

# Effect of Benzene, Toluene, Xylene on the Semen Quality and the Function of Accessory Gonad of Exposed Workers

GuoBing XIAO\*, CuiBao PAN, YaoZhang CAI, Hui LIN and ZhanMing FU

Institute of Occupational Medicine, Ningbo Municipal Centers for Disease Control and Prevention, 37 Yongfeng Rd. Ningbo 315010, P. R. China

*Received August 20, 1999 and accepted February 7, 2001*

**Abstract:** The effects on semen and the function of accessory gonad of workers after short and long term exposure to benzene, toluene, and xylene were examined. The semen and blood of 24 married workers exposed to benzene, toluene, and xylene were collected. Routine sperm characteristic, acrosin activity, and Lactate dehydrogenase C4 (LDH-C4) relative activity were detected. The results showed that benzene, toluene, and xylene were found in the blood and semen of some ex-workers at workplaces where the air concentration of benzene, toluene, and xylene exceeded the maximum allowable concentration (MAC). No such solvents were detected in the blood and semen of workers of the control group. The sperm vitality and sperm motility decreased in the exposed workers. The mean acrosin activity,  $\gamma$ -GT activity and LDH-C4 relative activity in the exposed workers were lower, and fructose concentration was higher than those in the control. There were negative correlations between sperm vitality, sperm activity, acrosin activity, or LDH-C4 relative activity and working history. These results suggest that the mixture of these solvents could affect the sperm and the function of accessory gonad. This might be one reason of the abnormal pregnancy outcome among the wives of workers exposed to benzene, toluene, and xylene.

**Key words:** Benzene, Toluene, Xylene, Semen, Function of accessory gonad

## Introduction

Exposure to n-hexane and thinners, particularly the components ethyl acetate and xylene, caused testicular damage in animals<sup>1</sup>. Exposure to carbon disulphide and ethylene glycol ethers has been linked to infertility<sup>2</sup>. Some studies suggest that there is an association between paternal occupational exposure to solvents and abnormal pregnancy outcome. Paternal exposure to solvents has been linked to spontaneous abortion<sup>3,4</sup>, low birth weight, and birth defects<sup>5</sup>. The mean length and weight of children of spray painters at birth were slightly lower than those of the electronics workers<sup>6</sup>. The highest risks were observed among painters and wood workers. Solvents, which were associated with

spontaneous abortion, include toluene, xylene, and miscellaneous solvents such as thinners<sup>5</sup>. The mixture of benzene, toluene, and xylene is widely used in various industries. Hazardous mixture exposure takes place in shoemaking, spray painting and paint manufacturing. Harmful effects of these solvents include haematotoxic and leukogenic effects. However, there is no any information about the effect of long-term exposure to the mixture on male reproductive function, especially on semen quality.

In this study, the effects of benzene, toluene, and xylene on semen quality of workers were investigated.

## Subjects and Methods

### *Subjects*

The study was conducted from June 1994 to July 1996 in

\*To whom correspondence should be addressed.

**Table 1. Characteristics of two groups examined (mean  $\pm$  SD, n=61)**

Group	n	Age (yr)	Work standing (yr)	Marriage (yr)	Smoking (yr)	Smoking quantity (pieces/d)	Drinking (yr)	Drinking volume (ml/d)
Exposed	24	33.18 $\pm$ 6.88	13.10 $\pm$ 5.97	8.16 $\pm$ 4.39	6.63 $\pm$ 8.11	8.47 $\pm$ 7.59	7.25 $\pm$ 8.17	210.53 $\pm$ 234.86
Control	37	31.78 $\pm$ 6.36	16.33 $\pm$ 8.48	10.26 $\pm$ 6.14	7.81 $\pm$ 9.15	5.94 $\pm$ 6.64	9.50 $\pm$ 6.61	159.38 $\pm$ 118.63

a city of Zhejiang province. The subjects were selected from married workers with at least one year of working history at shoemaking, spray painting, or paint manufacturing, and were exposed to high airborne concentration of benzene, toluene, and xylene (n=56). The mean concentrations of benzene, toluene, xylene in work airborne were 103.34 (0~7070.3), 42.73 (0~435.8), 8.21 (0~133.1) mg/m<sup>3</sup>, respectively. In addition, 40 age- and occupational-matched non-exposed controls with similar physical activity were selected from managers. Each subject was interviewed about reproductive history, tobacco and alcohol use, and detailed past and present occupational and medical histories, and was invited to donate blood and semen samples. Only 24 of 56 exposed workers volunteered to donate blood and semen samples and 37 non-exposed workers (of 40 workers) volunteered to donate blood and semen samples. The characteristics of these two groups of workers were shown in Table 1. There were no significant differences in characteristics between the exposure workers and the control.

