens LM (1988) Perinatal mortality and mother's marital status at birth in subsequent siblings. Early Hum Dev 18:199–214
- 22. Taskinen K, Lindbohm ML, Hemminki K (1986) Spontaneous abortions among women working in the pharmaceutical industry. Br J Ind Med 43:199–205
- Taskinen H, Kyyronen P, Hemminki K, Hoikkala M, Lajunen K, Lindbohm ML (1994) Laboratory work and pregnancy outcome. J Occup Med 36:311–319
- Tzoumaka-Bakoula C, Lekea-Karanika V, Matsaniotis N, Golding J (1989) The Greek national perinatal survey. Paediatr Perinat Epidemiol 3:43–51, 1989
- 25. Valentini A, Agnesi R, Dal Vecchio L, Di Tommaso A (1993) Modificazioni della composizione dei collanti utilizzati nel comparto calzaturiero: non più polineuropatie? In: 56° Congresso Nazionale Società Italina di Medicina del Lavoro e Igiene Industriale; II; 1169–72. SGE Editoriali, Padova
- 26. Windham GC, Shusterman D, Swan SH, Fenster L, Eskenazi B (1991) Exposure to organic solvents and adverse pregnancy outcome. Am J Ind Med 20:241–259