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Semen Study of Papaya Workers
Exposed to Ethylene Dibromide

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16. Abstract (Limit: 200 words) A cross sectional semen and cytogenetic study was performed on male workers exposed to ethylene-dibromide (106934) (EDB) in the papaya fumigation industry in Hawaii. Semen analyses were conducted on 46 men in six fumigation facilities with an average length of employment of 5 years and airborne exposures to EDB ranging from 16 to 213 parts per billion. Statistically significant decreases in sperm count per ejaculate and the percentage of viable and motile sperm and increases in the proportion of specific morphological abnormalities were observed among exposed men when compared with controls. Semen volume and sperm concentration were also lower in the exposed group. No effect of exposure to EDB on sperm velocity, the overall proportion of sperm with normal morphology or YFF bodies was noted. The authors conclude that based on the decreases in sperm count, viability and motility and increases in certain types of morphological abnormalities among workers exposed to EDB, EDB may increase the risk of reproductive impairment in workers at exposure levels near the NIOSH recommended limit of 45 parts per billion and far below the current OSHA standard of 20 parts per million.			
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ABSTRACT

Ethylene dibromide (EDB) is widely used as a scavenger in leaded gasoline and in pesticides used for timber, grain, soil and fruit fumigation. It is a highly reactive alkylating agent with mutagenic and carcinogenic properties. Evidence from animal studies suggests that EDB may affect spermatogenesis and fertility in males, but adequate studies of its reproductive toxicity in humans are lacking. The National Institute for Occupational Safety and Health (NIOSH) has conducted a cross-sectional semen and cytogenetic study of male workers exposed to EDB in the papaya fumigation industry in Hawaii. The results of the semen study are presented here. Semen analyses were conducted on 46 men in six fumigation plants with an average length of employment of 5 years and airborne exposures to EDB ranging from 16 to 213 ppb (plant averages for full-shift breathing zone samples); the comparison group consisted of 43 unexposed men from a nearby sugar refinery. Statistically significant decreases in sperm count per ejaculate and the percentage of viable and motile sperm and increases in the proportion of specific morphological abnormalities (tapered heads, absent heads and abnormal tails) were observed among exposed men in comparison with controls after consideration of smoking, caffeine and alcohol consumption, subjects' age, abstinence, age of the sample at analysis and other potentially confounding variables. Semen volume and sperm concentration were also lower in the exposed group than the unexposed group but these differences were not statistically different after correction for confounding factors. No effect of exposure to EDB on sperm velocity, the overall proportion of sperm with normal morphology or YFF bodies was observed. These data strongly suggest that EDB may increase the risk of reproductive impairment in workers at exposure levels near the NIOSH recommended limit of 45 ppb (as an 8 hour time weighted average) and far below the current OSHA standard of 20 ppm.

I. INTRODUCTION

There has been increasing concern in recent years about the potential mutagenic, carcinogenic and reproductive effects of ethylene dibromide (EDB). Approximately 90 million kg. of EDB are produced annually in the United States. EDB is used principally 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; an unknown number of temporary and permanent agricultural workers are potentially exposed to EDB during the application of EDB-containing pesticides and the handling of EDB-contaminated food products (NIOSH, 1977). NIOSH recommended a standard of 130 ppb as a 15 minute average exposure in the 1977 criteria document (NIOSH, 1977), which was reaffirmed in 1981 following further evidence of EDB's carcinogenicity in animals (NIOSH, 1982). In September 1981, California OSHA adopted the 130 ppb NIOSH recommendation as a standard. In December 1981, OSHA announced advanced notice of proposed rulemaking to reduce the current standard of 20 ppm (8 hour time weighted average) and 30 ppm ceiling limit to 0.1 ppm as an 8-hour TWA. Hearings have been conducted, at which NIOSH recommended an 8 hour TWA of 45 ppb in addition to the 15 minute limit (NIOSH, 1984a), but the standard has not yet been promulgated. Finally, the Environmental Protection Agency (EPA) proposed the cancellation of the registration of EDB for use on stored grain,

for soil and timber fumigation, and for the post-harvest fumigation of citrus and tropical fruits and vegetables (EPA, 1983). The use of EDB on stored grain and for soil and timber fumigation was suspended in 1984; its use as fumigant for exported tropical fruits is still permitted.

Despite conclusive evidence from animal studies that EDB is a mutagen, a carcinogen and a spermatotoxin, few studies of workers exposed to EDB have been conducted. NIOSH researchers have therefore conducted a cross-sectional combined cytogenetic and semen study among male workers with chronic exposure to EDB in the treatment of papaya for fruit fly infestation. This report describes the study of semen characteristics; the results of the cytogenetic study are reported separately.

II. OVERVIEW OF REPRODUCTIVE EFFECTS OF EDB IN MALES

(i) Animal Studies

Oral administration of EDB has been shown to produce decreases in sperm concentration and percentage motility and increases in abnormal sperm morphology in bulls after two or more weeks of oral dosing with 2-4 mg/kg of EDB (Amir and Volcani, 1965; Amir 1973, 1975; Amir and Lavon, 1976; Amir et. al., 1977). DNA, lipoprotein and amino acid changes in the spermatozoa were also observed (Amir 1973; Amir et. al., 1977). Amir (1973) concluded that EDB affected both the process of spermatogenesis and

the maturation of spermatozoa in the testis and epididymis.

Dose-dependent increases in abnormally shaped sperm with extensive nuclear abnormalities and decreases in percentage motility were observed in rams given from 7.8-13.5 mg/kg EDB subcutaneously for 12 days (Eljack and Hrudka, 1979) but not after oral administration at comparable doses (Bondi and Alumot, 1966). Measurements of sperm velocity were not conducted in these animal experiments. Edwards et. al. (1970) reported reduced average litter sizes in untreated female rats and mice when mated with males given 10 mg/kg i.p. for 5 days. Short et. al. (1979) also investigated the effect of exposure to EDB on litter sizes among untreated female rats mated with males exposed to 19, 39 and 89 ppm EDB by inhalation for 10 weeks. Decreases in testicular size and litter sizes were observed among the highest exposure group. The contributory effects of increased maternal morbidity observed in this group cannot, however, be excluded.

(ii) Human Studies.

There have been four previous reports of sperm characteristics and one fertility study of male workers exposed to EDB. Twelve workers potentially exposed to EDB at an anti-knock fuel additive manufacturing plant showed a significant decrease in median but not in mean sperm counts and significantly elevated levels of luteinizing hormone in comparison with seven controls (NIOSH, 1978). All except one of the 38 air samples taken during the investigation indicated non-detectable levels of EDB. No

statistically significant differences in sperm concentration or motility were found among 44 soil and citrus fumigators with varied estimated potential exposure to EDB (Griffiths et. al., 1978). Ter Haar (1980) reported the distribution of sperm concentration among manufacturing workers divided according to estimated exposure to more than 0.5 ppm (n=19) and less than 0.5 ppm (n=40). A higher percentage of the former group had sperm concentrations of less than 40 million per ml (42% vs. 20% in the latter group), but no statistical analyses were performed on the data and no control subjects were included.