#### *Sample collection and analysis*

Participants were requested to abstain from ejaculation for 48 hours before the semen sample collection. The samples collected by masturbation was delivered to the laboratory immediately, and it was incubated at 37°C until liquefaction completed. Whole semen analysis, including liquefaction time, semen pH value, sperm concentration, total sperm count, percentage vitality, and sperm activity, was completed within one hour after liquefaction according to the World Health Organization (WHO) protocol. The acrosin activity was determined according to the method of Huang Yufeng<sup>7</sup>. Seminal fructose was detected by the method of resorcin<sup>8</sup>. Seminal  $\gamma$ -Glutamyltransferase ( $\gamma$ -GT) activity was determined according the colorimetry, which International Federation of Clinical Chemistry (IFCC) recommended<sup>7</sup>. The relative activity of LDH-C4 was determined by gelose electrophoresis.

From each subject, a 5-ml venous blood sample was withdrawn in a plastic syringe, and was analyzed for benzene, toluene, and xylene in blood by headspace chromatographic method. At the same time, the benzene, toluene, and xylene

in semen were also detected by headspace chromatographic method<sup>9</sup>.

#### *Statistics*

All results are analyzed on COMPAQ computer supplied with a STATISTICA (Release 5.0) package of statistics using  $\chi^2$ , student t test, and multiple regression. The data used in multiple regression included the exposed workers and control workers, and the exposure work duration of control workers is assumed as zero in this process.

## **Results**

The results of routine semen test of the two groups were shown in Table 2. The mean sperm vitality, sperm activity and acrosin activity reduced in the exposed workers ( $P < 0.01$ ). The  $\gamma$ -GT activity and LDH-C4 relative activity in exposed were lower than those in the control workers.

Benzene, toluene, and xylene were detected only in the blood and semen of the exposed workers, but these solvents were not found in control group (Table 3). The mean white blood cell number and platelet number in exposed workers were  $5.66 \pm 1.21 (\times 10^9 \text{ counts/L})$ ,  $120.1 \pm 26.9 (\times 10^9 \text{ counts/L})$ , respectively.

In the analysis of relationship between the biochemical parameter and the factors of exposure showed that semen quality was defined as a dependent and the factors related to exposure were defined as an independent. After multiple regression, we found the relationship between liquefaction time and toluene concentration in semen; sperm vitality and work history, sperm motility and work history, sperm concentration and benzene concentration in blood were obvious (Table 4).

## **Discussions**

#### *Effect of benzene, toluene, and xylene on fertility*

Benzene, toluene, and xylene are used in various industries. Subacute exposure of male rats to a high level (2000 ppm) of toluene vapor can elicit mild toxic changes in the kidneys, thymus, and reproductive organs of males. The results

**Table 2. Results of routine sperm test of two groups (mean  $\pm$  SD)**

Group	n	Liquefaction time (min)	pH value	Volume of semen (ml)	Vitality (%)	Sperm activity (grade)	Sperm density ( $\times 10^9$ spermatozoa/L)
Exposed	24	34.29 $\pm$ 16.97	7.28 $\pm$ 0.20	2.34 $\pm$ 1.47	58.95 $\pm$ 15.60	2.52 $\pm$ 0.96**	85.84 $\pm$ 75.88
Control	37	27.43 $\pm$ 11.76	7.35 $\pm$ 0.29	2.90 $\pm$ 0.95	72.63 $\pm$ 6.98	3.17 $\pm$ 0.75	82.26 $\pm$ 45.58

  

