A 34-year-old male laboratory worker suffered from asthenospermia and fertility problems. He was suspected of having been exposed to solvents used at work due to a malfunction of the ventilation system in his laboratory from August 1996 to April 1997. A laboratory walk-through and air and bulk sample collection were performed to determine the possible exposure levels of chemical hazards in his job. The scenario was reconstructed to simulate the worker's previous exposure during the ventilation shutdown period. It was found that the worker was possibly exposed to chloroform at levels of 10 or 50 times higher than the permissible exposure limit or the threshold limit value of 2 hr/day, 5.5 days/week, and 4.25 weeks/month for 8 months. Because chloroform is known to be spermatoxic, the possibility of chloroform causing the worker's asthenospermia cannot be ruled out. Further study on spermatoxicity of chloroform is warranted. Key words: asthenospermia, chloroform, occupational exposure, sperm.

**Field Study**

The patient, a laboratory technician, used infrared spectrophotometry (IR) to analyze the purity of petrochemical products. He received an average of 40–50 samples daily in 5-mL glass vials. Both before and after sample analysis, he was required to clean three types of IR specimen holders with isooctane, chloroform, and tetrahydrofuran (THF). The washing times for these three types of specimen holders were 25–60 sec with isooctane, 100–200 sec with chloroform, and 120–300 sec with THF, depending on the type of specimen holder used and the sample's viscosity. The patient always wore the gloves (polybutadiene latex) during this procedure. He also wore a respiratory mask equipped with a charcoal cartridge when he judged that the ventilation was not efficient. The charcoal cartridge was replaced on an irregular basis. However, the patient reported that he could still smell the organic solvents even when worn the respirator. He began work at about 0830 hr, he took a break from 1130 to 1230 hr, and he worked until 1700 hr, a workday of approximately 6.5–7.0 hr.

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Normally, the task of cleaning with solvents should be done in an exhaust ventilation hood. The patient started this job in October 1994, but due to pump failure during August 1996–April 1997, the ventilation system was shut down (Figure 1). The patient performed his routine procedures under the same ventilation hood, with the hood door open wide, and he used surrogate exhaust ventilation (a wall fan) beside the hood during the ventilation shutdown period (Figure 2). The patient recorded the number of samples and the types of specimen holders cleaned in a daily laboratory logbook during exhaust ventilation (a wall fan) beside the exhaust ventilation hood (Figure 2). The patient recorded the number of samples and the types of specimen holders cleaned in a daily laboratory logbook during the ventilation shutdown period. We could not perform the experiment to determine the accumulation of solvents in the field due to ethical concerns, but we could estimate this accumulation. If we estimate the ventilation conservatively, for example, 1% of the daily ventilation remaining overnight (0.51 m3/hr), the chloroform levels in the air could build up to 450 ppm through all-night accumulation; this is 100-fold higher than the exposure levels during the day. Because of the shutdown of the ventilation system and the wall fan at night, the solvent concentrations could not be effectively reduced by the beginning of the next workday. This indicates that the subject was possibly exposed to the chloroform at levels of 10 or 50 times higher than the permissible exposure limit (50 ppm) or the threshold limit value (10 ppm), respectively, for 2 hr/day, 5.5 days/week, and 4.25 weeks/month for 8 months.

Results

We conducted and assessed the exposure levels of chloroform during the ventilation shutdown and during regular operation of the exhaust ventilation system. The exposure level of chloroform was 8.5 ppm by active sampling and 4.6 ppm by passive sampling during the shutdown of the ventilation system; however, the exposure level of chloroform was below the detection limit (< 0.15 ppm) when the exhaust ventilation system operated properly.

In our reconstruction of the exposure scenario during the ventilation shutdown, we estimated that 12.11 g chloroform evaporated during 8 hr at the same conditions in which the patient worked during the ventilation shutdown for a period of 8 months. In the field study, we determined that the area of the hood door was 2,025 cm2 (45 cm × 45 cm), the velocity of the wall fan (surrogate exhaust) was 7.0 cm/sec, and the volume of the laboratory was 28.30 m3 (Figure 2). We also estimated that the airborne chloroform concentration was 21.92 mg/m3 (i.e., 4.48 ppm) during the period when the ventilation system was not working; this is consistent with Harte (2). This estimate is similar to the result of the air monitoring in the field study (4.6–8.5 ppm), indicating the exposure level of chloroform during the ventilation shutdown period was around 5–10 ppm. At night for building security, all the laboratory windows were closed and the wall fan was turned off. To determine how this affected the laboratory, we turned off the wall fan and closed the windows for 5 min; we found only trace air velocity at the edge of the window, which is consistent with the findings in the literature (3).

Discussion and Conclusion

Before the shutdown of the ventilation system, the patient’s semen analysis was normal. Except for the ventilation shutdown, we could identify no other occupational or environmental hazards that were associated with the sudden reduction in the patient’s sperm motility. Drugs, drinking alcohol, smoking tobacco, or surgery probably did not cause the condition because these were unchanged during the period of May 1996–July 1997.

Many chemicals have been shown to be male reproductive hazards (4). Carbon disulfide and lead were found to damage the density, morphology, and motility of sperm (4.5). Exposure to DBCP causes oligospermia, azoospermia, and testicular atrophy (6.7). Boron, cadmium, carbaryl, dibromoethane, kepone, toluenediamine, methyl mercury, and dinitrotoluene have been reported to be detrimental to either the density or the morphology of sperm (4.7–9);

Table 1. Consecutive results of semen analysis.

| Test | 9 July 1997 | 6 August 1997 | 15 October 1997 |
|------|-------------|--------------|-----------------|
| Semen analysis | | | |
| Volume (mL) | 4.0 | 5.5 | 3.0 |
| Count (million/mL) | 68.6 | 73.8 | 90.6 |
| W BC | 15–20/HPF | 12–15/HPF | 1–2/HPF |
| Morphology | | | |
| Motility (at 30 min after ejaculation) | | | |
| Rapid | 17% | 10% | 32% |
| Medium | 6% | 1% | 6% |
| Slow | 3% | 0% | 2% |
| Static | 74% | 89% | 60% |
| Path velocity (µm/sec) | 35 | 40 | 50 |

HPF, high-power field under light microscope.

