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THE EFFECTS OF ETHYLENE DIBROMIDE ON SEMEN QUALITY: A COMPARISON OF SHORT-TERM AND CHRONIC EXPOSURE

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Abstract — Two occupational field studies were conducted to determine the effects of ethylene dibromide (EDB) exposure on male reproductive potential. The first study was a longitudinal study of 10 EDB-exposed forestry employees and 6 unexposed men conducted in Colorado in the summer of 1983. The exposure time was approximately 6 weeks. The second study was a cross-sectional study of 46 EDB-exposed papaya workers and 43 unexposed men conducted in Hawaii in December 1983 in which the average term of employment was about 5 years. In the longitudinal study, sperm velocity decreased in all 10 exposed men and in only two unexposed men. Semen volume was also decreased in 9 of the 10 exposed men (there was no change in the other man); only two unexposed men had a decrease in their semen volume. The longer term EDB exposure resulted in decreases in sperm motility and viability, suggesting that the short term exposure may slow sperm velocity, but longer exposures cause immotility and cell death. An apparent decrease in semen volume that was observed in the longitudinal study was not statistically significant in the cross sectional study of workers having chronic exposure. However, a significantly higher semen pH was observed in the exposed men compared to the unexposed men in the cross-sectional study. The results from both studies suggest that the accessory sex glands may be affected by EDB exposure.

Key Words: Ethylene dibromide, EDB, Semen analysis, Occupational exposure, Sperm.

INTRODUCTION

Ethylene dibromide (EDB; also called dibromoethane) has been widely used in the United States as a scavenger in leaded gasoline and as an active component of approximately 100 pesticides used as soil, fruit, grain, and timber fumigants. The National Institute for Occupational Safety and Health (NIOSH) estimated in 1977 that approximately 108,000 workers (principally in manufacturing and formulating industries) were potentially exposed to EDB (1). An unknown number of agricultural workers have also been potentially exposed to EDB, although this number is declining due to restrictions placed on the use of EDB as a pesticide by the Environmental Protection Agency (EPA) since 1983 (2). The Occupational Safety and Health Administration (OSHA) has proposed a reduction in their current permissible exposure level from 20 ppm (20,000 ppb) to 100 ppb as an 8-hour time-weighted average (TWA) (3). The NIOSH recommended standard is 45 ppb (8-hour TWA) with a 15-minute ceiling limit of 130 ppb (4). EDB is a highly reactive alkylating agent, mutagenic in a number of genotoxicity assays, and carcinogenic in animals (1,5).

Animal studies have demonstrated effects of EDB on semen quality and fertility in several species. Decreases in sperm concentration and percent motility and increases in abnormal sperm morphology have been observed in bulls after oral administration (6–10). Dose-dependent increases in abnormally shaped sperm and decreases in the percentage of motile sperm were observed in rams after subcutaneous dosing (11) but not after oral administration at comparable doses (12). Edwards et al. (13) reported reductions in average litter size in untreated female mice mated with males given 10 mg/kg EDB intraperitoneally for 5 days, and in untreated female rats mated with males exposed to 39 ppm EDB by inhalation for 10 weeks (14). Hurtt and Zenick (15) evaluated EDB exposure in rats and found pronounced spermatogenic effects and decreased seminal vesicle weights.

There have been few previous attempts to

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evaluate fertility or semen quality among male workers exposed to EDB. In one published study of birth rates of children of wives of EDB-exposed men compared to U.S. national rates, a statistically significant decrease in the observed/expected birth ratio was found for one of four plants where EDB was used (16). In previous investigations of sperm characteristics (17–20), small sample sizes, lack of specification of the methods of sample collection and analysis, or lack of comparison groups and exposure data have resulted in inconclusive findings. Further, previous investigators have not examined a range of sperm characteristics (including viability, motility, and morphology) that may affect male fertility.

Two independent studies were conducted to assess the effects of EDB exposure on semen quality. The first study was a longitudinal study of short term EDB exposure conducted on temporary forestry employees hired by the state of Colorado from May to July, 1983. The second was a cross-sectional study of male workers exposed to EDB used in the treatment of papaya for fruit fly infestation on the island of Hawaii in December, 1983. This report describes the results of these studies and compares the male reproductive effects observed following short-term and chronic EDB exposure.

