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Reduction of Sperm Motility in a Male Laboratory Worker Exposed To Solvents: A Case Study

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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 spermatotoxic, the possibility of chloroform causing the worker's asthenospermia cannot be ruled out. Further study on spermatotoxicity of chloroform is warranted. *Key words:* asthenospermia, chloroform, occupational exposure, sperm. *Environ Health Perspect* 109:753–756 (2001). [Online 13 July 2001]
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Case Report

A 34-year-old male laboratory worker came to the medical center on 21 June 1997 complaining that his wife had difficulty becoming pregnant. Both he and his wife, a 32-year-old healthy bank accountant, had unremarkable past histories. They had been married for 18 months; their sexual activity was normal and they used no contraception, but the patient's wife had not become pregnant. He consulted the urology clinic of the National Cheng Kung University Medical College and received a complete physical examination and semen analysis. He was found to be a well-developed and well-nourished man. He had no history of trauma, inguinal hernia, cryptorchidism, or surgery. He had mumps at 8 years of age, with no complications. The patient denied having had a high fever in the past 12 months, as well as having been exposed to extreme heat or radiation. He smoked less than 10 cigarettes and drank less than 4 cups of beer per week. His blood pressure was 100/84; his physical examination was unremarkable, except that his right testis was slightly smaller than the left testis. No varicocele was identified. Serum aspartate aminotransferase (glutamic-oxaloacetic transaminase) was 40 U/L, and glutamic-pyruvic transaminase was 109 U/L (normal < 55). Both hepatitis B surface antigen and hepatitis C virus antibody were negative. Bilirubin, alkaline phosphatase, and creatinine were within normal limits. His cholesterol and triglycerides were elevated at 219 mg/dL and 227 mg/dL, respectively. Abdominal sonography showed a moderate fatty liver. Serum concentrations of luteinizing hormone (3.0 mIU/mL; normal 1.5–9.2), follicle-stimulating hormone (7.9 mIU/mL; normal 1–14), testosterone (4.7 ng/mL; normal 3–10), and prolactin (5.1 ng/mL; normal 3.1–16.5) were all within normal limits. The patient's semen analysis (9 July 1997; Table 1) showed normal volume, sperm count, and morphology, but poor motility. Anti-sperm antibody was negative.

The patient was married in December 1995. A complete fertility test was performed for the patient and his wife as screening on 28 May 1996 in a local hospital. Results of tests for the patient and for his wife were within normal limits. The patient had normal semen appearance, volume, and sperm count. Ninety-two percent of sperm were normal in morphology. At 30 min after ejaculation, 95% of sperm were motile at a normal speed, and at 60 min, 30% were motile.

In June 1997, test results showed a reduction in sperm motility (asthenospermia) compared to the test from 1 year earlier. The patient wondered if the change was related to his elevated exposure to solvents, which resulted from the shutdown of the ventilation system in his laboratory during August 1996–April 1997. A team, organized to investigate this potential exposure, included an occupational physician, a urologist, and an industrial hygienist. The team members conducted a laboratory walk-through to understand the patient's work area and responsibilities. Air and bulk samples were collected to determine the possible exposure levels of chemical hazards in his job.

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 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 wore the respirator. He began work at about 0830 hr, he took a break from 1130 to 1330 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 the ventilation system shutdown.

Environmental Monitoring

Our goal was to determine the patient's potential exposure to chemicals known to affect male fertility, namely, dibromochloropropane (DBCP), lead, boron, cadmium, carbaryl, kepone, methyl mercury, toluene, toluenediamine, and dinitrotoluene. We conducted environmental monitoring using both active and passive sampling to estimate the possible exposure levels of solvents in periods when the ventilation system was shut down and during normal ventilation operation.

We collected air samples using the NIOSH 1003 method of sampling organic compounds (*1*). For active sampling, we used a tube containing 100–50 mg active charcoal (SKC 226-01; SKC, Eighty Four, PA, USA) as adsorbent. For passive sampling, we used badges containing active charcoal (3M 3500 Organic Vapor Monitor; 3M, St. Paul, MN, USA) to monitor the exposure levels of air hazards for longer periods: one badge was used for 2 hr during a ventilation shutdown and another badge was used for the whole work shift. For active sampling, we asked the patient to wear the badge (personal pump) for 20 min with a precalibrated flow rate of 100 and 200 mL/min during ventilation shutdown and for the whole work shift, respectively. Area (active) samples were also collected for both conditions. Sample tubes were immediately frozen and stored at < 4°C when sampling was finished.