Finally, semen analyses were performed and serum gonadotropic hormone levels measured for 16 soil fumigators and 39 residents of an area in Hawaii with EDB contaminated well water and compared with 28 controls from an uncontaminated area by Rogers et. al (1981). No differences in mean sperm concentration, percentage motility or morphology were noted between the three groups, but a higher proportion of workers and potentially exposed residents (44 and 39% respectively) had sperm concentrations of less than 40 million per ml, compared with 18% of the controls. Six of the potentially exposed residents also had abnormally low testosterone levels (less than 3 ng per ml); no differences in LH or FSH levels were found. A higher proportion of workers and potentially exposed residents had impaired fertilization capacity in the zona free hamster egg fertilization assay.

In each of these reports, small sample sizes, lack of specification of the methods of sample collection and analysis and inadequate control groups and/or exposure data mean that the results of these studies are unsatisfactory as a basis for conclusions. NIOSH has recently conducted a longitudinal study of 12 timber fumigators with short term exposure to EDB at levels comparable to the NIOSH recommended standard of 130 ppb, using the same methodology and range of semen characteristics as measured in the present study (NIOSH, 1984b). A statistically significant decrease in sperm velocity and a marginally significant decrease in semen volume was observed among exposed workers when pre- and post-exposure samples were compared. No other effects on semen quality were observed.

In a study of EDB-exposed workers (Wong et. al., 1980), the number of live births among wives of white male workers during a 19 year study period were compared with U.S. national birth rates. A statistically significant decrease in the observed/expected birth ratio was found for one of the four plants. However, the higher rate of vasectomies at this plant and other methodological limitations in this study preclude any firm conclusions with respect to the effect of EDB on male fertility.

III. DESCRIPTION OF WORKFORCE AND EXTENT OF EXPOSURE

EDB is used as a post-harvest fumigant in Hawaii to treat fruit fly infested papaya intended for export, principally to the U. S. mainland and Japan. EDB is introduced into a fumigation chamber in pure liquid form in a closed system and evaporated by heating. Harvested papaya are loaded in the fumigation chamber in bins by forklift operators and fumigated for 2-3 hours. Following unloading of the chamber, treated fruit are sorted, graded, and packed for transport. There are currently six papaya treatment plants on Hawaii, all located in or near Hilo, Hawaii, employing a total of approximately 75 males and 115 females. Most of the plants consisted of a single work area where sorting and packing of fumigated fruit was done, with a fumigation chamber often opening into the same room. Packers and sorters generally work inside all day and rarely enter the fumigation chamber. Forklift drivers and fumigators frequently enter the chamber but also work outside for periods of the day. No respirators or other personal protective equipment are used.

NIOSH conducted two industrial hygiene surveys of these facilities a year prior to and during the semen and cytogenetic study. (A complete description is given in a separate report [NIOSH, 1984c]). Personal (breathing zone) samples were taken according to NIOSH Standard Method P&CAM 260. Samples were collected in all plants on at least two separate days. Workers were sampled for a full 8 hour shift unless their duty cycle indicated a shorter sampling

period. Several short term (14-75 minutes) measurements of exposures during entry into the fumigation chamber by fumigators and forklift drivers were also conducted. The results are summarized in Table 1. Full shift exposures ranged from 16 to 213 ppb and were generally below the proposed OSHA standard of 100 ppb as an 8 hour time weighted average and far below the current standard of 20 ppm (20,000 ppb). Numerous individual samples exceeded the NIOSH recommended 8 hour TWA of 45 ppb and the ceiling limit of 130 ppb. One - way analysis of variance showed no significant differences between job categories or plants, although exposures were higher in Plant 6 (mean 148 ppb) where packing and sorting were done in the fumigation chamber itself. The geometric mean of the short term exposures for forklift drivers was 87 ppb and for fumigators, 40 ppb; exposures ranged from 12 - 262 ppb. The lower full shift values for these workers may be attributed to the fact that they also spend more time outside the plant than packers and sorters. The airborne levels of EDB measured in consecutive years are broadly comparable; reported changes in work practices and reductions in the amount of pure EDB used in the fumigation process suggest, however, that exposures may have been higher in previous years.

In summary, the exposure data show a) that men in each job category have closely comparable 8 hour time weighted average exposure to EDB, with higher short-term exposures among forklift drivers when entering or leaving the fumigation chamber and b) that men in 5 of the 6 plants have comparable 8 hour time-weighted average exposures across job categories, with the exception of men in plant 6, whose full shift exposures were higher than those in any of the other five plants.

IV. SEMEN STUDY DESIGN AND METHODS

a) Selection of Participants

(i) Exposed workers

Personnel lists of all male workers currently employed at five of the six plants (the sixth was located during the field study itself) were obtained during a site visit by one of the study team (JMR). Letters were sent to each man explaining the purpose of the study and details of the samples to be collected. Each man was subsequently interviewed on a one to one basis by two of the study team (during a second site visit) to solicit participation. 72 percent of currently employed workers from the first five plants agreed to participate; approximately 33 percent of those at the sixth plant, who were contacted only at the time of the field survey, agreed to participate. Two workers with less than two months' employment were excluded.

(ii) Unexposed Workers

Workers in a sugar processing plant adjacent to one of the papaya treatment plants in Hilo were identified as a suitable control group of similar ethnic background, socioeconomic status and age range. A walk-through survey of the plant was conducted by the industrial hygienists and established that these workers had minimal exposure to chemical and physical hazards, with the exception of garage mechanics and truck drivers who had potential exposure to diesel fumes and oils, and a small number of field workers who had occasional exposure to pesticides.. Letters were circulated to each of the approximately 200 male workers explaining the purpose of the study and soliciting participation. The first 50 workers who volunteered to participate were selected as controls.

b. Questionnaire and Semen Collection

Each participant was given a detailed consent form and a questionnaire was administered by personal interview to provide data on personal and demographic data, medical history, smoking and drinking habits, work history and current and previous exposures to chemical and physical hazards. Each participant was given a coded, sterile glass jar and thermos flask with written and verbal instructions on the method of sample

collection. Subjects were asked to collect the semen specimen at home by masturbation into the glass jar, after a minimum of 2 days and preferably less than 5 days of abstinence, and to place the sample in the thermos flask and bring it to the laboratory within one hour of collection. The date and time of ejaculation, abstinence period and spillage (if any) were recorded on the jar label by the subject.

c) Semen analysis methods

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 the NIOSH laboratories. All samples were processed and analyzed in blind fashion by the investigators.