MATERIALS AND METHODS

Semen analysis

The semen analysis for each of these studies was conducted by the same research team using the identical methods within a single year. Subjects were asked to collect a semen specimen at home by masturbation into a coded, sterile glass jar, after a minimum of 2 days and preferably less than 5 days of abstinence. The sample was placed in a thermos container (Aladdin, Model #7100, Nashville), and brought to the laboratory within 1 h of collection. The date and time of ejaculation, abstinence period, and spillage (if any) were recorded on the jar label by the subject. Appointments for semen sample delivery were scheduled to insure that the analyses could be conducted before sample age would become a confounding factor.

Semen analyses were conducted in two phases. Video recordings, viability assessments, sperm counts, volume and pH measurements, fixation of slides, and cryopreservation of seminal plasma were conducted at the field study location. Morphology and morphometry analyses of slides and motility and velocity analyses of video tapes were conducted at NIOSH laboratories. All samples were processed and analyzed in blind fashion by the investigators.

Sperm velocity and motility. Seven microliters of the semen sample were placed on a glass slide under a coverslip and placed on a microscope stage warmed to 37°C by a heat curtain. Five to eight fields selected arbitrarily were video recorded using a 25× phase objective, a video recorder, vertical enhancer, and high resolution camera and monitor. The time from ejaculation to videotaping (sample age) was recorded. Video tapes were analyzed using a semiautomatic image analysis system with video overlay in digitizing tablet. For sperm velocity measurements, thirty motile sperm from each sample were randomly selected, the path of each was digitized, and the start and stop times (to the nearest 0.01 second) for each tracing were recorded. Sperm velocity was measured both as velocity along the actual sperm path and straight line (point-to-point or distance) velocity (21,22).

The percentage of motile sperm was determined by marking all sperm observed in one video frame, then advancing the video tape to identify motile and nonmotile sperm. This process was repeated for a minimum of five fields, so that an average of 200 sperm were scored.

Semen pH and volume. The pH of the semen sample was determined using a pH meter equipped with a gel filled plastic pH electrode. The total volume was measured using a 5 mL plastic disposable syringe.

Sperm morphology and morphometry. Four air-dried smears were prepared from each whole semen sample, fixed in absolute ethanol for 10 minutes, and stored for later analysis. Slides were stained in Papanicolau stain according to World Health Organization semen analysis guidelines (23). Sperm morphology was scored according to Zaneveld and Polakoski (24) reading 200 cells on each of two slides. The remaining two slides were used for objective analysis of sperm head shape (morphometry) using a 63× dry objective and video camera with a 4× enlarger to evaluate 100 sperm on each of two slides. Individual sperm heads were outlined using a digitizing tablet; the software allowed calculations of area, perimeter, length, width, width/length ratio, and 4π (area)/perimeter^2 (Pi factor).

Sperm viability. Viability by stain exclusion, modified from Eliasson and Treichl (25) was determined by mixing 100 μL of semen with 100 μL of 0.5% (w/v) eosin y stain in Tyrodes buffer. A 7 μL

sample was placed on a slide and 200 sperm were classified as unstained (viable) or stained (nonviable). Viability by hypoosmotic swelling (26) was analyzed by mixing 100 μL of semen with 1.0 mL of a solution containing 150 milliosmolar sodium citrate and 150 milliosmolar fructose. After an incubation of at least 30 minutes (after which further swelling does not occur), 7 μL were placed on a slide, and 200 sperm were classified as swollen (viable) or unswollen (nonviable) using differential interference contrast (DIC) microscopy.

Sperm concentration. One hundred microliters of semen were mixed with 100 μL of distilled water. Five microliters of this suspension were placed on a Makler Chamber and the sperm were counted using DIC microscopy. A replicate was prepared and counted for each sample. The mean of the two counts was used for further calculations.

Questionnaire

A detailed questionnaire was administered by personal interview to provide personal and demographic data, medical history, smoking and drinking habits, work history, and current and previous exposures to known or suspected chemical or physical hazards.

The longitudinal study of short term exposure

Fifteen workers were engaged in the task of applying EDB insecticide to felled pine trees to kill developing insects before they reached sufficient maturity to leave the host tree and attack nearby healthy trees. Infested trees were identified by another crew earlier in the year and the timber was cut to length, stacked, and covered with polyethylene sheets before the fumigation crew arrived.

