

Some altered concentrations of elements in semen of workers exposed to trinitrotoluene

H X Liu, W H Qin, G R Wang, Z Z Yang, Y X Chang, Q G Jiang

Abstract

Objectives—A cross sectional study was performed to find the concentrations of elements contained in the semen of workers exposed to trinitrotoluene (TNT).

Subjects and methods—Semen of exposed workers in two TNT plants located in He-Nan Province in 1992 were examined.

Results—The average TNT concentrations in the workplace, except the packing site, were found to have exceeded the maximal allowable concentration (MAC, 1 mg/m³); skin contaminations of male workers exposed to TNT were higher after a shift than in controls, and correlated with the total blood concentrations of TNT, 4-amino-2, 6-dinitrotoluene (4A), and 2-amino-4, 6-dinitrotoluene (2A). Cu, Zn, Na, Mg, and Se concentrations were significantly decreased, but K, Ca, Co, Mn and Li contents were not significantly changed in the semen of workers exposed to TNT. Compared with the control group, the percentage of liquifying time of semen, the sperm malformation incidence, and viability in the men exposed to TNT were all significantly changed.

Conclusions—Men exposed to TNT have decreased concentrations of some elements in semen and altered semen physiology.

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Keywords: trinitrotoluene; semen; elements contained in semen

In recent years, we have found that the testis is one of the target organs affected by toxicity induced by trinitrotoluene (TNT). The TNT was actively reduced in the rat testicular microsome system *in vitro*.¹ Rat testicular Cu and Zn concentrations were significantly reduced, but serum Zn concentration was significantly increased, whereas serum caeruloplasmin activity was decreased after six weeks of exposure to TNT.² After TNT (50-200 mg/kg) was given orally for six weeks all experimental groups showed an increased proportion of morphologically abnormal sperm.³ Rat testicular enzyme activities were also disturbed: the activities of glucose-6-phosphate dehydrogenase, acid phosphatase, and lactate dehydrogenase were decreased after eight weeks exposure in two experimental groups (50 and 200 mg TNT/kg body weight),

whereas sorbitol dehydrogenase and non-specific esterase activities had decreased after six weeks of exposure in these same two groups.⁴ Rat testicular non-specific esterase activity increased after six and eight weeks exposure but only in the 200 mg TNT/kg group. This enzyme activity in rat serum from the 200 mg TNT/kg group was also increased after six weeks exposure, but after eight weeks exposure it was increased in all dose groups (50, 100, and 200 mg TNT/kg).⁵ Rat testicular δ -aminolaevulinic acid dehydratase activity did not significantly change in rat testicular homogenate but it was significantly increased in rat serum after 100 mg TNT/kg were given orally for four weeks. The mean (SD) concentrations of superoxide radical, H₂O₂, and the product of lipid peroxide in Leydig cells incubated with 0.56 and 1.12 mmol TNT l⁻¹ were 0.140 (0.011) and 0.198 (0.020), 14.9 (1.4) and 17.4 (1.0), and 2.82 (0.13) and 3.73 (0.17) *v* controls 0.062 (0.008) nmol l⁻¹, 13.8 (1.2) nmol, and 1.31 (0.05) nmol, respectively. These exposed values were higher than those of the controls. These end points did not significantly change in Sertoli cells incubated with TNT.⁶ Testicular Zn in rats in the (TNT + Zn) group (193 (115) μ g/g testis weight) was significantly higher than in the TNT group (179 (14) μ g/g testis weight), but was not significantly different from the control and the Zn groups. Rat testicular sorbitol dehydrogenase activity and serum testosterone concentration in the (TNT+Zn) group were not significantly different from those in the control, Zn, and TNT groups. Therefore Zn had, at least partly, an antagonistic effect on male reproductive toxicity induced by TNT.⁷

In a pilot study more sexual disorders—such as, impotence, loss of libido, and sexual hypoaesthesia—were found in male workers exposed to TNT (63.5%) than in the control group (5.4%); compared with the control group, the volume of semen and percentage of motile spermatozoa were significantly decreased, and the incidence of sperm malformation significantly increased in the exposed workers. Mean (SD) serum testosterone concentration (480.0 (120.0)) in men exposed to TNT was significantly lower than in the control group (585.0 (95.0) ng/100 ml).⁸

The present study was designed to seek further support for male reproductive toxicity induced by TNT. This was a cross sectional study performed in two civil explosive manufacturing plants located in He-Nan Province in 1991 to ascertain the reproductive and sexual functions of male workers exposed to

He-Nan Institute of Occupational Disease, Zheng-Zhou, He-Nan Province, China

H X Liu
W H Qin
G R Wang

Beijing Medical University, Beijing, China

Y X Chang
Q G Jiang

Correspondence to: Professor Q G Jiang, Department of Occupational Health, School of Public Health, Beijing Medical University, Beijing 100083, China.