(i) Sperm velocity and motility

Seven microliters of the semen sample were placed on a glass microscope slide and covered with a 22 mm square glass coverslip. The sample was placed on a microscope stage warmed to 37°C by a heat curtain (Model #ASI 400, Nicholson Precision Instruments, Bethesda, Maryland). Five to eight fields selected arbitrarily

were video recorded using a 25x phase objective (Carl Zeiss Inc., NY). The video equipment consisted of a Panasonic NV 8200 video recorder, time-date generator (Panasonic WJ-810), vertical enhancer (Hitachi VE 102), MTI black and white high resolution camera and Hitachi high resolution monitor (model VM 1290). The individual sample number was voice recorded concurrently on the videotape. The time from ejaculation to videotaping (sample age) was recorded. Video tapes were analyzed using a Zeiss Videoplan (Carl Zeiss Inc., NY) semiautomatic image analysis system with video overlay and digitizing tablet. For sperm velocity measurements, thirty motile sperm from each sample were randomly selected and the path of each was digitized (Albertson et. al., 1984). The start and stop times (to the nearest 0.01 second) for each tracing were recorded from the time-date data generated during semen recording and superimposed on the videotape.

Sperm velocity estimates involve two approaches; velocity along the actual sperm path (Overstreet et. al., 1981; Jenks et. al., 1982) and straight line (point to point or distance) velocity. The ratio of path length velocity to distance velocity, termed the forward progression ratio, can be used to describe the motility pattern of the sperm. The ratio is a number between 0-1, where numbers closer to 1 indicate a more linear progressive path. The software of the microcomputer permitted calculations of total path length and the

point to point (straight-line) distance traveled by each tracked sperm. Both the path length and straight line distance were divided by the elapsed time to calculate path length velocity and straight line distance velocity. The forward progression of each sperm was represented by the ratio of straight line distance to path length.

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 non-motile sperm. This process was repeated for a minimum of five fields, so that an average of 200 sperm were scored.

(ii) Semen pH and volume.

The pH of the semen sample was determined using an Orion (model 701) pH meter equipped with a gel filled plastic pH electrode (model E-5M, Fisher Scientific). The volume was measured using a 5 ml plastic disposable syringe.

(iii) Sperm morphology and morphometry.

Four air-dried smears were prepared from each whole semen sample, fixed in absolute ethanol for ten minutes, and stored for later

analysis. Air-dried slides were stained in Papanicolau stain according to the WHO semen analysis guidelines (Belsey et. al., 1980). Sperm morphology was scored according to Zaneveld and Polakoski (1977), reading 200 cells on each of 2 slides. The remaining two slides were used for objective analysis of sperm head shape (morphometry) using the Videoplan system (Schrader et. al., 1984). A 63x dry objective and HMI video camera with a 4x enlarger were used to evaluate 100 sperm on each of 2 slides. Individual sperm heads were outlined using the digitizing tablet and the software allowed calculations of area, perimeter, length, width, width/length ratio, and $4\pi (\text{area})/\text{perimeter}^2$ (Pi factor) as indices of sperm head shape.

(iv) Sperm viability.

Viability by stain exclusion [modified from Eliasson and Treichl (1971)] was determined by mixing 100 μ l of semen with 100 μ l of 0.5% (w/v) eosin y stain in Tyrodes buffer (Difco). Seven μ l of this suspension were placed on a microscope slide and 200 sperm were counted and classified as unstained (viable) or unstained (nonviable). Viability by hypoosmotic swelling (Jeyendran et. al., 1984) was analyzed by mixing 100 μ l 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 ul were placed on a slide, and 200 sperm were classified as swollen (viable) or unswollen (nonviable) using differential interference contrast (DIC) microscopy.

(v) Sperm concentration.

100 ul of semen were mixed with 100 ul of distilled water. Five microliters of this suspension were placed on a Makler Chamber (Sefi-Medical Instruments Haifa, Israel) and the sperm were counted using DIC microscopy. Replicates were prepared and counted for each sample.

(vi) YFF bodies.

The YFF sperm assay is a quantification of the incidence of sperm with two fluorescent bodies (YFF bodies) and has been proposed as a measure of Y chromosomal nondisjunction. Semen samples were analysed by a contractor according to the method of Kapp and Johnson (1980). Semen smears were stained with quinacrine dihydrochloride and scored under fluorescent microscopy for the number of sperm with zero, one and two fluorescent bodies, expressed as a percentage of the total number of sperm counted. Only normal (i.e. oval) sperm heads were counted. In the majority

of cases, 500 sperm were counted; in a few cases 100, 240 or up to 520 sperm were counted, depending on the number of normal sperm observed on the smear. For 45% of the subjects, one slide per individual was read; for 43%, two slides per individual, including eight slides which were read twice to assess reader repeatability, and for 12%, 4 slides per individual were read.

d. Statistical analysis.

Data for each semen characteristic were tested for normality using the Shapiro-Wilk statistic for sample sizes of less than 51 (SAS, 1982). In the case of data that were not normally distributed, the least severe transformation that did not significantly depart from normality was used. In the case of sperm concentration, a square root transformation was used. Natural log transformations were used for semen volume, distance velocity, and area and width of sperm heads. Logistic transformations were used for proportions, i.e percentage motility, viability, sperm progressing at less than 25 $\mu\text{m}/\text{second}$, YFF bodies and the proportion of sperm with normal (oval) morphology.

In order to examine whether the proportion of abnormal sperm in any given category were significantly different in the exposed and unexposed groups, after correction for potential confounders, data for all abnormal sperm classifications were analyzed using the FUNCAT procedure (SAS, 1979).

Since the data are multinomial (i.e. the frequency of responses in one category are not independent of the frequency in other categories), this procedure permits the simultaneous analysis of the frequency of responses in each category of abnormal sperm (in comparison to the proportion of normal forms), using exposure and other potential confounders as independent variables in a least squares linear model to produce approximate chi-square statistics. Since sperm cells are the unit of analysis in this case rather than the subject, the subject's identification number was entered into the the model to test for within - subject variability.

All other variables were analyzed using two-sided t tests and least squares linear regression analysis.

A conventional conservative approach to the analysis of multiple comparisons is to employ a correction factor [usually that of Bonferroni (see Neter and Wasserman, 1974)] for the acceptable level of statistical significance depending on the number of comparisons, since one in twenty comparisons will be statistically significant by chance at $p = 0.05$. In order to reduce the likelihood of a false positive result one approach is to take groups of multiple or closely correlated measurements [in this case (i) sperm viability by vital stain and hypoosmotic stress and percentage motility (ii) sperm velocity by path length and distance velocity and percentage of sperm progressing at less than 25 $\mu\text{m}/\text{second}$ and

(iii) morphometry measurements, using area, length and width of head as representative measurements], and to perform a test of the overall significance of the independent variables included in the regression model on the group of dependent variables, using the MANOVA procedure (SAS, 1979). In the case of abnormal sperm morphology classifications, where a large number (200 per subject) of observations were simultaneously analyzed, the significance level of independent variables in the overall model is adjusted for the number of comparisons (in this case, 9; i.e. 8 degrees of freedom). A level of statistical significance of the chi-square test of $p = 0.001$ was considered adequate evidence of the effect of exposure on individual categories of abnormal sperm.