The fumigation of felled trees was accomplished by one of two methods. For the smaller wood piles, an EDB emulsion was carried in 5 gal jerry cans to the site, and a calculated volume (2.0 gal per cord) of emulsion was poured through a slit in the plastic covering onto the stacked wood. When completed, the slit was sealed with adhesive tape. For larger piles which were occasionally near a roadway, insecticide was applied by spraying the emulsion under pressure by a hose supplied from a nearby tank truck. The EDB content of the emulsion, whether delivered by pouring or spraying, was approximately 4% by volume. The Colorado Forestry Service indoctrinated each worker about the known dangers of EDB and the appropriate handling of the chemical during each assigned task. Protective gloves were distributed to the workers; however, these were not always worn. Respirators were distributed to the workers and were occasionally worn during the filling of the trucks and the spraying of the emulsion.

To determine EDB airborne concentrations, two types of sampling pumps were used to collect samples at low and high sampling rates over periods ranging from 15 min to 8 h. The high-rate pumps were used for short-term samples which ranged from 15 min to 1 h in duration, and the low rate pumps were used for the 8-h samples. Standard 150 mg activated charcoal tubes were used as the collection media for all breathing zones (BZ) samples. Samples were collected by attaching the charcoal sample tube to the worker's collar which was connected by tubing to a sampling pump worn on the worker's belt. The 8 h samples were collected from 15 workers for 3 days. The charcoal tubes were analyzed for EDB using gas chromatography and an electron capture detector.

Ejaculates from 12 men (ages 20 to 32, mean 25.1) were collected and examined 1 to 2 weeks before exposure and during the last week of exposure to EDB. A control group population of 6 men (ages 20 to 35, mean 26.5) was also evaluated at the same sampling times. These men were also forestry workers, but their work duties did not allow contact with the EDB fumigation operations.

The cross-sectional study of chronic exposure

Six plants which were engaged in the fumigation of papaya for fruit fly infestation, employing a total of approximately 75 male workers, were investigated. Two industrial hygiene surveys of these facilities were conducted a year prior to, and during, the semen and cytogenetic study, and are described in detail elsewhere (27). Air samples were conducted in all plants on at least two separate days. Workers were sampled for a full 8-h shift unless their duty cycle indicated a shorter sampling period. Several short term (14–75 min) measurements of exposure concentrations during entry into the fumigation chamber by fumigators and forklift drivers were also conducted. A total of 82 full-shift and 19 short-term samples were collected.

Letters were sent to each male worker (all of whom were potentially exposed) explaining the purpose of the study. Each man was subsequently interviewed confidentially to solicit participation. Seventy-two percent of currently employed workers from the first five plants agreed to participate and provided semen samples. Approximately 33% of those at the sixth plant, who could be contacted only at the time of the field study, agreed to partici-

Table 1. EDB exposure personal breathing zone air samples (parts per billion)

Exposure	Longitudinal study Acute exposure	Cross-Sectional study Chronic exposure
Time-weighted average	60	88
Peak exposures	2165	262
Skin exposure	Extensive	Moderate

pate. The overall participation rate was 64%, suggesting that the participation rate may be improved by the provision of explanatory information prior to the study. Four exposed workers were subsequently excluded due to medical conditions causing azoospermia or due to employment of less than 2 months. The average duration of employment for exposed workers was 5 years (4.9 ± 3.6 years). Workers in a nearby sugar processing plant with no EDB exposure were identified as a control group of similar ethnic background, socioeconomic status, and age range. The first 50 workers who volunteered to participate (for payment) were selected; 43 men provided semen samples.

RESULTS

The longitudinal study of short term exposure

To study potential peaks in exposure during specific operations, short-term samples were collected over durations of 15 min to 1 h. These data are summarized in Table 1. The short-term exposures while filling the truck tanks ranged from nondetectable levels to 2165 ppb. Spraying log piles resulted in short-term exposures from 57 to 525 ppb. The short term exposures while pouring the EDB emulsion on the log piles ranged from 8 to 184 ppb. It should be noted that the NIOSH recommended ceiling limit for 15-min exposure is 130 ppb.