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TNT. It included a questionnaire survey of reproductive history and symptoms, a work history, an industrial hygiene investigation, a routine semen analysis, and measurement of the concentrations of 10 elements in semen.

Materials and methods

PRODUCTION PROCESS AND POPULATION STUDY
The production process was the same in these two plants and was similar to that described previously.⁹ There are five jobs in the explosives production area: ball milling, mixing, cooling and drying, loading, and packing, but workers engaged in these five jobs often changed their work with each other.

Unexposed workers had no known exposure to chemicals and lived in the same city as the workers exposed to TNT. Table 1 shows the characteristics of semen contributors, both exposed to TNT and unexposed.

HEALTH QUESTIONNAIRE

The same health questionnaire and definition of sexual dysfunction were used as in previous work.⁹

AIR, SKIN, AND BIOLOGICAL MONITORING

Area air samples were taken by industrial hygienists in these two factories twice every year. The TNT in air was sampled on a commercial pervinyl chloride filter membrane at a flow rate of 20 l/min. The sampling volume was 40 l of air. A 95% ethanol solution (10 ml) was used to dissolve the TNT. After adding 2.5% NaOH (0.1 ml) to 5.0 ml of this 95% ethanol solution, a colorimetric estimation was carried out.¹⁰

Samples from skin were collected after the shift to assess TNT contamination during two to four successive days: the skin (2.5 × 4 cm²) of the back of the hand, neck, and chest was wiped with ethane on cotton balls, 10 ml of 95% ethanol solution was then used to dissolve the TNT from the balls. Colorimetric analysis was then performed as already mentioned.

Venous blood samples from each of the exposed and unexposed workers were analysed for TNT and its metabolites 4-amino-2, 5-dinitrotoluene (4A) and 2-amino-4, 6-dinitrotoluene (2A) by the high performance liquid chromatography (HPLC) method of Lui *et al.*¹¹ To a 0.5 ml blood sample 1.0 ml of H₂O was added, and after adding 0.15 ml of concentrated HCl, the blood sample was put into boiling water for 45 minutes. After cooling and neutralisation to pH 8.0, the blood sample was extracted twice with ether. The ether extract was evaporated to dryness in a 40°C water bath. To dissolve the TNT, 4A, and 2A 0.25 ml of methanol was added to the evaporating dish. For final measurement 0.25 ml of H₂O was added to the sample. Warian-Sy-5060 HPLC equipped with a UV detector was used with a 300 × 4 mm reversed phase C₋₁₈ microbore column. The mobile phase was methanol: water (50:50) at a flow rate of 1.0 ml/min. Column temperature was 30°C and UV wavelength was 235 nm. Detection limits of TNT, 4A, and 2A were 0.014, 0.017, and 0.018 µg/ml respectively. Average recovery rates of these three chemicals were 82.8%, 88.6%, and 93.9%, respectively, at both 0.5 and 1 µg/ml.

SOME ELEMENTS IN SEMEN, AND SEMEN ANALYSIS

Subjects were each required to produce a semen specimen at the clinic by masturbating into a clean jar, and the semen samples thus collected were analysed at once. These subjects were asked to abstain from sexual activities for a minimum of three days before the collection of their samples. The concentration of Se in semen was analysed by fluorospectrophotometry,¹² concentrations of Zn and Cu in semen were measured as previously described.² Other elements were measured by flame or graphite stove atomic absorption spectrophotometry.¹² Sperm counts, volume and viability measurements, and morphological analysis of semen slides were conducted by the methods described previously.⁹

STATISTICAL METHODS

Percentages of sexual dysfunction (table 5) and abnormal semen analysis (table 6) were compared by Fisher's test. Other data were analysed with two tailed Student's *t* test. Correlation coefficients of the data were found with a routine formula.¹³

Results

The atmospheric monitoring in plants A and B showed that of the 173 air samples collected in 1992 in both plants, 51% of the samples from plant A and 48% of the samples from plant B exceeded the maximal allowable concentration (MAC, 1 mg/m³) in China (table 2).

Skin concentrations of TNT in male exposed workers were higher at the end of the shift in plant A than in plant B. The geometric mean of TNT concentrations from hand, neck, and chest in workers from plant A were 36.12, 11.28, and 7.97 µg/cm², respectively (table 3).