Group	n	Total sperm count ( $\times 10^9$ spermatozoa per ejaculation)	Acrosin activity (U/L)	$\gamma$ -GT (U/L)	Fructose (g/L)	LDH-C4 (%)
Exposed	24	173.44 $\pm$ 155.71	18.02 $\pm$ 7.24***	1714.43 $\pm$ 873.88*	3.09 $\pm$ 2.05	13.48 $\pm$ 3.64***
Control	37	232.09 $\pm$ 141.47	30.74 $\pm$ 10.05	2418.97 $\pm$ 411.92	2.03 $\pm$ 0.74	19.14 $\pm$ 2.10

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. control

**Table 3. Results of biological monitoring in exposed workers ( $\mu\text{mol/L}$ , geometric mean)\***

sample	n	benzene			toluene			xylene		
		No. of positive	range	content*	No. of positive	range	content*	No. of positive	range	content*
blood	24	13	0.40~51.32	4.40	11	0.30~17.17	1.42	11	0.38~ 4.50	1.32
semen	17	12	0.17~ 8.54	1.85	6	0.11~ 0.40	0.22	10	1.12~33.84	5.67

\*Positive sample only.

**Table 4. Multiple regression results of semen variables**

Dependent variable	Variable	Beta	B	SE B	Constant	Sig T
Liquefaction time	Semen-Toluene	0.2571	4.6689	2.3035	28.9597	0.0472
Sperm vitality	Working duration	- 0.5663	- 0.9621	0.1942	72.3617	0.0001
Sperm activity	Working duration	- 0.3896	- 0.0463	0.0152	3.1633	0.0036
Sperm concentration	Blood-benzene	0.4868	0.0049	0.0012	0.7660	0.0002
Acrosin activity	Working duration	- 0.5768	- 0.7689	0.1790	29.2822	0.0012
LDH-C4 relative activity	Drinking volume	- 0.2352	0.0616	- 0.4494	20.7387	0.0008
	Working duration	- 0.1788	0.4634	- 0.4512		0.0008
	Semen-Toluene	- 0.0101	0.0031	- 0.3716		0.0035
Fructose	Semen-benzene	0.0532	0.0120	0.4947	2.4234	0.0001
$\gamma$ -GT	Semen-benzene	- 19.0218	5.5794	- 0.3527	144.5212	0.0012
	Blood-xylene	- 30.2810	7.5835		- 0.4086	0.0002

demonstrated that toxic effects on the fertility and reproduction were not only in females but also in males exposed to toluene vapor<sup>10</sup>. In male rats thinner, particularly the components ethyl acetate and xylene, was reported to interfere with the functions of the testes and accessory reproductive organ; but toluene has no effect on these functions<sup>11</sup>.

In our studies, we found that benzene, toluene, and xylene exist in the blood or semen of many male exposed workers. The concentration of benzene, toluene, xylene in the blood was 51.32, 17.17, and 4.50 ( $\mu\text{mol/L}$ ) in uppermost,

respectively. Otherwise, those in semen were 8.54, 0.40 and 33.84 ( $\mu\text{mol/L}$ ), respectively. The metabolic products of these solvents are hydroxybenzenes. In view of the mutagenicity of benzene, these solvents, which existed in body, especially in semen, directly or indirectly affect reproductive system, especially on germ cells. The percentage of semen liquefaction time exceeded 30 min elevated and the sperm vitality and activity decreased in the exposed workers.

It is remarkable that, in men, the rate of sperm production is much lower than that in many animal species and the

proportion of morphologically abnormal forms is much higher than that in mice, for example. The reasons for these differences are not known. Endogenous factors (genetic factors and prenatal influence) have been shown to play a role, but occupational and other environmental exposures may also adversely affect sperm production in men<sup>2)</sup>. Some studies showed that smoking is associated with lowered semen quality<sup>12-14)</sup>, and impairs the secretory function of accessory sex glands in man<sup>14)</sup>. Moreover, smokers were found to have sperm volumes significantly smaller than non-smokers of the same age. No additional effects on sperm parameters were found. Cigarette smoking revealed no detrimental effect on spermatogenesis<sup>15)</sup>. Some reports appear that age contributed to the decline in semen quality in men over 40 years old<sup>16)</sup>. A statistical difference was seen between men over 50 years of age compared with younger men, but only for the hyposmotic swelling test (HOS) scores and velocity. No statistical differences were found on any of the other parameters evaluated by computer-assisted semen analysis (CASA)<sup>17)</sup>.