Skin absorption was recognized as a major potential route of exposure in this work setting, therefore the use of dermal dosimeters was attempted in this study. This methodology is still in the experimental stage and no quantitative exposure estimates were obtained. However, extensive skin exposure was noted by observation of the workers. The personal characteristics of the 12 exposed and 6 unexposed men in the semen study were evaluated from the questionnaires and physical exam. All but three of these workers had lived in the state of Colorado during the preceding year. One exposed and one control group subject reported previous urogenital illness and two exposed subjects had evidence of a moderate varicocele on physical exam. No changes in medication usage or personal habits (smoking, clothing, and use of hot baths) were reported by the participants between the first and second samples, with the exception of one exposed man who had taken antibiotics prior to, but not during, the study period. Changes in alcohol and beverage consumption were minimal. No changes in health status were reported, with the exception of seven exposed and four unexposed men who had had influenza like symptoms within the 3 months preceding the first sample (none with accompanying fever), but not during the period of study.

Due to missing data on abstinence and sample age, two individuals were eliminated from the statistical analyses; therefore, statistical evaluations were based on 10 exposed and 6 control men. Three sets of statistical analyses of the semen characteristics were performed using the Wilcoxon rank sum test. Comparisons were made between the control and exposed groups before exposure, after exposure, and finally a comparison of the observed changes from the preexposure to postexposure period between the exposed and control groups.

There were no significant distributional differences in the semen characteristics for pH, viability by hypoosmotic swelling, or sperm concentration (sperm/mL and sperm/ejaculate) using the three sets of statistical analyses. The median distribution of the morphology and morphometry characteristics was consistent between groups and collection times. Statistical analysis of the observed changes between groups found significant differences in semen volume ($p = 0.03$), length of abstinence ($p = 0.03$), age of the semen sample ($p = 0.003$), and the distance (point-to-point) velocity ($p = 0.004$).

The distance velocity of the exposed group decreased significantly ($p = 0.004$) in relation to the control group. The observed changes for the 10 exposed and 6 control subjects are illustrated in Figure 1. Every exposed man had a decrease in sperm velocity, while only two unexposed men showed a decrease. Even though there was a significant difference in sample age, there was no significant correlation between sperm velocity and sample age. The median velocity of the preexposure and postexposure groups were not significantly different from the respective control groups. There was a significant correlation (Spearman rank $p < 0.05$) between the preexposure value and the net change in velocity for an individual. The m-ranking procedure with the preexposure value as the covariant was performed and the decrease in sperm velocity was

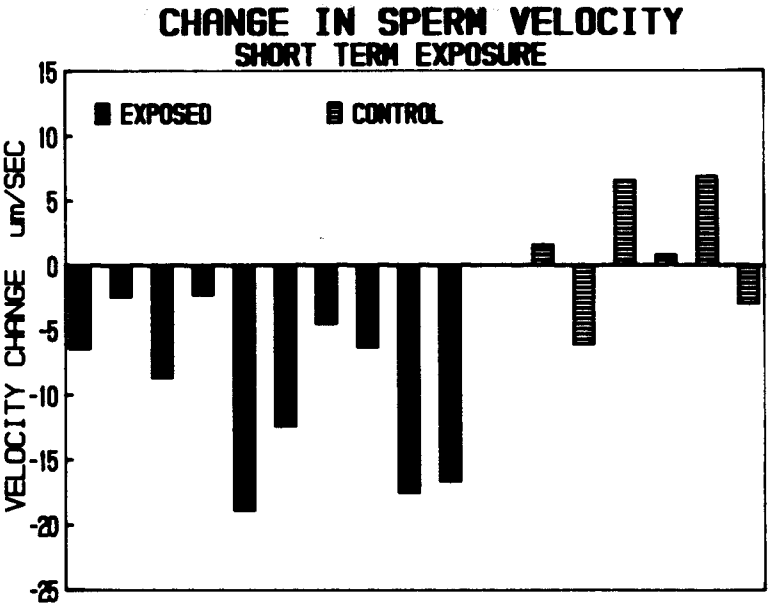

Fig. 1. Change in sperm velocity in the EDB study of short-term exposure. The solid bars represent the change in average sperm velocity for each exposed man. The dashed bars represent the change in the average sperm velocity for each unexposed man.

still significant ($p = 0.01$). Due to the relationship between the path length velocity and the distance velocity, the same procedures were performed on the path length velocity; however, no significant differences were observed for the Wilcoxon rank sum test ($p = 0.14$) or for the m-ranking procedure ($p = 0.21$). There were no significant differences in the ratio between the path length and distance velocities.