Table 1 Characteristics of semen contributors exposed and unexposed to TNT

Groups	Semen samples (n)	Mean (SD) age (y)	Mean (SD) duration of exposure or length of service (y)	Smoking (%)	Drinking (%)
Control A	35	28.5 (8.1)	5.7 (2.9)	60.4	37.3
Plant A	35	21.1 (8.5)	4.3 (2.2)	65.4	39.5
Control B	43	30.8 (8.9)	10.3 (6.9)	64.3	40.1
Plant B	50	35.0 (9.5)	12.5 (7.4)	69.4	36.1

Table 2 The TNT Air Concentrations (mg/m³) during 1992 in plants A and B

Job	Samples n	MAC exceeded	Concentration (mg/m ³)
		n (%)	Geometric mean (range)
Plant A:			
Ball milling	17	11 (65)	4.27 (0.06-42.8)
Mixing	18	4 (22)	0.44 (0.02-5.70)
Cooling and drying	18	14 (78)	2.51 (0.29-23.7)
Loading	17	14 (82)	1.56 (0.31-8.80)
Packing	17	1 (6)	0.41 (0.06-2.03)
Plant B:			
Ball milling	22	13 (59)	1.13 (0.17-26.0)
Mixing	19	13 (68)	1.36 (0.15-13.6)
Cooling and drying	9	6 (67)	1.32 (0.30-5.06)
Loading	18	8 (44)	1.01 (0.19-3.96)
Packing	18	1 (6)	0.35 (0.12-4.94)

Table 3 Skin contaminations with TNT at the end of the shift ($\mu\text{g}/\text{cm}^2$) in workers from plants A and B in 1992

Job	Samples n	Back of hand	Neck	Chest
		Geometric mean (range)	Geometric mean (range)	Geometric mean (range)
Plant A:				
Ball milling	4	127 (39.2-200)	24.1 (5.58-45.0)	16.0 (8.67-33.3)
Mixing	4	49.6 (20.5-113)	12.4 (5.13-28.3)	5.60 (3.00-10.8)
Cooling and drying	2	8.76 (5.33-12.2)	6.76 (6.35-7.17)	9.32 (5.33-15.3)
Loading	4	47.8 (4.42-51.2)	13.9 (8.52-17.5)	9.20 (3.92-20.8)
Packing	4	25.4 (4.00-50.0)	6.49 (5.00-8.52)	4.18 (1.50-5.56)
Plant B:				
Ball milling	4	3.24 (1.52-6.91)	6.14 (2.48-11.5)	4.25 (1.81-7.71)
Mixing	4	6.77 (3.39-12.7)	3.84 (1.18-5.00)	5.40 (0.62-16.6)
Cooling and drying	2	79.0 (5.33-153)	10.6 (7.17-14.0)	8.48 (3.35-13.6)
Loading	4	66.8 (2.27-124)	24.9 (12.5-42.9)	11.8 (5.33-22.9)
Packing	4	47.7 (3.82-156)	3.22 (0.15-8.00)	3.64 (0.15-6.55)

Table 4 Blood TNT and its two metabolites ($\mu\text{g}/\text{ml}$) in male workers exposed to TNT

Chemicals	Plant A		Plant B	
	n	X (SD)	n	X (SD)
TNT	34	0.161 (0.048)	59	0.091 (0.075)
4A	34	0.404 (0.218)	59	0.286 (0.132)
2A	34	0.126 (0.056)	59	0.169 (0.090)

Table 5 Sexual functions in male workers exposed to TNT from plants A and B and control workers

Groups	n	Sexual dysfunctions			Sum (%)
		Loss of libido	Prosermia	Impotence	
Control	72	2	6	0	8 (11.1)
Exposed	42	9	6	1	16 (38.1)**

** P < 0.01.

Biological monitoring was similar to environmental monitoring. The blood concentrations of TNT, 4A, and 2A combined in workers from plants A and B were 0.691 and 0.546 $\mu\text{g}/\text{ml}$ respectively (table 4). The skin contamination of TNT after the shift in exposed male workers showed a significant correlation with the total blood concentration of TNT, 4A, and 2A:

$$Y = 0.559 + 0.0127X \quad \text{plant A}$$

$$Y = 0.3516 + 0.02607X \quad \text{plant B}$$

Table 6 Semen characteristics of workers exposed to TNT from plants A and B and control workers

Characteristics	Control (n = 50)		Exposed (plant A, n = 49)		Control (n = 50)		Exposed (Plant B, n = 48)	
	n	%	n	%	n	%	n	%
Volume (< 1.5 ml /ejaculation)	9	18.0	10	20.4	10	20	11	22.9
Liquefying time (> 60 min)	2	4.0	10	20.4**	1	2	13	29.1**
Sperm concentration (< 6 × 10 ⁷ .ml ⁻¹)	14	28.0	20	40.1	10	20	16	33.3
Higher incidence of abnormal morphology (> 25%)	0	0.0	11	22.4**	0	0	13	27.1**
Viability (<60%)	6	12.0	22	44.9**	4	8	25	52.0**

** P < 0.01.

Table 7 Concentrations of Cu, Zn, Na, Mg and Se ($\mu\text{g}/\text{ml}$) in semen from workers exposed to TNT (mean (SD))