There are a number of factors which may potentially affect semen quality, such as the age of the subject, other environmental agents, drugs (both medicinal and personal) and medical conditions, such as diabetes and urogenital disorders. Abstinence time and the age of the sample at analysis must also be considered. The following potentially confounding variables and effect modifiers were considered a priori for inclusion in the regression models: tobacco smoking (current, ex- and non-smokers), marijuana use (times per week), alcohol consumption (drinks per week), caffeine consumption (cups of coffee and/or tea per day), history of urogenital disorders and prescription medication use in the preceding year, history of fever in the preceding three months, abstinence time, sample age and age and ethnic background of the subject. Treatment of

potentially confounding variables was done by considering variables for inclusion in the regression model using a forward and backward stepwise technique with exposure forced into the model; variables remained in the model if the p value of the coefficient was less than 0.1. Assessment of factors for modification of the exposure effect was done by the introduction of two factor interaction terms with the exposure variable (Kleinbaum and Kupper, 1978). The means for the semen characteristics, adjusted for the variables in the final model, are calculated by the LSMEANS procedure for linear regression models (SAS, 1979).

V. RESULTS OF SEMEN STUDY

Semen samples were received from a total of 48 exposed and 43 control subjects (96% and 86% respectively of those who agreed to participate). Two exposed men were found to be azoospermic. Subsequent medical examination of these men determined the cause to be Klinefelter's syndrome and bilateral cryptorchidism respectively, and they were eliminated from further analysis. The personal characteristics of the exposed and unexposed groups are given in Table 2. Exposed subjects ranged from 20 to 61 years of age; unexposed men ranged from 18 to 60 years of age. The mean ages of the two groups were significantly different ($p < 0.01$); a higher proportion of exposed workers were

of 26 years of age or less (46% compared to 14% in the unexposed group). The ethnic background of the majority of participants was Asian, Pacific Islander or of mixed race (the latter classification was used where the subject reported more than one ethnic group as his racial background). A higher proportion of the exposed group were White or Filipino (see Table 2). The two groups did not differ significantly with respect to recent fever, or alcohol, caffeine, cigarette or prescription medication consumption, but a significantly higher proportion of exposed workers reported the use of marijuana ($p < 0.05$). Three exposed workers reported a history of urogenital disorders (varicocele, testicular trauma and venereal disease). Eight unexposed and three exposed workers reported having problems having children; of eight cases which resulted in a doctor's visit, the woman's condition was cited as the cause in six and cited as unidentified in two cases (one exposed and one unexposed).

The employment characteristics of participants are shown in Table 3. The average length of employment of exposed and unexposed workers was approximately 5 and 11 years respectively. About half of the exposed workers, particularly those in the smaller plants, reported multiple jobs assignments; these workers were classified according to whether their jobs involved frequent or rare entry into the fumigation chamber (see Table 3).

a). Analysis of data by EDB exposure.

The means and standard deviations for each of the semen characteristics in the exposed and unexposed groups are presented in Tables 4 to 8, giving the p values for two-sided t tests or chi square tests performed on the data (using suitable transformations of data which were not normally distributed as described in Section IV above). A significant differences was observed between the two groups for semen pH ($p = 0.018$). The mean sperm concentration was significantly lower in the exposed group; it is also worth noting that a significantly higher proportion of exposed men had sperm concentrations in the oligospermic range (defined here as a sperm concentration of less than 20 million per ml)(21.7% vs 4.7%; $p = 0.028$). There were no azoospermic men (other than the two previously excluded for medical reasons as described above). The sperm count per ejaculate, semen volume and the percentage of motile sperm, viable sperm by vital stain and normal (oval) sperm were also lower in the exposed group, but the differences were not statistically significant. The percentage of viable sperm was significantly lower in the exposed group for hypoosmotic swelling ($p = 0.013$) but not for the vital stain method. Significant differences were observed between groups for three categories of abnormal sperm morphology: absent heads, tapered heads and abnormal tails ($p = <0.001$, <0.001 and 0.009 respectively). No differences were observed between the groups for sperm velocity, sperm morphometry or YFF bodies.

b) Analysis of potentially confounding variables and effect modifiers.

The results of the regression analyses are presented in Tables 9 to 14, giving the variables included in the final models and the adjusted means of the semen characteristics corrected for the confounders in the model.

(i) Semen pH, volume, sperm concentration and count.

The data in Table 9 show that the semen pH of exposed subjects remained significantly more alkaline ($p = 0.028$) than that of unexposed subjects after correction for marijuana use and recent fever as the only significant confounders. The adjusted mean for semen volume, corrected for the effects of abstinence and subject's age as significant confounders, remained lower in the exposed group than in the unexposed group, but as in the case of exposure considered alone, this difference did not reach statistical significance ($p = 0.125$) (Table 9). Similarly, sperm concentration remained lower in the exposed group after correction for abstinence, the subject's age and ethnic background, and caffeine consumption as significant confounders, but in contrast to the case of exposure considered alone, this difference was not significant ($p = 0.153$). When sperm count per ejaculate was considered, however, the effect of exposure was statistically significant ($p = 0.009$) after correction for abstinence as the only significant confounder.

Note: one exposed subject had a reported abstinence of 60 days (compared to the average of approximately 6 days) and a low sperm concentration of 17 million/ml and volume of 3.5 ml; inclusion of this subject resulted in an apparently significant relationship between exposure (when analyzed as an interaction term with abstinence) and a decrease in sperm concentration and volume at longer abstinence times. Since there was otherwise a significant positive correlation between increasing abstinence time and increasing sperm concentration and volume ($p < 0.001$ and 0.016 respectively), it was concluded that the apparent effect of exposure at different levels of abstinence could be attributed to this individual's outlying abstinence value. The regression model is presented for the analysis where he was excluded. Two of the three exposed subjects who had a previous history of urogenital disorders (venereal disease and varicocele) also had sperm concentrations of less than five million/ml. Inclusion or exclusion of these subjects did not alter the lack of a significant exposure effect on sperm concentration after correction for confounders, or alter the results for other semen characteristics. Examination of the frequency distributions of previous illnesses in the two groups (other than influenza with accompanying fever and urogenital disorders) indicated few individuals with previous conditions (e.g. diabetes) that may potentially affect semen

quality or testicular function; semen profiles for these individuals were within the normal range. As an additional check, regression analyses were performed with and without such individuals in the models, and did not alter the results).

(ii) Sperm viability, motility and velocity.