The patient was asked to have one ejaculation 4 days before semen collection and no ejaculation between that and the semen collection. Semen was analyzed in the fertility laboratory by computer-assisted semen analysis (Version 10 HTM-Ivos Specification; Hamilton-Thorne Research, Beverly, MA, USA).
In the field study, we found that considerable amounts of isooctane, THF, and chloroform were used by the patient. Isooctane and THF have not been associated with male reproductive hazards. Chloroform has been reported to cause morphologic abnormality in mouse spermatozoa at a concentration of 400 ppm (10), but effects have not been reported at lower concentrations. In addition, reports on chemical-induced reduction in sperm motility have been relatively limited. Methyl mercury has been reported to affect sperm motility (11), and impairment of sperm motility after exposure to lead has been reported in rats (12). Mechanistic studies showed that lead affects intracellular membranes (13) and impairs mitochondrial functions, which is related to sperm motility (14). In vivo exposure to lead in rats significantly reduced Ca²⁺ATPase activity, resulting in an increase in intracytosolic calcium and high levels of lipid peroxidation in nerve terminals (15). Changes in intracellular Ca²⁺ homeostasis have often been associated with mitochondrial mechanisms, likely caused by the inhibitory effect of Pb²⁺ on Ca²⁺ uptake into mitochondria (16) and promotion of Ca²⁺ efflux from mitochondria (15). Because abnormal morphology in sperm has often been associated with reduced motility (17,18), it is possible that chloroform also causes reduced motility, as seen in our patient.

The proven causes of isolated asthenospermia include artifactual asthenospermia, axonemal defects (immotile cilia syndrome), protein–carboxyl methylase (PCM) deficiency, anti-sperm antibody, necrospermia, and culture-proven infection (19). In our hospital, a tertiary referral center, the semen collection was performed under a carefully controlled procedure according to the World Health Organization laboratory manual (20) and analyzed by computer-assisted sperm analysis (Version 10 HTM-IVOS Specification; Hamilton-Thorne Research, Beverly, MA, USA). Sperm motility was categorized into rapid, medium, slow, or static. When comparing these two methods, the sum percentage of rapid/medium/slow motility sperm from computer-assisted sperm analysis should be identical to the percentage of total motile sperm by the manual method. Obviously, the semen samples in July and August 1997 had lower percentages of total motile sperm (26% and 11%, respectively) compared to the sample in May 1996 (92%) after 30 min. Artifactual asthenospermia was unlikely. Actually, the first and second semen analyses performed in our hospital were compatible with each other. These three separate semen analyses showed that no more than 20% of motile sperm were attached to the immunobeads, no culture-proven seminal tract infection was noted, and no obvious necrospermia was observed under supravital stain. Thus, the diagnoses of anti-sperm antibody, infection, and necrospermia could be excluded. Although we did not study the axonemal ultrastructure of the sperm tail by electron microscopy or PCM activity, the recovery of sperm motility in the third semen sample suggests that the cause of asthenospermia is not related to axonemal defect or PCM deficiency. Therefore, no specific causes of asthenospermia could be identified in this patient except overexposure to solvents. White blood cells were found in the patient's semen. We evaluated the patient's pyospermia but found no seminal tract infection. At least one prospective study has demonstrated that there is no relationship between the presence of leukocytes in asymptomatic individuals and fertility status (21). Currently, there is no convincing evidence suggesting that alterations in seminal plasma constituents caused by infection lead to asthenospermia (19). Therefore, we believe that the presence of leukocytes without bacterial infection, as witnessed in our patient, was probably not the cause of his asthenospermia. In contrast, we could not exclude the possibility that the leukocytes in semen were caused by overexposure to solvents.

Because no stirring, wiping, or other operations in the simulation experiment, which were necessary in the regular operation, can increase the evaporation rate, we may have underestimated the evaporation of chloroform and other solvents in the simulation experiment. In rats, the reported effective spermatoxic dose of chloroform was 400 ppm, which is higher than the permissible exposure level (10 ppm of the permissible exposure limit-time-weighted average in Taiwan (22) and 50 ppm of the permissible exposure limit-ceiling in the United States (23)). However, effects associated with even lower exposures cannot be ruled out. It is important for toxicologists to continue to study the effects of chloroform on sperm motility.

During our environmental monitoring, the chloroform concentration was approximately 5–10 ppm in the daytime during the ventilation shutdown, which was verified by the simulation study. We were unable to completely reconstruct the exposure setting because of ethical considerations. However, following the same reconstruction principle of the daily exposure, we found that even with a conservative estimate of all-night accumulations, the chloroform concentrations at
the beginning of the workday could be as high as 10 times the daily exposure level. Therefore, the patient could have been exposed to ambient levels of chloroform that were spermatotoxic.

The patient, from another city, drove 1 hr by car each time he came to our hospital. Although he stopped coming to our hospital after the field study, he reported by telephone that results of another semen analysis showed significantly improved sperm motility. To protect workers from potential reproductive hazards, further investigation is needed to determine chloroform effects on sperm motility at various dose ranges, including dose levels near the permissible limits. Further human epidemiology studies or animal assays are needed to verify this hypothesis.

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