In our study, most subjects are lower than 40 years old, and the age, smoking and drink habit of exposure workers is similar to that of control workers. In addition, we only selected the subject who has one child at least for avoiding a selection-bias. It is impossible to exclude the possibility that the relationship between the exposure to the three solvents and the deterioration of the semen quality is explained by a selection-bias.

The acrosome of mammalian spermatozoa contains high amounts of acrosin, which is believed to be essential for gamete fusion, particularly for binding to, and penetration of, the zona pellucida. In addition, its activation from proacrosin seems to be associated with the capacitation process. Furthermore, it might facilitate cervical mucus penetration and intrauterine sperm migration by releasing kinins from kininogen, as well as participating in the acrosome reaction and in chromatin decondensation in the oocyte. Acrosin is an acrosomal protease believed to play a major role in fertilization<sup>18, 19)</sup>.

Lactate dehydrogenase C4 (LDH-C4), the specific isozyme of LDH produced by germ cells. It exists in spermatocyte, spermatoon, and sperm only, but not in spermatogonium and Sertoli cell. Activities of total lactate dehydrogenase play an important role in providing energy for cell metabolism, lactate dehydrogenase-C4, an isoenzyme of lactate dehydrogenase supposed to be specific for germinal epithelium activity<sup>20, 21)</sup>. The relative activities of LDH-C4 directly affect sperm motility.

In this study, the relative activity of LDH-C4, and the mean

activity of acrosin in the exposed workers were remarkably lower than that in the control workers ( $P < 0.001$ ). The results suggested that these solvents could affect the fertility.

After multiple regressions, we found some relationships between biochemical markers and the factors of exposure. The results revealed that the deterioration of the semen quality was related to high concentration of the three solvents in the blood and/or semen, and support further that the mixture of these solvents could affect the male reproductive system in human.

Seminal fructose provides energy for the action of sperm. The higher the percent of disassemble of fructose is, the stronger the sperm acts. The concentration of fructose not only images the secretion of spermatophore, but also indirectly reflects the increment of testicle<sup>8)</sup>. In this study, the level of fructose in semen in the exposed workers is little higher than that of the control workers, but no significant difference was found. And also, a positive correlation was found between seminal fructose concentration and benzene concentration in semen. We don't know whether that is due to the decrease of sperm vitality or/and sperm motility, which result in a fall of disassemble of fructose.

The activity of  $\gamma$ -GT reflects the function of prostate<sup>7)</sup>. Lower level of  $\gamma$ -GT implied that deterioration of the function of prostate. The fall of the activity of  $\gamma$ -GT in exposure workers suggested that these solvents could impair the function of prostate.

#### *Possible mechanism of benzene, toluene and xylene on fertility*

There are many ways for solvents affect fertility of human<sup>2, 3)</sup>. But, I think the possible mechanism of benzene, toluene and xylene on fertility may be including following:

- (1) Benzene, toluene, and xylene appear in the blood and semen suggests that these solvents permeate blood-testis barrier. So, the effect of benzene, toluene, and xylene on male reproductive system could exert by influencing testicle itself.
- (2) In our other study<sup>22)</sup>, the average plasma levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) of workers exposed to the mixture of solvents were significantly lower than those of non-exposed workers ( $P < 0.01$ ). FSH primarily stimulates Sertoli cells, which nurture the developing germ cells. The production of germ cells in the seminiferous epithelium is under endocrine control. The abnormality of the stimulating hormone might affect the sperm production. The mixture of these solvents affects the function of accessory gonad. Apparently, the finding of benzene, toluene, and xylene in semen may explain the adverse effect.

- (3) Benzene, toluene and xylene could be transferred to the body of the wife of the exposed workers through the semen and/or smeary work clothes, which could affect the development of ovum and embryo.
- (4) In addition, these solvents could affect central nervous system, and then result in the deterioration of the semen quality.

In this study, we found that sperm vitality, motility and acrosin activity were decreased in the workers exposed to benzene, toluene, and xylene. The results suggest benzene, toluene, and xylene could affect the quality of semen and sperm by directly influencing the function of testicle and/or the function of accessory gonad. These findings might explain the abnormal pregnancy outcome among the man exposed to solvents. Further studies are, however, required to confirm this finding.

### Acknowledgement

This research was supported by Ningbo youth science fund.