The age of the subject was the only significant confounder for sperm viability and motility (Table 10). When age was included in the model, the effect of exposure on the percentage of viable sperm (by vital stain and hypoosmotic swelling) and the percentage of motile sperm was found to be significant both when considered separately ($p = 0.014$, 0.003 and $p < 0.001$ respectively) and as a group of characteristics in a multivariate analysis ($p = 0.003$), in contrast to an apparent exposure effect only on vital stain viability when confounders were not considered. Alcohol consumption was the only significant confounder for sperm velocity (Table 11); adjusting for this did not alter the lack of a significant exposure effect (path length velocity, $p = 0.462$, distance velocity, $p = 0.336$) and percentage of sperm progressing at less than $25 \text{ } \mu\text{m}/\text{second}$ ($p = 0.153$).

(iii) Sperm morphology and morphometry.

With respect to sperm morphology and morphometry, the overall percentage of normal sperm (i.e. sperm with oval heads and normal tails) was marginally but not significantly lower in the exposed group ($p = 0.091$) after correction for subjects' age and ethnicity as the only confounding factors (Table 12). When all abnormal morphology classifications were considered simultaneously using the FUNCAT procedure, however, the exposed group had a significantly higher proportion of sperm with abnormal tails ($p = <0.001$), absent heads ($p = <0.001$) and tapered heads ($p = <0.001$) when corrected for age and ethnicity as in the case when exposure alone was considered (Table 13). [Note: inclusion of the subject's identification number in the model to test for within - subject variability (see Section IV) did not alter these results and was not therefore included in the final model].

When representative sperm head morphometry measurements were considered (area, length and width of head) the overall effect of exposure on morphometry was not significant after adjusting for the subject's age and ethnicity when tested by the multivariate MANOVA procedure ($p = 0.098$) (Table 14). However, exposure was associated with a decrease in sperm head width ($p = 0.037$) which agrees with the finding of a significantly increased proportion of tapered heads among the exposed group using sperm morphology analysis.

(iv) YFF bodies.

The proportion of sperm with YFF bodies did not differ significantly between the exposed and unexposed groups either before or after consideration of potentially confounding variables ($p = 0.720$). Since none of the factors included in the analysis were found to be significant confounders or effect modifiers, no regression model is presented for these data.

c) Analysis by job and duration of exposure.

To determine if a dose-response relationship between the effect of duration of exposure and semen quality could be observed, data were analyzed for each semen characteristic by years of exposure in comparison to controls, correcting for significant confounders. No significant linear trend with increasing duration of exposure was observed for any semen characteristic with the exception of sperm viability by vital stain ($p 0.023$) and hypoosmotic swelling ($p 0.006$) and percentage motility ($p 0.025$); in these cases longer duration of exposure was associated with poorer sperm viability and motility. Overall, therefore, no consistent effect of duration of exposure was observed. Similarly, no differences in semen characteristics between workers who had potentially higher (peak) exposures (fumigators, forklift drivers and workers in plant 6; $n = 18$) and other exposed workers ($n = 28$) were found when compared to unexposed workers. This may be due to the close comparability of full shift exposure levels, although small differences may not be detectable with this sample size.

VI. DISCUSSION.

Statistically significant effects of exposure to EDB were observed with respect to semen pH, sperm count per ejaculate, the percentage of viable and motile sperm and certain types of morphological abnormalities (tapered heads, absent heads and abnormal tails) after consideration of smoking, caffeine and alcohol consumption, subjects' age, abstinence and age of sample, history of urogenital illness and other potentially confounding factors. Semen volume, sperm concentration and the proportion of normal sperm were also lower in the exposed group than the unexposed group, but these differences were not statistically significant after correction for confounding factors. No effect of exposure on sperm velocity or YFF bodies was observed. These findings suggest that chronic exposure to EDB, at levels comparable to or in excess of the NIOSH recommended standard of 45 ppb as at 8 hour time weighted average and 130 ppb at a 15 minute ceiling limit but far below the current OSHA standard of 20 ppm, adversely affect semen quality.

No consistent effect of duration of exposure on semen quality was observed, although the sample size may be insufficient to detect such an effect. This finding provisionally suggests, however, that unless permanent damage has occurred to primary spermatogonial cells or to, for example, the hormonal control of testicular function, the effect of EDB on spermatogenesis reflects current rather than cumulative exposure due to the constant turnover of sperm production. This is consistent with the observation in animals that recovery

of normal spermatogenesis occurs after cessation of exposure (Edwards et. al., 1970) and the fact that EDB is rapidly metabolized in biological tissues (Edwards et. al., 1970).

Methodological considerations.

When performing multiple statistical comparisons on dependent variables, there is an increased likelihood of a false positive result if the acceptable level of significance of the nominal p values for each test is not adjusted. As stated in the methods section, a strongly conservative approach would be to treat each measurement independently (23 in this case) and apply a Bonferroni correction factor to obtain the acceptable level of significance. In this study, groups of multiple tests were tested for the significance of independent variables where appropriate by multivariate techniques which give p values adjusted for the number of comparisons and improve the confidence with which a given effect may be ascribed to exposure. In the case of sperm viability and motility the effect of exposure on this group of characteristics was still highly significant ($p = 0.005$) (Table 10); similarly, the effect of exposure on the overall proportion of abnormal sperm shapes was also highly significant ($p = <0.001$) (Table 13). The effect of exposure on semen pH and sperm count per ejaculate, for which no multivariate test was performed, may be considered to be of marginal statistical significance depending on what adjustment to the p value is considered appropriate. However, leaving aside strict statistical interpretations, the finding of a lower sperm count among exposed workers, which appears to be a function of an effect both on semen

volume and sperm concentration, strongly suggests that EDB may affect sperm and seminal fluid production. This finding is further supported by the observation that a higher percentage of exposed workers have sperm concentrations of less than 20 million per ml (21.7 % compared to 4.7 % among unexposed workers).

The number of subjects studied may limit the ability to detect small changes in certain semen characteristics due to exposure even if such differences are really present. In the case of sperm concentration, a sample of 46 exposed and 43 unexposed men was sufficient to have had an 80% chance of detecting a 32% or greater decrease in mean sperm concentration in the exposed group compared to the mean of our unexposed group at a one-sided alpha level of 0.05 (using a square root transformation of the data). In the case of semen volume, our sample size permitted an 80% chance of detecting a 45% decrease in mean volume in the exposed group compared to the mean of our unexposed group (using a log transformation of the data); similarly, for sperm velocity, an approximately 10% decrease in path length or distance velocity could have been detected in exposed workers. (Note: in each case the standard deviation assumed is calculated from the pooled variances observed in each group). Thus, smaller true differences between these groups for these semen characteristics could not be determined with confidence in the present study population.

