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Effects of Occupational Exposure to Low-Level Carbon Disulfide (CS₂) on Menstruation and Pregnancy*

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Abstract: In a retrospective cohort study, 265 female workers exposed to CS₂ and 291 non-exposed female workers, as well as 530 pregnancies were investigated focusing on the status of menstruation and the term and outcome of pregnancy. The average CS₂ concentration to which the workers had been exposed for the 15 years prior to the study was 1.7–14.8 mg/m³. The result showed that exposed female workers had a higher incidence rate of menstrual disturbance than the non-exposed women (35.9% vs. 18.2%, RR=2.0, $p<0.01$), and an exposure-response relationship between the CS₂ level and the incidence rate of menstrual disturbance was revealed. No significant difference was found between the exposed and non-exposed groups in terms of rates of toxemia, emesis gravidarum, spontaneous abortion, stillbirth, premature and overdue delivery, or congenital malformation. The birth weights for the two groups were similar. The possible effect of confounding factors was controlled during both the survey and data analysis. These results show that exposure to CS₂ at around 10 mg/m³ might affect the function of the female reproductive system.

Key words: Carbon disulfide—Menstruation—Pregnancy

INTRODUCTION

Existing data indicate that certain types of dysfunction in the female reproductive system might be associated with long-term and relatively high levels of exposure to carbon disulfide (CS₂).^{1–4} However, few comprehensive studies on reproductive

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disorders resulting from exposure to low levels of CS₂ have been documented so far. Therefore, in order to clarify the possible deleterious effects of low-level exposure to CS₂ on menstruation and pregnancy, we conducted a retrospective cohort study on female workers from five viscose rayon plants and a reference factory.

MATERIALS AND METHODS

SUBJECTS

Menstruation A survey on menstruation was restricted to subjects who presented no menstrual disorder at the beginning of exposure to CS₂ (work). The survey included 265 women who had been exposed to CS₂ since 1964 or later for at least 1 year by the end of 1985 at five viscose rayon plants in Shanghai (plant Nos. 1, 3, 4, 5 and 12). Another 291 age-matched non-exposed female workers from a thread factory were chosen as the control group. All of these plants were operating on a shift-work schedule, and both the working conditions (e.g., temperature, noise) and work load were comparable. The following information was obtained from

Table 1. Comparison of background factors between the two groups

Factors	Exposed (n=265)		Non-exposed (n=291)	
	n	rate (%)	n	rate (%)
1. Education below senior high school	248	93.6	281	96.6
2. Age at starting work (years)				
15-19	101	38.1**	191	65.6
20-29	135	50.9	94	32.3
30-39	27	10.2	0	0.0
≥40	2	0.8	6	2.1
3. Exposure duration ^a (years)				
1-4	104	39.4**	70	24.1
5-9	88	33.3	84	28.9
≥10	72	27.3	137	47.1
4. Born. mother	180	68.3**	154	52.9
5. Using birth control ^b	128	48.3**	103	35.4
6. Change in living environment	2	0.8	5	1.7
7. Use of drugs	0	0.0	2	0.7
8. Disease	2	0.8	4	1.4
9. Surgery	1	0.4	3	1.0
10. Exposure to other chemicals	12	4.5	7	2.4

** p<0.01

^a For control, work duration, one of the exposed women failed to remember the exact duration of exposure.

^b Includes every type of birth control method, among which 96.9% of exposed women and 95.1% of non-exposed women used IUD.
items 5-10 were all concerned with the status before the menstrual disorder occurred.

each worker by an interviewer: occupational history (job types, type of chemical exposure and exposure duration), menstrual history (age at menarche, days of menstrual cycle, duration and quantity of menses and symptoms during menstruation) and factors that might affect menstruation, such as age, reproductive history and methods of birth control employed, changes in living conditions (such as a change of residential area), previous diseases, drugs taken etc. The background information for the two groups is shown in Table 1. Any menstrual abnormalities were diagnosed by the interviewer based on the following documented criteria described in a textbook of clinical gynecology. Workers who manifested any one of the following symptoms for a continuous period of at least 3 months were considered to have suffered from menstruation disorder.