The change in abstinence time of the control was significantly different ($p = 0.03$) from the change in the exposed group. The median length of abstinence did not change from the first to second sample in the exposed group, while the control group had a median increase of 2 days. There was a significant decrease ($p = 0.03$) in the volume of semen from the exposed workers. Nine of the ten exposed men had a decrease in semen volume (the tenth man had no change). Only two of the six unexposed men had a decrease in semen volume (Figure 1). While abstinence is believed to be a confounder of volume (28), there was not a significant correlation between change in abstinence time and change in volume.

The cross-sectional study of chronic exposure

The results of this study are published in detail elsewhere (29) and are summarized here. The levels of exposure are presented in Table 1. Table 2 sum-

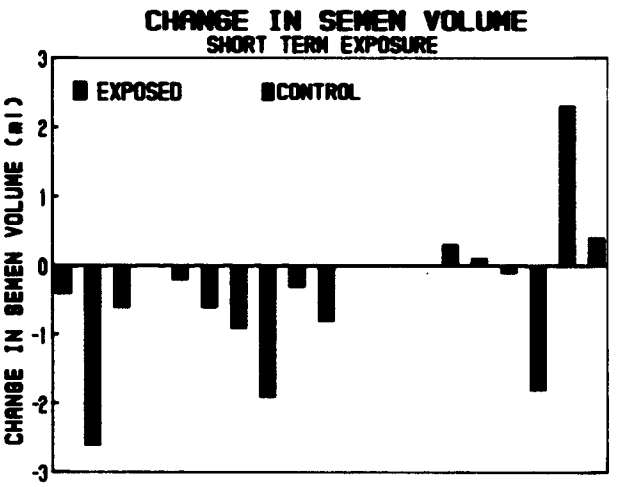

Fig. 2. Change in semen volume in the EDB study of short-term exposure. The solid bars represent the change in semen volume for each exposed man. The dashed bars represent the change in the semen volume for each unexposed man.

Table 2. The significant effects of EDB chronic exposure (mean ± standard deviation)

Variable	Control	Exposed	p
Sperm count/ejaculate (millions)	204 ± 199	134 ± 138	0.009
Sperm viability (%)			
Stain exclusion	73.0 ± 8.7	68.1 ± 15.8	0.003
Hypoosmotic swelling	74.3 ± 9.1	68.5 ± 12.9	0.001
Sperm motility (%)	44.9 ± 14.2	40.9 ± 18.2	0.031
Sperm morphology (%)			
Tapered head	2.5 ± 3.2	4.2 ± 6.2	0.001
Absent head	1.4 ± 1.3	2.1 ± 1.8	0.001
Abnormal tails	5.7 ± 4.5	6.5 ± 10.7	0.001
Sperm head width (micrometers)	2.63 ± 0.25	2.56 ± 0.24	0.040
Semen pH	7.90 ± 0.22	8.01 ± 0.24	0.030

marizes the statistically significant results of this study. Both mean semen volume and sperm concentration were lower in the exposed group than among unexposed workers, but the differences were not statistically significant after consideration of potentially confounding variables. The mean sperm count per ejaculate was, however, significantly lower among exposed workers after adjustment for abstinence (Table 2). The percentage of viable sperm by both stain exclusion and hypoosmotic stress was significantly lower among exposed men after adjustment for subject's age (Table 2). Sperm velocity, however, did not differ significantly between the two groups. With respect to sperm morphology, there was no significant difference in the overall proportion of normal forms between the two groups, but exposed workers showed a statistically significant increase in the proportion of specific abnormal forms [tapered heads, absent heads, and abnormal tails (Table 2)]. The presence of tapered heads was also reflected in a significant ($p = 0.04$) decrease in average head width by morphometric analysis among exposed workers (Table 2). Several other factors were significantly related to one or more semen characteristics and to exposure in this population (including abstinence and subject's age), emphasizing the need to control for potential confounders in the analysis. The time from sample collection to laboratory analysis (average 40 min) was not significantly related to a decrease in sperm viability, motility, or velocity. This observation is supported by the findings of Makler et al. (30), who found that sperm velocity did not decline significantly for at least 2 h after ejaculation, and indicated that on-site collection of samples is not a prerequisite for the measurement of these characteristics provided that samples are brought to the laboratory and analyzed within approximately 2 h of ejaculation.