In the case of YFF bodies, the sample size was sufficient to detect an approximately 26% difference between the two groups at an alpha level of 0.05

and 80% power. However, it should be noted that considerable variation in these measurements, probably due to the subjective nature of the scoring method, was observed. Taking the readings for the 50 subjects for whom at least two slides were read, the absolute differences in percentage of YFF bodies between pairs of slides ranged from 0 to 2.2%, with a mean and S.D. of $0.6 \pm 0.46\%$. This represents an approximately 50% variation on average from the mean of 1.2 - 1.3% in the two groups. Further, for 8 slides which were read twice, an absolute difference of 0.2 - 0.4% was observed. Such variation may thus obscure any differences due to exposure or other factors even if they are present. In view of these technical difficulties, and the fact that considerable controversy exists as to the meaning of these fluorescent bodies (Wyrobek, personal communication), the results of this assay should be interpreted with extreme caution.

It is possible that the results of the study may be biased if systematic differences exist between the two groups with respect to participants and non-participants; for example, if men with suspected reproductive problems were more likely to participate in the exposed group than in the unexposed group or vice versa. All currently exposed workers were given individual explanations of the purpose of the study prior to the field phase and the participation rate was high with the exception of the sixth plant located during the field study (overall, approximately 64 % of the total available cohort provided semen samples). There were no clear differences between participants and nonparticipants with respect to demographic characteristics, and reasons given for non participation were generally unrelated to factors

which may potentially affect semen quality. Random selection of 50 volunteers from the control population minimized the possibility of selection bias in this group. Subsequently, observed differences between the two groups with respect to age, history of urogenital disorders and other potentially confounding variables were treated during statistical analyses as described above and two of the exposed participants who were azoospermic due to medical conditions unequivocally unrelated to EDB exposure were eliminated from the study. Thus, while the possibility of selection bias cannot be ruled out, it is unlikely that it could explain the results of this study.

Other confounding factors.

A number of other factors were found to be significant confounders in this study. It is well established that abstinence time is closely correlated with both semen volume and sperm concentration (see, for example, Schwartz et. al., 1979), although the potential effect on other semen characteristics has not been thoroughly investigated. In this population as well, longer abstinence times were significantly associated with an increase in semen volume and sperm concentration but not with any other semen characteristic. Semen pH, sperm viability, motility and velocity are affected by the age of the sample at analysis (Makler et. al., 1981). In this study, the average time from ejaculation to videotaping for viability, motility and velocity was approximately 40 minutes and none exceeded two hours, and no effect on these semen characteristics was observed, supporting the view that changes in these

parameters can be minimized by analysis of samples within approximately two hours. The exact time that the semen pH was measured was not recorded in this study, and it is possible that some drift in pH may occur from ejaculation time to the time of analysis. Although no systematic difference in time between exposed and unexposed subjects' samples is likely (due to intermixing of samples on a given day and processing in blind fashion), the results for semen pH should be interpreted with caution.

Several investigators have examined the effect of cigarette smoking on sperm concentration, motility and morphology (Vicizian, 1969; Evans et al., 1981; Rodriguez-Rigau et. al., 1982) but the results are equivocal. Marijuana use (among heavy users) has also been associated with depression of sperm count or motility and increases in abnormal sperm morphology (see review by Wyrobek et.al., 1983). No effect of cigarette smoking or marijuana use on semen quality was observed in this study, except for an association between marijuana and more alkaline semen pH, although both were found to be significantly related to cytogenetic changes in this population (Steenland et al., in press)

Alcohol consumption has been associated with decreases in sperm concentration and motility among heavy users (see review by Wyrobek et. al., 1983). In this study, alcohol consumption appeared to be associated with an increase in sperm velocity; whether this is a causal or spurious effect remains to be established. Caffeine has been found to alter sperm motility in vitro

(Dougherty et. al., 1976, Harrison et. al., 1980). No data are available on the effect of caffeine consumption on semen quality in vivo, although it rapidly distributes into the seminal fluid at concentrations almost identical to those in blood (Beach et. al., 1984). No effect of caffeine was seen in this group, with the exception of a marginally significant association with decreasing sperm concentration. The subject's age, on the other hand, was found to be significantly negatively associated with semen volume, sperm viability and percentage of motile and normal sperm, and positively associated with sperm concentration after correction for other potential confounders. Ethnicity was also found to be related to differences in certain types of sperm morphology in this group. No other potentially confounding variables were found to explain a significant amount of variation in semen quality and none of these variables were found to be effect modifiers when analysed as interaction terms with exposure.

Comparison of results with other studies.

The results of this study may be compared with those of previous animal and human data on the effects of EDB on semen quality. Decreases in sperm concentration and, percentage motility and increases in abnormal sperm morphology have been observed in EDB-exposed bulls (Amir et al 1977) and rams (Eljack and Hrudka 1979); none of the animal studies to date have, however, evaluated the effects of EDB exposure on semen volume, pH or sperm velocity. Previously available studies on human semen quality are, when taken together,

suggestive of an effect of EDB on sperm concentration but are inadequate to evaluate potential effects on other semen characteristics. The NIOSH longitudinal study of short term exposure to EDB (NIOSH 1984b), in which the range of semen characteristics measured in this study were evaluated with the same methodology used in this study, provide the most comparable human data. In that study, a small group of 12 workers was exposed to levels of EDB comparable to those in the present study but for only six weeks. A statistically significant decrease in sperm velocity and a marginally significant decrease in semen volume were noted when pre and post-exposure semen samples were compared, but no other statistically significant differences were observed. The lack of observed effects on the majority of semen characteristics may be due to an insufficient sample size to detect, for example, small changes in sperm concentration (although intrapersonal variation in this and other characteristics is less than that between individuals), and the fact that potential effects on earlier stages of spermatogenesis would not be observable over the time period of exposure. The lack of effect of EDB on sperm velocity in the present study, in contrast to the longitudinal study, may suggest a transient early effect of EDB following short term exposure which is not observable after long term exposure, when the percentage of motile sperm appears to be significantly reduced but not the velocity of the remaining (motile) sperm. The effect of EDB on semen quality is also consistent with the observation that certain other alkylating agents, such as some cancer chemotherapy agents, and the structurally similar pesticide dibromochloropropane (DBCP) also affect spermatogenesis (see Wyrobek et al.,

1983a,b). In the case of DBCP, its spermatotoxicity is clearly considerably greater than that of EDB, since significant damage to spermatogonial cells, sufficient to cause sterility, can result from comparatively short term exposure.

Biological significance of changes in semen quality.