Suppression of menses: Women who had reached the age of 18 years or more without experiencing menarche, or those who had experienced cessation of menses for over 3 months.

Menorrhagia: Women with notable abdominal pain during menstruation, requiring the help of pain-killers.

Menstrual irregularity: Women in whom the menstrual cycle was shorter or longer by 7 days than the normal range of 28–30 days.

Abnormal duration: Women in whom the duration of menstruation was shorter than 3 or longer than 7 days.

Abnormal quantity: Women for whom the consumption of sanitary napkins needed during each menstrual period was either less than 0.5 or more than 3 packages.

The course of menstrual disorder among different sufferers may vary greatly. The prevalence at a definite time may not have well represented the overall incidence of disorder in the investigated populations over the length of the observation period. Accordingly, the accumulated incidence was introduced into the analysis. Women who had suffered from a menstrual disorder after exposure (work) were considered to represent cases of incidence.

$$\text{Accumulated incidence} = \frac{\text{no. of cases of menstrual disturbance occurring after exposure}}{\text{no. of subjects without menstrual disturbance before exposure (work)}}$$

Term and outcome of pregnancy For all married women, the reproductive history after starting work (exposure) was investigated. There were a total of 530 pregnancies during the exposed (working) period, comprising 253 exposed and 277 non-exposed pregnancies (induced abortions were not included). Details of any complications during the term of pregnancy as well as the outcome of pregnancy were sought individually. The main indices were toxemia, emesis gravidarum, spontaneous abortion, stillbirth, premature and overdue deliveries, congenital malformation and birth weight. Factors that might have affected the term and outcome of pregnancy, such as age, lifestyle, disease, drugs taken, genetic

background and nutrition status (judged by dietary habit during pregnancy and the monthly income of the family, being divided into 3 degrees: good, intermediate and bad) during pregnancy were also listed (Table 2). The definitions of diagnostic criteria were as follows:

Toxemia: Blood pressure over 130/90 mmHg, appearance of proteinuria, or edema of over second degree during the fifth–ninth month of pregnancy.

Table 2. The main background factors in the two groups of women who had experienced pregnancy

Factors	Exposed	Non-exposed
Female workers:		
1. Han race	100.0%	100.0%
2. Age at pregnancy <30 years	83.4%	84.1%
3. Primipara	71.2%**	82.0%
4. Nutrition status during pregnancy		
Good	21.3%**	38.3%
Intermediate	60.5%	44.4%
Bad	18.2%	17.3%
5. X-rayed during pregnancy	2.4%	3.3%
6. X-rayed within the first 3 months of pregnancy	0.4%	1.8%
7. Disease during pregnancy	11.1%	14.5%
8. Disease within the first 3 months of pregnancy	5.9%	5.8%
9. Drugs taken during pregnancy	4.4%	2.9%
10. Drugs taken within the first 3 months of pregnancy	1.2%	1.4%
11. Smoking during pregnancy	0.0%	0.0%
12. Drinking during pregnancy	0.0%	0.4%
13. Exposure to other chemical	2.0%	0.4%
14. Congenital and inheritable diseases	0.0%	0.4%
15. Family history of congenital and inheritable diseases	0.0%	1.1%
16. Consanguineous marriage	0.8%	0.4%
Husband		
17. Han race	100.0%	99.6%
18. Exposure to other chemicals	2.7%	2.2%
19. Drinking	12.3%	10.8%
20. Smoking (packs)		
0	55.3%	55.6%
<0.5	24.1%	26.4%
<1	20.6%	17.0%
≥1	0.0%	0.4%
21. Congenital and inheritable disease	0.0%	0.4%
22. Family history of congenital and inheritable disease	0.4%	1.1%

* $p < 0.05$

** $p < 0.01$

Emesis gravidarum: Anorexia or vomiting occurring during months 1–4 of pregnancy necessitating absence from work.

Premature delivery: Delivery occurring 3 weeks before the predicted delivery date with a baby birth weight of less than 2500 g.

Overdue delivery: Delivery taking place 2 weeks after predicted delivery date.

The identification of spontaneous abortion, stillbirth and congenital malformation was established according to the clinical diagnosis made by the gynecologist at the hospital referred to.

