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

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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, y-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 animals1). Exposure to carbon disulphide and ethylene glycol ethers has been linked to infertility2). 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 abortion1,3), low birth weight, and birth defects5). The mean length and weight of children of spray painters at birth were slightly lower than those of the electronics workers6). 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 thinners5). 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
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/m³, 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 Yufeng⁷). Seminal fructose was detected by the method of resorcine⁸). Seminal γ-Glutamyltransferase (γ-GT) activity was determined according the colorimetry, which International Federation of Clinical Chemistry (IFCC) recommended⁹). 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 method⁹).

Statistics

All results are analyzed on COMPAQ computer supplied with a STATISTICA (Release 5.0) package of statistics using χ², 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 γ-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±1.21 (×10⁹ counts/L), 120.1±26.9 (×10⁹ 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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Work standing (yr)</th>
<th>Marriage (yr)</th>
<th>Smoking (yr)</th>
<th>Smoking quantity (pieces/d)</th>
<th>Drinking (yr)</th>
<th>Drinking volume (ml/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37</td>
<td>31.78 ± 6.36</td>
<td>16.33 ± 8.48</td>
<td>10.26 ± 6.14</td>
<td>7.81 ± 9.15</td>
<td>5.94 ± 6.64</td>
<td>9.50 ± 6.61</td>
<td>159.38 ± 118.63</td>
</tr>
</tbody>
</table>
demonstrated that toxic effects on the fertility and reproduction were not only in females but also in males exposed to toluene vapor. 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.

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 (µmol/L) in uppermost, respectively. Otherwise, those in semen were 8.54, 0.40 and 33.84 (µmol/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

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### Table 2. Results of routine sperm test of two groups (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Liquefaction time (min)</th>
<th>pH value</th>
<th>Volume of semen (ml)</th>
<th>Vitality (%)</th>
<th>Sperm activity (grade)</th>
<th>Sperm density (× 10^9 spermatozoa/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>24</td>
<td>34.29 ± 16.97</td>
<td>7.28 ± 0.20</td>
<td>2.34 ± 1.47</td>
<td>58.95 ± 15.60</td>
<td>2.52 ± 0.96**</td>
<td>85.84 ± 75.88</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>27.43 ± 11.76</td>
<td>7.35 ± 0.29</td>
<td>2.90 ± 0.95</td>
<td>72.63 ± 6.98</td>
<td>3.17 ± 0.75</td>
<td>82.26 ± 45.58</td>
</tr>
</tbody>
</table>

### Table 3. Results of biological monitoring in exposed workers (µmol/L, geometric mean)*

<table>
<thead>
<tr>
<th>sample</th>
<th>n</th>
<th>benzene</th>
<th>toluene</th>
<th>xylene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive</td>
<td>range</td>
<td>content*</td>
<td>No. of positive</td>
</tr>
<tr>
<td>blood</td>
<td>24</td>
<td>13</td>
<td>0.40–51.32</td>
<td>4.40</td>
</tr>
<tr>
<td>semen</td>
<td>17</td>
<td>12</td>
<td>0.17–8.54</td>
<td>1.85</td>
</tr>
</tbody>
</table>

*Positive sample only.

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

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Variable</th>
<th>Beta</th>
<th>B</th>
<th>SE B</th>
<th>Constant</th>
<th>Sig T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquefaction time</td>
<td>Semen-Toluene</td>
<td>0.2571</td>
<td>4.6689</td>
<td>2.3035</td>
<td>28.9597</td>
<td>0.0472</td>
</tr>
<tr>
<td>Sperm vitality</td>
<td>Working duration</td>
<td>-0.5663</td>
<td>-0.9621</td>
<td>0.1942</td>
<td>72.3617</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sperm activity</td>
<td>Working duration</td>
<td>-0.3896</td>
<td>-0.0463</td>
<td>0.0152</td>
<td>3.1633</td>
<td>0.0036</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>Blood-benzene</td>
<td>0.4868</td>
<td>0.0049</td>
<td>0.0012</td>
<td>0.7660</td>
<td>0.0002</td>
</tr>
<tr>
<td>Acrosin activity</td>
<td>Working duration</td>
<td>-0.5768</td>
<td>-0.7689</td>
<td>0.1790</td>
<td>29.2822</td>
<td>0.0012</td>
</tr>
<tr>
<td>LDH-C4 relative activity</td>
<td>Drinking volume</td>
<td>-0.2352</td>
<td>0.0616</td>
<td>-0.4494</td>
<td>20.7387</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>Working duration</td>
<td>-0.1788</td>
<td>0.4634</td>
<td>-0.4512</td>
<td>0.0008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Semen-Toluene</td>
<td>-0.0101</td>
<td>-0.0031</td>
<td>-0.3716</td>
<td>2.4234</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fructose</td>
<td>Semen-benzene</td>
<td>0.0532</td>
<td>0.0120</td>
<td>0.4947</td>
<td>2.4234</td>
<td>0.0001</td>
</tr>
<tr>
<td>γ-GT</td>
<td>Semen-benzene</td>
<td>-19.0218</td>
<td>5.5794</td>
<td>-0.3527</td>
<td>144.5212</td>
<td>0.0012</td>
</tr>
<tr>
<td>Blood-xylene</td>
<td>30.2810</td>
<td>7.5835</td>
<td>-0.4086</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
a 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 men2. Some studies showed that smoking is associated with lowered semen quality12–14), and impairs the secretory function of accessory sex glands in man16). 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 spermatogenesis15). Some reports appear to explain the association of semen quality in men over 40 years old16). 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)17).

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 fertilization18, 19).

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 activity20, 21). 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 incretion of testicle8). 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 sperm motility, which result in a fall of disassemble of fructose.

The activity of γ-GT reflects the function of prostate27). Lower level of γ-GT implied that deterioration of the function of prostate. The fall of the activity of γ-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 human2, 3). 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 study22), 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 might affect 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

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References


8) Yu S (1997) Clinical basic laboratory medicine. 314–