DISCUSSION

Most occupational field studies usually involve a toxicant exposure over several years in which testicular alterations can often be determined by shifts in sperm concentration or sperm cell morphology. However, in a study involving short-term employment (e.g., summer employment, as in this study), spermatogenic function is difficult to study because a complete spermatogenic cycle (at least 74 days long (31)) may not have occured from the time of exposure to employment termination. The semen characteristics traditionally analyzed in field studies, e.g., sperm concentration and morphology, would not be expected to be altered in the ejaculate until at least one or possibly more spermatogenic cycles have occurred after the onset of exposure. However, alterations in sperm cell function could occur in the mature spermatozoa, and these characteristics could be detected soon after the initial exposure. Sperm velocity, the progressive motility ratio, and sperm viability are indices of sperm cell function that might be considered to be early markers of toxic effects.

The cross-sectional study of chronically exposed papaya workers was conducted to determine whether or not the differences detected in the longitudinal study of workers with short-term EDB exposure also occurred following chronic exposure.

Sperm motility is one of the most important aspects of sperm function (32) and is considered one of the best indicators of fertility (33). A shift in sperm motility characteristics is, therefore, a major concern in reproductive toxicology. The major effect detected in the pine tree fumigator study was a decrease in sperm velocity in men after short-term exposure to EDB.

While the sperm velocity in the papaya study was not significantly decreased, the overall number

of motile sperm was decreased. It appears that while sperm in the longitudinal study were swimming more slowly, many of the sperm in the chronic study were immotile (i.e., dead as determined by the viability assessed), so these results are not inconsistent.

In the longitudinal study of EDB exposed forestry workers, semen volume was also significantly decreased after exposure to EDB. This decrease does not appear to be statistically related to abstinence time. In the cross-sectional study of papaya workers, semen volume was also decreased, though not significantly after consideration of cofounders. Also, in the latter study the semen pH was significantly higher in the exposed workers compared to the unexposed men. These differences related to exposure suggest that the accessory sex glands have in some way been affected. We are currently conducting research on the seminal plasma from the cross-sectional study to determine which gland(s) may be affected.

The papaya workers study also detected spermatogenic effects. There were significant increases in abnormal forms and sperm count/ejaculate was decreased. Such effects could not be detected in the short-term study because the spermatogenic cycle is longer than the exposure period.

The results of these studies complement each other and suggest that the reproductive toxicant EDB has multiple sites of action.