The rates of occurrence of toxemia, emesis, spontaneous abortion and stillbirth were expressed as percentages of cases among total pregnancies, while rates of occurrence of premature delivery, overdue delivery and malformation were defined as percentages of cases affected among the total of living births.

EXPOSURE

A standardized regimen of environmental monitoring has been set for all viscose rayon plants in Shanghai since 1970. Air samples from various worksites of the plants were taken and analyzed once a week using the diethylamine-copper acetate method⁵⁾ by the Department of Industrial Hygiene at each plant. Based on these recorded data, the monthly median concentration of CS₂ in the environment was calculated for each plant and the average yearly concentrations were obtained.

In order to compare the real exposure level of workers and the fixed-site environmental concentration of CS₂, a parallel sampling study was conducted in plant No. 12. Air samples from every worksite were taken and analyzed twice per 8-h shift for two shifts. Data obtained from 153 air samples were calculated both in terms of fixed-site concentration and time-weighted average (TWA). Based on the time spent at each worksite judged by on-site inspection, the time-weighted average (TWA) concentration of CS₂ exposure (mg/m³) was computed according to the following formula:

$$\text{TWA (mg/m}^3\text{)} = \frac{c_1 t_1 + c_2 t_2 + \dots + c_i t_i}{8}$$

c_i : CS₂ concentration in the air of the worksite i

t_i : time spent by the worker at worksite i per 8-h shift

STATISTICAL ANALYSIS

t-test, chi-square test, Mantel-Haenszel chi-square and expanded Mantel-Haenszel chi-square tests, multiple linear regression as well as the COX model were applied. During the COX model analysis, years of exposure (work) for each worker were regarded as worker person-years.

QUALITY INSURANCE OF THE STUDY

Design of the questionnaire form and selection of the diagnostic criteria More than one question was required to reflect the same item investigated. Subjects

were asked the question again if the answer was incomplete or unclear. Interviewees were asked only to recall the symptoms and the relevant date when the symptoms occurred. The diagnosis and the relation between any menstrual disorder and CS₂ exposure were evaluated by the interviewer based on the critical criteria. In this way, possible bias was considered to be diminished to a certain extent.

Training of the interviewers Three interviewers were included in this survey. They were all medical workers and had been well trained before the investigation.

Checking of the investigation form Each form was checked by the designer of the form and reinquiry was conducted if necessary.

Table 3. CS₂ concentration (mg/m³) at workplace in various plants

Year	Plants				
	No. 12	No. 3	No. 1	No. 4	No. 5
70-74	30.6 (50%)	10.5 (31%)	14.5 (33%)	5.9 (25%)	
75-79	12.2 (40%)	9.0 (33%)	6.3 (22%)	7.9 (21%)	2.1 (4%)
80-85	4.7 (21%)	3.5 (10%)	3.3 (15%)	0.7 (4%)	1.5 (3%)
Average	14.8	7.0	6.1	4.5	1.7

Each number (%) in parentheses is:

those above MAC of 10 mg/m³
total determinations

Table 4. Comparison of CS₂ concentration (mg/m³) by fixed-site sampling and TWA 8-h

Job classification	No. of samples	Fixed site conc.	TWA 8-h
Sulfur dissolution	8	29.5	6.2
fiber-bundling	15	27.3	6.1
Carbon addition	2	23.9	3.5
Filtering	12	22.5	8.4
Sulfur precipitation	8	20.9	5.8
Fiber-cutting	8	14.6	5.4
Viscose-aging	8	11.9	10.7
Rayon-refining	24	8.3	3.5
Sulfur addition	4	8.1	1.0
Fiber-spinning	16	6.4	4.7
Viscose dissolution	8	4.0	3.5
Carbon-baking	8	3.4	0.3
Acidification	8	3.1	1.8
Sulfur-refining	8	1.9	0.7
Xanthation	12	0.9	0.8
Measuring	4	0.8	0.7
Average	153	11.7	3.9

RESULT

Concentration of CS₂ in the air

As shown in Table 3, the average CS₂ concentration in the early years of the 1970s was mostly above 10 mg/m³ (the present MAC adopted in China). The level of CS₂ has decreased gradually since 1975, and the average level (median) has been below 10 mg/m³ throughout the 1980s. Among the factories surveyed, Plant no. 12 had the highest concentration and no. 5 the lowest.