There is considerable evidence from clinical studies that low sperm concentration, poor motility and abnormal morphology may be closely correlated (Bartak, 1973; Rodriguez-Rigau et. al., 1981; Steinberger and Rodriguez-Rigau, 1983;). The question arises as to whether exposure to EDB results in a primary effect on some aspect of testicular function and semen quality (e.g. semen pH, or sperm morphology) which gives rise to secondary effects, for example, on sperm motility. Sperm viability and percentage motility were closely correlated in this sample, since in both cases the percentage of viable or motile sperm is calculated from the total number of sperm observed, and all non-viable sperm are, by definition, non-motile, but viable sperm are not necessarily motile. Among both the exposed and unexposed groups in this study, a decrease in sperm motility was significantly correlated with a decrease in velocity and an increase in sperm with abnormal tails, and, in the exposed group only, with an increase in sperm with microcephalic heads. Sperm viability and motility were also significantly (positively) correlated with the overall percentage of normal sperm shapes in the exposed group. These data and previous findings suggest that an effect on one sperm characteristic

(such as sperm morphology) is likely to result in changes in certain other sperm characteristics, such as viability and motility.

There are several lines of established evidence which link poor semen quality with impaired fertility, and some evidence to suggest that alterations in semen quality may be associated with adverse reproductive outcomes where conception has been successful. Subfertility has long been associated with low sperm counts, although it is not unusual for pregnancies to occur where the sperm concentration is consistently below 20 million per ml (Smith and Steinberger, 1977). The importance of sperm motility and morphology as well as the number of sperm has been increasingly recognized as indicative of fertility potential in humans (Eliasson, 1978; Steinberger and Rodriguez-Rigau, 1983). Alterations in semen quality have also been linked with adverse reproductive outcomes. For example, several studies have found an association between low sperm concentration and motility or increased abnormal sperm morphology and spontaneous abortions (Furuhjelm et. al., 1962; Czeizel et. al., 1967; Joel, 1966) although a more recent study (Homonnai et. al., 1980) failed to demonstrate such an association. Finally, there is also evidence from animal data, particularly the mouse sperm morphology assay, that there is a very high correlation between chemicals with known germ cell mutational activity and their ability to produce sperm head morphological changes; this suggests that agents that produce morphological changes in sperm in this species should be regarded as suspect germ cell mutagens in mammals (Wyrobek et al., 1983b). Overall, these data support the hypothesis that

chemically induced alterations in semen quality increase the risk of impaired fertility and may increase the risk of adverse reproductive outcomes. In the present study, eight exposed and three unexposed workers reported in the questionnaire having had problems having children, but in no case was the cause cited as the male's in the eight cases where a doctor was consulted (one case in each group was of unidentified cause). The number of participants is clearly insufficient, however, to quantitatively evaluate fertility or reproductive outcomes in relation to exposure.

VII. CONCLUSIONS

There is a considerable body of research to support the view that impairment of one or more aspect of semen quality is associated with impaired fertility and, less certainly, with adverse reproductive outcomes. Our findings of decreases in sperm count, viability and motility and increases in certain types of morphological abnormalities among workers exposed to EDB suggest, therefore, an increased risk of reproductive impairment among this group of workers at levels of exposure near the NIOSH recommended standard of 45 ppb and far below the current OSHA standard of 20 ppm.

TABLE 1

EDB Exposure by Job Category and Plant
(parts per billion)

Full Shift Samples

Job Category	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
Sorter/ ('83)	23(2.0)[8]	55(3.3)[8]	70(1.4)[8]	49(3.6)[6]	72(1.9)[9]	148(2.1)[7]
packer ('82)	72(1.6)[5]	95(1.3)[4]	61(1.5)[8]	na	na	na
Forklift('83)	39(1.3)[2]	68(1.2)[2]	213(1.2)[2]	152(1.0)[2]	16(10.5)[2]	na
driver ('82)	89() [1]	125(1.0)[2]	145(1.4)[2]	na	na	na
Fumigator	na	na	116(n=2)	na	na	na

Short Term Samples

Forklift('83)	26(4.0)[2]	195(1.4)[3]	na	na	na	na
driver						
Fumigator('83)	12(3.2)[4]	118(3.6)[3]	262(-)[1]	69(2.8)[2]	na	na
('82)	27(1.6)[2]	39(4.3)[4]	na	na	na	na

Data shown is geometric mean (geometric standard deviation)[number of men sampled]

na=not available(samples not taken at these plants).

TABLE 2

Characteristics of exposed and unexposed workers

Characteristic	Unexposed (n=43)	Exposed (n=46)
Age (years) (mean $\pm$ SD)	34.4 $\pm$ 10.1	28.9 $\pm$ 10.2
Ethnicity (%)		
White	21.0	13.0
Filipino	49.0	28.0
Japanese	14.0	11.0
Hawaiian	2.0	15.0
Mixed(Asian, Pacific Islander, White)	14.0	33.0
Cigarette Smokers (%)		
Current Smoker	37.2	41.3
Ex-Smoker	34.9	19.6
Non-Smoker	27.9	39.1
Alcohol consumption (drinks/week)(mean $\pm$ SD)	9.1 $\pm$ 9.1	11.9 $\pm$ 15.2
Caffeine consumption (cups tea/coffee/day (mean $\pm$ SD)	2.0 $\pm$ 2.1	1.9 $\pm$ 3.2
Use of prescription medication in preceding year (%)	48.8	41.3
Use of marijuana (%)	20.9	41.3
History of urogenital disorders (%)	0.0	6.5
Fever in preceding 3 months (%)	4.7	15.0
Length of abstinence (days) (mean $\pm$ SD)	4.2 $\pm$ 4.9	5.8 $\pm$ 9.9
Time from semen sample collection to analysis (Minutes) (Mean $\pm$ SD)	39.0 $\pm$ 17.1	39.4 $\pm$ 18.1

TABLE 3

Employment characteristics of exposed and unexposed workers

	Unexposed	Exposed
Length of employment (yrs) (mean, S.D.)	11.0 $\pm$ 8.1	4.9 $\pm$ 3.6

Current job description: exposed workers

Job description	Percentage of workers (n=46)
Fumigator	6.5
Forklift driver	23.9
Packer/Sorter	10.9
Shipping	8.7
Miscellaneous (inc. entry into fumigation chamber) ¹	26.1
Miscellaneous (no entry into fumigation chamber) ¹	23.9

¹ Includes workers (principally in smaller plants) with multiple job assignments e.g. forklift driver and packer.