We analyzed samples collected in the charcoal tubes and badges using a gas chromatograph/flame ionization detector (GC/FID) equipped with an auto-sample injector (HP 5890 series II/HP 3397; Hewlett Packard, Avondale, PA, USA). We used a DB-624 column (30 m × 0.32 mm, film thickness 1.8 µm, J&W #123-1334; J&W Scientific, Folsom, CA, USA) in the analysis. Before instrumental analysis, we used carbon disulfide to dissolve the samples; this procedure took at least 30 min with occasional agitation.

To estimate the possible evaporation rate during the ventilation shutdown, we conducted a simulated experiment. We removed lids from containers of routinely used solvents, and we turned off the ventilation and turned on the wall fan. We weighed the solvents before and after the experiment and determined the solvent loss by the difference of the solvent weights. We calculated the volume of the laboratory by multiplying the length, width, and height of the room.

Results

We reconstructed 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 cm^2 (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 m^3 (Figure 2). We also estimated that the airborne chloroform concentration was 21.92 mg/m^3 (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*).

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 m^3/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.

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.[a]

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
WBC	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.
[a]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).

however, our patient was not significantly exposed to any of these chemicals.

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 Ca^{2+}ATPase activity, resulting in an increase in intrasynaptosomal calcium and high levels of lipid peroxidation in nerve terminals (*15*). Changes in intracellular Ca^{2+} homeostasis have often been associated with mitochondrial mechanisms, likely caused by the inhibitory effect of Pb^{2+} on Ca^{2+} uptake into mitochondria (*16*) and promotion of Ca^{2+} 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 spermatotoxic 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

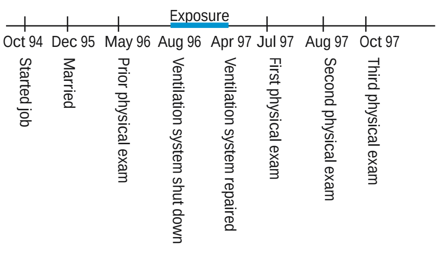

Figure 1. Time line showing events of the 3-year period in which the patient was examined for infertility.

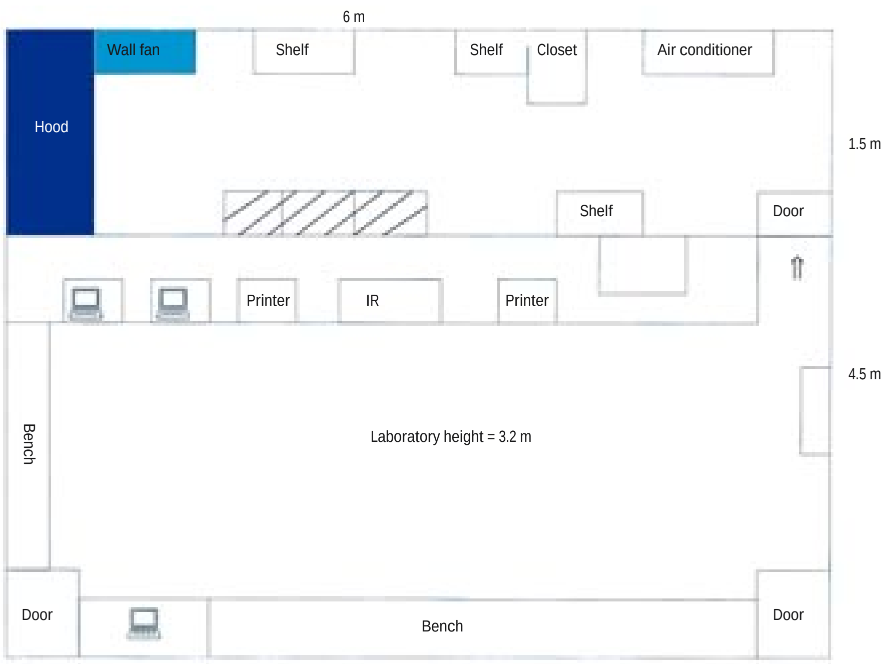

Figure 2. Schematic diagram of the laboratory where the patient works.

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 > 60%. After recently receiving artificial insemination (from her husband) the patient's wife became pregnant.