Since the time of workers spent at operation worksites is usually much shorter than that of workers in control rooms, the TWA 8-h values of CS₂ at 16 worksites were all lower than that of the fixed-site concentration. However, both types of value tend to show a similar distribution pattern among different worksites (Table 4). The linear regression equation is expressed as: $Y = 3.2353 + 2.1508X$ ($r = 0.6542$), where Y is the CS₂ concentration for fixed-site sampling and X is the TWA 8-h concentration.

MENSTRUAL DISTURBANCE

The incidence rate of menstrual disturbance in the exposed group was significantly higher than that in the non-exposed group with a relative risk (RR) of 2.0 (Table 5). Irregularity of menstruation was the most common disturbance, followed by abnormal quantity of breeding. Both were significantly higher in the exposed group than in the non-exposed group (Table 6).

Based on the CS₂ concentration in the work environment, the five plants were grouped into "low contamination" with an average CS₂ concentration of 3.1 mg/m³ which included Plant Nos. 4 and 5; "intermediate contamination" at 6.5 mg/m³

Table 5. Incidence of menstrual disturbance

Group	No. of Subjects	Cases	Rate (%)	RR
Exposed	265	95	35.9**	2.0
Non-exposed	291	53	18.2	

** $p < 0.01$

Table 6. Type of menstrual disturbance

Symptoms	Exposed (n=265)		Non-exposed (n=291)	
	No.	rate (%)	No.	rate (%)
Menstrual disturbance	95	34.9**	53	18.2
Suppressed	5	1.9	4	1.4
Painful	13	4.9	8	2.8
Irregularity of cycle	55	20.8**	23	7.9
Abnormal duration	25	9.4	20	6.9
Unusual bleeding	43	16.2**	19	6.5

** $p < 0.01$

which included Plant Nos. 1 and 3; and "high contamination" at 14.8 mg/m³ for Plant No. 12. An association between the exposure level and the incidence of overall menstrual disorders as well as some of the specific symptoms of menstrual

Table 7. Relationship between menstrual disturbance & exposure level

Symptoms	CS ₂ concentration (mg/m ³)			
	0.0	3.1	6.5	14.8
No. of subjects	291	134	66	65
Abnormal cases	53 (15.2%)	43 (32.1%)	23 (34.9%)	29 (44.6%)
Suppressed	4 (1.4%)	0 (0.0%)	2 (3.0%)	3 (4.6%)
Painful	8 (2.8%)	2 (1.5%)	2 (3.0%)	9 (13.9%)
Irregular cycle**	23 (7.9%)	22 (16.4%)	12 (18.2%)	21 (32.3%)
Abnormal duration	20 (6.9%)	14 (14.5%)	5 (7.6%)	6 (9.2%)
Unusual bleeding**	19 (6.5%)	17 (12.7%)	15 (22.7%)	11 (16.9%)

** $p < 0.01$, $p < 0.05$ ($2 \times c$ linear regression).

Table 8. Incidence of menstrual disturbance by stratified analysis

Stratum		Group	Total	Cases	Rate (%)	RR
Reproduction	yes	Exposed	180	55	30.5	2.0**
		Non-exposed	154	27	17.5	
	no	Exposed	84	39	46.4	
		Non-exposed	137	26	20.0	
Birth control	yes	Exposed	128	49	38.3	1.9**
		Non-exposed	103	21	20.4	
	no	Exposed	137	46	33.6	
		Non-exposed	188	32	17.0	
Age (years)	10-19	Exposed	100	12	12.0	2.2**
		Non-exposed	197	7	3.6	
	20-29	Exposed	220	62	28.2	
		Non-exposed	283	31	12.3	
	30-39	Exposed	104	14	13.6	
		Non-exposed	115	14	12.2	
	≥ 40	Exposed	20	6	30.0	
		Non-exposed	8	1	12.5	
Exposure duration (years)	1-4	Exposed	264	47	17.8	2.7**
		Non-exposed	291	19	6.5	
	5-9	Exposed	160	33	20.6	
		Non-exposed	221	8	3.6	
	10-14	Exposed	72	9	12.5	
		Non-exposed	137	18	13.1	
	≥ 15	Exposed	16	5	31.3	
		Non-exposed	78	8	10.3	

** $p < 0.01$ (Mantel-Haenszel chi-square test).

One of the women with abnormal menstruation could not remember when her menstruation began. Also, with regard to the relation of disorder with age, reproduction and exposure duration for this subject, data were omitted from this analysis.