### References

- 1) Sallmen M, Lindbohm ML, Anttila A, Kyyronen P, Taskinen H, Nykyri E, Hemminki K (1998) Time to pregnancy among the wives of men exposed to organic solvents. *Occup Environ Med* **55**, 24–30.
- 2) Lähdetie J (1995) Occupation- and exposure-related studies on human sperm. *J Occup Environ Med* **37**, 922–30.
- 3) Lindhohm ML, Hemminki K, Bonhomme MG, Anttila A, Rantala K, Heikkilä, Rosenberg MJ (1991) Effects of paternal occupational exposure on spontaneous abortions. *Am J Pub Health* **81**, 1029–33.
- 4) Taskinen H, Anttila A, Lindhohm ML, Sallmén M, Hemminki K (1989) Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents. *Scand J Work Environ Health* **15**, 345–52.
- 5) Lindhohm ML (1995) Effects of parental exposure to solvents on pregnancy outcome. *J Occup Environ Med* **37**, 908.
- 6) Höglund GV, Iselius EL, Knave BG (1992) Children of male spray painters: weight and length at birth. *Br J Ind Med* **49**, 249–53.
- 7) Huang Y (1993) Experimental diagnostic handbook of andrology. 2nd ed, 63–122, Southeast University Publishing Company, Nanjing, China.
- 8) Yu S (1997) Clinical basic laboratory medicine. 314–5, The Peoples Medical Publishing House, Beijing.
- 9) Foo SC, Phoon WO, Khoo NY (1988) Toluene in blood after exposure to toluene. *Am Ind Hyg Assoc* **49**, 255.
- 10) Ono A, Sekita K, Ogawa Y, Hirose A, Suzuki S, Saito M, Naito K, Kaneko T, Furuya T, Kawashima K, Yasuhara K, Matsumoto K, Tanaka S, Inoue T, Kurokawa Y (1996) Reproductive and developmental toxicity studies of toluene. II. Effects of inhalation exposure on fertility in rats. *J Environ Pathol Toxicol Oncol* **15**, 9–20.
- 11) Yamada K (1993) Influence of lacquer thinner and some organic solvents on reproductive and accessory reproductive organs in the male rat. *Biol Pharm Bull* **16**, 425–7.
- 12) Vine MF, Tse CK, Hu P, Truong KY (1996) Cigarette smoking and semen quality [see comments]. *Fertil Steril* **65**, 835–42.
- 13) Chia SE, Ong CN, Tsakok FM (1994) Effects of cigarette smoking on human semen quality. *Arch Androl* **33**, 163–8.
- 14) Pakrashi A, Chatterjee S (1995) Effect of tobacco consumption on the function of male accessory sex glands. *Int J Androl* **18**, 232–6.
- 15) Holzki G, Gall H, Hermann J (1991) Cigarette smoking and sperm quality. *Andrologia* **23**, 141–4.
- 16) Merino G, Carranza-Lira S (1995) Semen characteristics, endocrine profiles, and testicular biopsies of infertile men of different ages. *Arch Androl* **35**, 219–24.
- 17) Check JH, Shanis B, Bollendorf A, Adelson H, Breen E (1989) Semen characteristics and infertility in aging. *Arch Androl* **23**, 275–7.
- 18) Suter L, Habenicht UF (1998) Characterization of mouse epididymal acrosin: comparative studies with acrosin from boar and human ejaculated spermatozoa. *Int J Androl* **21**, 95–104.
- 19) Schill WB, Topfer-Petersen E, Heissler E (1988) The sperm acrosome: functional and clinical aspects. *Hum Reprod* **3**, 139–45.
- 20) Vanithakumari G, Govindarajulu P (1991) Seminal plasma enzymes in relation to fertility in human males. *J Reprod Biol Comp Endocrinal* **1**, 127.
- 21) Aydin S, Yilmaz Y, Odabas O, Sekeroglu R, Tarakcioglu M, Atilla MK (1997) A further study of seminal plasma: lactate dehydrogenase and lactate dehydrogenase-X activities and diluted semen absorbance. *Eur J Clin Chem Clin Biochem* **35**, 261–4.
- 22) Cai YZ, Xiao GB, Wang TY, Fu ZM (1996) Effects of benzene, toluene, xylene on male sexual hormone. *Chin J Pub Health* **15**, 198–9.