REFERENCES

1. National Institute for Occupational Safety and Health. Criteria for a recommended standard. Occupational exposure to ethylene dibromide. Cincinnati, OH: NIOSH Pub. No. 77-221; 1977.
2. Environmental Protection Agency. Ethylene Dibromide: Position Document No. 4. EPA Office of Pesticides Programs, Washington, DC; September 27, 1983.
3. U.S. Department of Labor. Occupational Safety and Health Administration. Notice of proposed rulemaking: occupational exposure to ethylene dibromide. *Federal Register* 1983; **48**:45956–6003.
4. National Institute for Occupational Safety and Health. Testimony on the Occupational Safety and Health Administration's proposed rulemaking on occupational exposure to ethylene dibromide; Washington, DC; February 9, 1984.
5. National Institute for Occupational Safety and Health. Ethylene dibromide (Revised). Current Intelligence Bulletin No. 37. NIOSH Pub. No. 82-105; 1981.
6. Amir, D. The sites of the spermicidal action of ethylene dibromide in bulls. *J Reprod Fertil* 1973; **35**:519–525.
7. Amir D. Individual and age differences in the spermicidal effect of ethylene dibromide in bulls. *J Reprod Fertil* 1975; **44**:561–565.
8. Amir D, Esnault C, Nicolte JC, Conrot M. DNA and protein changes in the spermatozoa of bulls treated orally with ethylene dibromide. *J Reprod Fertil* 1977; **51**:453–456.
9. Amir D, Lavon U. Changes in total nitrogen, lipoproteins and amino acids in epididymal and ejaculated spermatozoa of bulls treated orally with ethylene dibromide. *J Reprod Fertil* 1976; **47**:73–76.
10. Amir D, Volcani R. Effects of dietary ethylene dibromide in bull semen. *Nature* 1965; **206**:99–100.
11. Elijack AH, Hrudka F. Pattern and dynamics of teratospermia induced in rams by parenteral treatment with ethylene dibromide. *J Ultrastr Res* 1979; **67**:124–134.
12. Bondi A, Alumot E. Effect of EDB fumigated feed on animals. Final Technical Report PL-480. U.S. Dept. Agriculture; Washington, DC; 1966.
13. Edwards K, Jackson H, Jones AR. Studies with alkylating esters II. A chemical interpretation through metabolic studies of the antifertility effects of ethylene dimethanesulphonate and ethylene dibromide. *Biochem Pharmacol* 1970; **19**:1783–1789.
14. Short R, Winston J, Hong C, Minor J, Lee C, Seifter J. Effects of ethylene dibromide on reproduction in male and female rats. *Toxicol Appl Pharmacol* 1979; **49**:97–105.
15. Hurtt M, Zenick H. The spermatotoxic effects of ethylene dibromide (EDB). *Toxicologist* 1985; **5**:114.
16. Wong O, Utidjian MD, Korten VS. Retrospective evaluation of reproductive performance of workers exposed to ethylene dibromide. *J Occup Med* 1980; **21**:98–102.
17. National Institute for Occupational Safety and Health. Health Hazard Evaluation Report No. HE 77-119-481. Hazard Evaluations and Technical Assistance Branch, Division of Surveillance, Hazard Evaluations and Field Studies, NIOSH, Cincinnati, Ohio; 1978.
18. Griffiths J, Heath R, Davido R. Spermatogenesis in agricultural workers potentially exposed to ethylene dibromide. Interim Report, Human Effects Monitoring Branch, EPA, Washington, DC; June 8, 1978.
19. Ter Haar G. An investigation of possible sterility and health effects from exposure to ethylene dibromide. In: Banbury Report No. 5, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1980.
20. Rogers BJ, Fujita JS, Najita L, Hale RW. Reduction of sperm concentration in a population exposed to ethylene dibromide (Abstract) Proc 6th Ann Mtg Andrology Soc *J Androl* 1981; **2**:35–36.
21. Overstreet JW, Katz DF, Hanson FW, Fonseca JR. A simple inexpensive method for objective assessment of human sperm movement characteristics. *Fertil Steril* 1979; **31**:162–172.
22. Jenks JP, Cosentino MJ, Cockett ATK. Evaluating sperm motility: a comparison of the Rochester motility scoring system versus videomicrography. *Fertil Steril* 1982; **38**:756–759.
23. Belsey MA, Moghissi KS, Eliasson A, Paulsen CA, Gallegos AJ, Prasal MRN. *Laboratory manual for the examination of human semen and semen cervical mucus interaction*. Singapore: Press Concern; 1980.
24. Zaneveld LJD, Polakoski KL. Collection and physical examination of the ejaculate. In: Hafez ESE, ed. *Techniques of human andrology*. New York: Elsevier; 1977:147–172.
25. Eliasson R, Treichl L. Supravital staining of human spermatozoa. *Fertil Steril* 1971; **22**:134–137.
26. Jeyendran RS, Van de Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil* 1984; **70**:219–228.
27. Clapp DE. Industrial hygiene survey of papaya fumigation facilities in Hawaii. Industrial Hygiene Section, Industrywide Studies Branch, Division of Surveillance, Hazard Evaluations and Field Studies, NIOSH, Cincinnati, Ohio; 1984.
28. Schwartz D, Laplanceh A, Jouannet P, David P. Within-subject variability of human semen in regard to sperm count, volume, total number of spermatozoa and length of abstinence. *J Reprod Fertil* 1979; **57**:391–395.
29. Ratcliffe JM, Schrader SM, Steenland K, Clapp DE, Turner T, Hornung RW. Semen quality in papaya workers with long

term exposure to ethylene dibromide. *Br J Ind Med* 1987; **44**:317–326.
30. Makler A, Zaidise I, Paldi E, Brandes JM. Factors affecting sperm motility. I: In vitro change in motility with time after ejaculation. *Fertil Steril* 1979; **31**:147–154.
31. Huckins C. Adult spermatogenesis: characteristics, kinetics, and control. In: Lipschultz LI, Howards SS, eds. Infertility in the male. New York: Churchill Livingston; 1983:113.
32. Alexander NJ. Male evaluation and semen analysis. *Clin Obstet Gynecol* 1983; **5**:463–482.
33. Wyrobek AJ, Gordon LA, Burhart JG, Francis MW, Kapp RW Jr, Letz G, Malling HV, Topham JC, Whorton MD. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res* 1983; **115**:73–148.