Table 9. Result of COX model analysis

Variable	B	Exp. (B)	SE (B)	B/SE	P
CS ₂ concentration (mg/m ³)	0.3901	1.48	0.0710	5.4917	0.001**
Reproductive status	-1.8676	0.15	0.2615	-7.1408	0.001**
Birth control status	0.8515	2.34	0.2426	3.5106	0.001**
Age (year)	0.0521	1.05	0.0214	2.4394	0.05*

**, * There was a significant relationship between the incidence rate of menstrual disturbance and the variables considered.

disturbance was revealed (Table 7).

Mantel-Haenszel and expanded Mantel-Haenszel chi-square tests were used to adjust for inequality of age, observation duration, reproductive and birth control status (Table 8). The final result and the outcome of the COX model analysis still showed that the rate of menstrual disturbance was highly related to the CS₂ level (Table 9).

TERM AND OUTCOME OF PREGNANCY

There was no difference between the comparison groups in terms of toxemia, emesis gravidarum, spontaneous abortion, premature and overdue delivery, or congenital malformation. Most of the RR values were below 1.5 (Table 10). No relationship between these effects and exposure level of CS₂ was found (Table 11). The birth weights of infants in the exposed and non-exposed groups were very similar (3280 g vs. 3205 g).

The pregnancy data were stratified according to the sequential number of pregnancies, age at pregnancy, etc. After the Mantel-Haenszel chi-square analysis (including expanded version), the result showed that there was no difference

Table 10. Comparison of term and outcome of pregnancy between exposed and non-exposed pregnancies

Item	Exposed		Non-exposed		RR
	No.	Rate (%)	No.	Rate (%)	
Total pregnancies	253		277		
Total living births	225		256		
Toxemia	42	16.6	44	15.9	1.1
Emesis gravidarium	28	11.1	25	9.0	1.2
Spontaneous abortion	19	7.5	16	5.8	1.3
Stillbirth	5	2.0	1	0.4	5.7
Premature delivery	4	1.8	5	2.0	0.9
Overdue delivery	14	6.2	11	4.3	1.5
Malformation	5	2.2	5	2.0	1.1

No significant differences in the rates were found between the exposed and non-exposed groups.

Four pregnancies out of each pregnant group had other outcomes such as ectopic pregnancy or hydatid pregnancy.

Table 11. Relationship between term and outcome of pregnancy and exposure level

Item	CS ₂ concentration (mg/m ³)			
	0.0	3.1	6.5	14.8
Toxemia	15.9%	9.0%	18.2%	25.0%
Emesis gravidarum	9.0%	11.0%	10.4%	11.0%
Spontaneous abortion	5.7%	13.0%	2.6%	5.3%
Stillbirth	0.4%	3.0%	1.3%	1.3%
Premature delivery	2.0%	1.2%	2.7%	1.5%
Overdue delivery	4.0%	6.0%	8.2%	3.0%
Malformation	1.9%	3.6%	1.4%	1.5%

No relationships were found between any of the rates and the CS₂ concentration.

Table 12. Multiple regression analysis of infant birth weights

Variable	Partial regression coefficient
No. of pregnancies	0.2514**
Exposure duration (years)	0.0250
Age of pregnancy (years)	-0.0176
CS ₂ concentration (mg/m ³)	-0.0076
Body weight before pregnancy (500 g)	0.0139**
Average income (Yuan/month. person)	0.0071**

** $p < 0.01$.

between the two groups of pregnancies for all the indices considered (Table omitted).