Groups	n	Cu	Zn	Na	Mg	Se
Plant A	35	0.295 (0.103)*	214 (119)*	2857 (280)**	125 (76.0)*	—
Control A	35	0.383 (0.232)	262 (116)	3349 (497)	166 (73.8)	—
Plant B	43	0.555 (0.432)**	296 (76.6)**	3248 (755)**	123 (49.7)**	0.084 (0.036)**
Control B	50	0.856 (0.455)	385 (175)	3749 (559)	188 (65.3)	0.149 (0.054)

P < 0.05; ** P < 0.01; v controls.

where, Y is the total blood concentration of TNT, 4A, and 2A, and X is the skin TNT contamination at the end of the shift; $r = 0.4502$ ($P < 0.052$) for plant A workers and $r = 0.904$ ($P < 0.001$) for plant B workers.

More sexual disorders, such as impotence, loss of libido, and hypoaesthesia, were found in the male workers exposed to TNT than in the control group; impotence or prosermia did not occur in the same workers (table 5). Compared with the control group, the liquefying time of semen was extended, the percentage of motile spermatozoa was significantly decreased, and the incidence of sperm malformation was significantly increased in workers exposed to TNT (table 6).

Concentrations of Cu, Zn, Na, and Mg in semen were significantly decreased in workers exposed to TNT from both plants A and B (table 7). Concentrations of Se in semen were significantly decreased in the plant B. Concentrations of K, Ca, Mn, and Li in semen were not significantly different between the two groups (data not shown).

Discussion

Both the air TNT concentrations in these two plants and skin concentrations of TNT at the end of the shift in exposed workers proved that workers exposed to TNT in these two plants have had very serious exposure. The results of air TNT concentration (table 2), skin contamination with TNT at the end of the shift (table 3), and blood TNT, 4A, and 2A concentrations (table 4) showed that the industrial conditions were more serious in plant A than in plant B. The fact that the total blood concentrations of TNT, 4A, and 2A were significantly related to skin contamination at the end of the shift supports the view that TNT is mainly absorbed through the skin.¹⁴ In measurements of blood TNT and its two metabolites, 4A and 2A, the highest one was 4A. This metabolite also had the highest concentration when TNT and its major metabolites were measured in the urine samples of workers

exposed to TNT.¹⁵ Therefore, blood concentration of 4A could also be used as an indicator in biological monitoring of workers exposed to TNT.

Compared with the control group, we confirm the previous results that male workers exposed to TNT show sexual dysfunction (impotence, loss of libido, and prostermia) and abnormal semen analysis (increased incidence of sperm malformation and liquefying time, decreased viability).⁸

Magnesium is one of the essential elements for the living body, as almost all fundamental cell reactions, such as protein biosynthesis and anaerobic energy production, require Mg⁺⁺ ions. Magnesium serves as an activator for many enzyme systems of metabolism within the cell. Most important among these enzymes are those that hydrolyse and transfer phosphate groups, including the enzymes concerned with reactions involving ATP.¹⁶ Therefore, lower Mg content in the semen of workers exposed to TNT may relate to the abnormal results of routine semen analysis on the workers exposed to TNT.

The semen Zn and Cu contents were very different (table 5) even in the two control groups located in two different cities. This may reflect the influence of different diets. Therefore, a local control group is needed for comparing the results of Zn and Cu metabolism in case these are influenced by chemical toxicity.

Zinc and Cu metabolism were disturbed in rats and workers exposed to TNT. Zinc concentrations in rat testes were significantly decreased, and in serum increased. Zinc concentrations in both hair and serum of male workers exposed to TNT were significantly increased.^{2 17} Semen Zn concentrations in workers exposed to TNT were significantly decreased in this study. The Cu content of rat testes, and serum and hair of exposed workers were all significantly decreased.² The Cu content of semen of exposed workers was also significantly decreased in this study. Therefore, Zn and Cu metabolism were disturbed by TNT toxicity.

The relation between hair, serum, blood, and semen concentrations of Zn and Cu in semen should be further studied. The mechanism of disturbed Zn and Cu metabolism induced by TNT toxicity has not yet been established. It was found that Zn and Cu metabolism were regulated by hormone concentrations. Serum testosterone, LH, and FSH concentrations were significantly changed by TNT toxicity.¹⁸ Therefore, disturbed sex hormone concentrations induced

by TNT toxicity in both exposed male workers and rats may affect, at least in part, the Zn and Cu metabolism.

The toxicant TNT produces oxidative stress.¹⁹ Its toxicity was synergic if the animal's diet was deprived of Se or vitamin E.^{20 21} It is not surprising to see that the Se content in workers exposed to TNT was far below that in control workers.

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