In summary, the patient's initial semen analysis was normal. After being highly exposed to chloroform, isooctane, and THF at work due to a malfunction of the ventilation system for 8 months, his semen analysis showed poor sperm motility. After the ventilation system was repaired, the patient's sperm motility was improved. Because chloroform is known to damage sperm (*8*), the possibility of chloroform causing significant reduction of sperm motility cannot be ruled out. The shutdown of the ventilation system may be the explanation for the reduction of the patient's 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.

Reference and Notes

1. NIOSH. Halogenated hydrocarbons: method 1003. In: NIOSH Manual of Analytical Methods (NMAM), 4th ed, (Eller P, Cassinelli M, eds). DHHS (NIOSH) 94-113. Washington, DC:National Institute for Occupational Safety and Health, 1994;1–7.
2. Harte J. Consider a Spherical Cow: A Course in Environmental Problem Solving. Mill Valley, CA:University Science Books, 1988.
3. Harrje DT. Building dynamics and indoor air quality. In: Indoor Air Pollution (Samet JM, Spengler JD, eds). Baltimore, MD:The Johns Hopkins University Press, 1991;68–81.
4. Letz G. Male reproductive toxicology. In: Occupational Medicine (LaDou J, ed). Norwalk, CT:Appleton & Lange Publisher, 1990;288–296.
5. Bonde JP, Giwercman A, Ernst E. Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design options. Occup Environ Med 53:511–519 (1996).
6. Whorton MD, Foliart DE. Mutagenecity, carcinogenecity and reproductive effects of dibromochloropropane (DBCP). Mut Res 123:13–30 (1983) .
7. Mottet NK, Shaw C-M, Burbacher TM. Health risks from increases in methylmercury exposure. Environ Health Perspect 63:133–40 (1985).
8. Topham JC. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mut Res 74:379–387 (1980).
9. Rosenthal SL. A review of the mutagenicity of chloroform. Environ Mol Mutagen 10:211–26 (1987).
10. Land PC, Owen EL, Linde HW. Morphologic changes in mouse spermatozoa after exposure to inhalational anesthetics during early spermatogenesis. Anesthesiology 54:53–56 (1981).
11. Mohamed MK, Burbacher TM, Mottet NK. Effects of methyl mercury on testicular functions in *Macaca fascicularis* monkeys. Pharmacol Toxicol 60:29–36 (1987).
12. Hilderbrand DC, Der R, Griffin WT, Fahim MS. Effect of lead acetate on reproduction. Am J Obstet Gynecol 115:1058–1065 (1973) .
13. Simons TJB. Lead-calcium interactions in cellular lead toxicity. Neurotoxicology 14:77–86 (1993).
14. Chaves E, Jay D, Bravo C. The mechanism of lead-induced mitochondrial Ca^{2+} efflux. J Bioenerg Biomembr 19:285–295 (1987).
15. Sandhir R, Julka D, Gill KD. Lipoperoxidative damage of lead exposure in rat brain and its implications on membrane bound enzymes. Pharmacol Toxicol 74:66–71 (1994).
16. Goldstein GW. Lead encephalopathy: the significance of lead inhibition of calcium uptake by brain mitochondria. Brain Res 136:185–88 (1977).
17. Green S, Fishel S. Morphology comparison of individually selected hyperactivated and non-hyperactivated human spermatozoa. Hum Reprod 14:123–130 (1999).
18. Donnelly GP, McClure N, Kennedy MS, Lewis SE. Direct effect of alcohol on the motility and morphology of human spermatozoa. Andrologia 31:43–47 (1999).
19. McConell JD. Abnormalities in sperm motility: techniques of evaluation and treatment. In: Infertility in the Male (Lipschultz LI, Howards SS, eds). 3rd ed. St. Louis, MO:Mosby-Year Book, Inc., 1997;254–259.
20. WHO. Collection and examination of human semen. In: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 3rd ed. New York:Cambridge University Press, 1992.
21. Tomlinson MJ, Barratt CL, Cooke ID. Prospective study of leukocytes and leukocyte subpopulations in semen suggests they are not a cause of male infertility. Fertil Steril 60:1069–1075 (1993).
22. Council of Labor Affairs, Executive Yuan, Taiwan, ROC. Permissible Exposure Limits for Airborne Hazardous Materials in the Work Place. Taipei, Taiwan:Council of Labor Affairs, Executive Yuan, 1995.
23. Air Contaminants. 29CFR § 1910.1000. Washington, DC:U.S. Department of Labor, 2001.