In attempting to identify the possible factors that might affect the birth weight of newborns, multiple linear regression analysis was conducted. The variables covered in the analysis were CS₂ concentration, exposure duration, number of pregnancies, mother's age, body weight before pregnancy and the nutrition status during pregnancy. The result showed that the birth weight was related neither to the exposure level nor to the exposure duration, and was likely to be affected by the mother's body weight, nutrition status and number of pregnancies (Table 12).

DISCUSSION

The level of CS₂ in the air of the working environment is influenced by many factors such as the amount of production, raw materials used, the operation process, the ambient temperature, and the function of the ventilation system used. In Shanghai, as previously reported, the CS₂ concentration at workplaces has been well controlled since the mid-1970s.⁽⁶⁾ The present study also showed that the concentrations of CS₂ at various plants were mostly above 10 mg/m³ in the first half of the 1970s. Afterwards, they decreased gradually and have been kept below

10 mg/m³ in most cases since 1980 (Table 3).

CS₂ is a chemical with multiple toxicity having remarkably adverse effects on both the cardiovascular and nervous systems.⁷⁻⁹⁾ Workers exposed to CS₂ levels as low as 3 ppm have been to manifest retinal microaneurysms and hemorrhages.¹⁰⁾ Liang You-xin also reported that Chinese workers with a CS₂ exposure level of 5.73 (1.9–15.5) mg/m³ showed a decrease of nerve conduction velocity.¹¹⁾ In addition, effects of CS₂ on reproductive function have been reported to be evident not only at high exposure levels, but also at low levels. Female workers exposed to a CS₂ level below 10 mg/m³ for less than 3 years were reported to have a rate of abnormal menstrual bleeding as high as 11.8%, while the rate for controls was only 2.8%.¹⁾ Agard Zhava investigated 177 women exposed to CS₂ levels below 10 mg/m³, and found that 40% of them suffered from abnormal menstruation, which was significantly higher than the figure for non-exposed workers.³⁾ The results obtained from the present study are obviously in agreement with these findings. Female workers with a duration of exposure for mostly less than 10 years (Table 1) to low-level CS₂ (1.7–14.8 mg/m³) had higher risk of menstrual disturbance than non-exposed women (Table 5). The total incidence rate of menstrual disturbance as well as some of the specific symptoms of such disturbance showed a close exposure-response relationship. The most common disturbance was irregularity of menstruation, in accord with the findings reported by previous authors. Furthermore, in our study, factors that might have affected the occurrence of menstrual disorder were taken into account in the statistical analysis. Upon equalizing the confounding effects of age, reproduction history and contraceptive measures used (IUD was used by more than 95% of female workers), a significant difference in the incidence rate of menstrual disorders still existed between the exposed and non-exposed groups.

There is no evidence to show that CS₂ at such a low level exerts deleterious effects on the term and outcome of pregnancy. This negative result is unlikely to have been due to the influence of confounding factors, since most of such factors were equal between the exposed and non-exposed groups (Table 2) and the remaining unequal factors were equalized by appropriate statistical techniques. However, the result did show that effects of low-level CS₂ on the function of the female reproductive system, mainly centering on menstruation status, were apparent. Although the mechanism responsible for the functional disturbance remains obscure, it is presumably due to a derangement of hormonal balance.³⁾

Given the existence of such an association between menstrual disorders and low-level of CS₂ exposure, it is important to consider the safe level of CS₂ at places of work for female workers of reproductive age. Should the set occupational exposure limit for a chemical probably causing reproductive dysfunction in female workers be different from that for male workers? With regard to this issue, experts have already put forward many opinions and views and a WHO expert group (1981) also concluded that there is a reason to recommend a tentative health-based

exposure limit of 3 mg/m³ (TWA 8-h) for women of child-bearing age.¹²⁻¹⁴⁾ The results obtained from our preliminary study basically coincide with the WHO recommendation i.e., that a CS₂ level of 10 mg/m³, the present MAC adopted in China, is unlikely to be safe enough for female workers. In order to avoid the risk of damaging the reproductive system, we would prefer to accept the health-based occupational exposure limit recommended by the WHO as a tentative standard for Chinese female workers of fertile age, although more work needs to be done both in the follow-up survey and in the verification of the recommended standard.

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