Current Job description: unexposed workers

Job description	Percentage of workers (n=43)
Sugar mill operative	53.5
Field worker (cane planters, cutters, Etc.)	4.7
Truck driver	11.6
Garage mechanics	25.6
Miscellaneous	4.7

TABLE 4

Semen characteristics in exposed and unexposed workers

Characteristic	Unexposed (n=43)		Exposed (n=46)		p ¹
	Mean	S.D.	Mean	S.D.	
Semen volume (ml)	2.78	1.54	2.57	1.39	0.419
Semen pH	7.90	0.22	8.02	0.24	0.018
Sperm Concentration (million/ml)	68.72	47.50	52.57	42.06	0.038
Sperm count (million/ejaculate)	204.80	199.20	134.82	138.49	0.056
Sperm viability by stain exclusion(%) ²	.72.98	8.74	68.07	15.76	.0.078
Sperm viability by hypoosmotic stress(%) ²	.74.30	9.09	68.46	12.86	0.013
Sperm motility (%) ²	44.93	14.22	40.94	18.02	0.374

S.D. = Standard Deviation of the Mean

1: p = p value of two-sided t test

2: n=45 in exposed group

TABLE 5

Sperm velocity in exposed and unexposed workers

Characteristic	Unexposed (n=43)		Exposed (n=45) ¹		p ²
	Mean	S.D.	Mean	S.D.	
Path length velocity (um/sec)	68.48	10.75	71.80	17.23	0.283
Distance velocity (um/sec)	43.18	7.57	42.94	11.38	0.645
Ratio of Distance/path length	0.67	0.11	0.63	0.96	0.152
Sperm progressing <25 um/sec(%)	0.20	0.13	0.23	0.13	0.274

S.D. = standard deviation of the mean

1: one exposed subject not included due to low sperm count.

2: p = p value of two-sided t test

TABLE 6

Sperm morphology classifications in exposed and unexposed workers¹

Category	Unexposed (n=43)		Exposed (n=46)		p ²
	Mean	S.D.	Mean	S.D.	
Oval (normal) heads (%)	80.16	9.52	77.88	14.13	0.386
Macrocephalic heads (%)	1.69	2.54	1.35	1.29	0.127
Microcephalic heads (%)	1.10	0.99	0.99	0.99	0.754
Tapered heads (%)	2.49	3.17	4.21	6.22	<0.001
Double heads (%)	0.83	1.47	1.03	0.92	0.151
Absent heads (%)	1.43	1.29	2.08	1.76	<0.001
Abnormal tails (%)	5.71	4.45	6.54	10.68	0.009
Immature forms (%)	5.64	4.71	5.29	3.73	0.592
Amorphous heads (%)	0.61	0.60	0.64	0.78	0.620

S.D. = standard deviation of the mean.

1: Mean % of 2 slides: 200 cells/slide.

2: p = p value of two-sided t test (oval forms) The p values for the chi square analysis of abnormal forms in comparison with oval forms considering only exposure in the model (FUNCAT procedure) are also given; note that this analysis does not compare the difference between means for each abnormal category as in the t test.

TABLE 7

Sperm morphometry in exposed and unexposed workers

Characteristic	Unexposed (n=43)		Exposed (n=46)		p ¹
	Mean	S.D.	Mean	S.D.	
Area (um ²)	9.058	1.291	8.817	1.066	0.344
Perimeter (um)	11.442	0.719	11.364	0.675	0.596
Length (um)	4.418	0.314	4.423	0.470	0.946
Width (um)	2.629	0.253	2.562	0.241	0.189

S.D. = standard deviation of the mean.

1: p = p value of two-sided t test

TABLE 8

Percentage of sperm with 0, 1 or 2 Y bodies ¹
in exposed and unexposed workers

Characteristic	Unexposed (n=43)		Exposed (n=46)		p ²
	Mean	S.D.	Mean	S.D.	
0 Y bodies	52.69	3.69	52.60	2.88	0.886
1 Y body	45.98	3.60	46.12	2.77	0.824
2 Y bodies	1.33	0.68	1.28	0.62	0.720

S.D. = standard deviation of the mean.

1: Based on the first slide read per subject.

2:p = p value of two-sided t test

TABLE 9

Effect of exposure on sperm concentration¹, semen volume² and semen pH after correction for potentially confounding variables and effect modifiers

Characteristic	Variables in final model	P	F	r ²	Adjusted means	
					Unexposed	Exposed
Sperm concentration (million/ml) ³	Exposure	0.153	7.20	0.305	59.39	48.37
	Abstinence	<0.001				
	Ethnicity	0.003				
	Age of subject	0.031				
	Caffeine	0.011				
Semen volume (ml) ³	Exposure	0.125	3.46	0.110	2.53	2.11
	Age of subject	0.037				
	Abstinence	0.016				
Sperm count (million/ejaculate) ³	Exposure	0.009	10.15	0.193	139.76	80.99
	Abstinence	<0.001				
Semen pH	Exposure	0.028	6.68	0.191	7.90	8.01
	Marijuana use	0.005				
	Fever(<3 mths)	0.036				

1: Using square root transformation

2: Using log_e transformation

3: Excluding subject with 60 days' abstinence

TABLE 10

Effect of exposure on sperm viability¹ and motility¹ after correction for potentially confounding variables and effect modifiers

Characteristic	Variables in final model	P	F	r ²	Adjusted means	
					Unexposed	Exposed
Sperm viability (% unstained)	Exposure	0.003	12.47	0.227	75.40	67.09
	Age of subject	<0.001				
Sperm viability (% swollen)	Exposure	<0.001	11.93	0.219	85.47	68.13
	Age of subject	<0.001				
% Motile sperm	Exposure	0.031	7.04	0.142	46.58	35.21
	Age of subject	<0.001				

MANOVA test of significance for hypothesis of no overall effect of independent variables on the group of dependent variables (percentage viable sperm by vital stain and hypoosmotic swelling and percentage motile sperm).

Exposure p = 0.005
Age of subject p < 0.001

TABLE 11

Effect of exposure on sperm velocity after correction for potentially confounding variables and effect modifiers

Characteristic	Variables in final model	P	F	r ²	Adjusted means	
					Unexposed	Exposed
Path length velocity (um/sec)	Exposure Alcohol	0.462 0.003	5.48	0.114	69.06	71.25
Distance velocity ¹ (um/sec)	Exposure Alcohol	0.336 <0.001	7.41	0.148	42.95	41.26
% Sperm progressing at less than 25 um/sec ²	Exposure Alcohol	0.153 0.013	3.86	0.083	16.17	20.52

MANOVA test of significance for hypothesis of no overall effect of independent variables on the group of dependent variables (path length velocity, distance velocity and sperm <2 um/sec)

Exposure	p= 0.298
Alcohol	p= 0.002

1 Using log_e transformation

2 Using logistic transformation

TABLE 12

Effect of exposure on proportion of normal (oval) sperm after correction for potentially confounding variables and effect modifiers

Characteristic	Variables in final model	P	F	r ²	Adjusted means	
					Unexposed	Exposed
Normal (oval) sperm						
	Exposure	0.091	3.19	0.101	82.72	78.87
	Age of subject	0.005				
	Ethnicity	0.380				

TABLE 13

Effect of exposure on abnormal sperm morphology classifications after correction for potentially confounding variables and effect modifiers.

Sperm morphology classifications	Variables in final model	Chi-square	p
Tapered heads	Exposure	39.89	<0.001
	Age of subject	1.12	0.291
	Ethnicity ¹	4.84	0.028
Absent heads	Exposure	17.14	<0.001
	Age of subject	2.44	0.119
	Ethnicity	17.87	<0.001
Abnormal Tails	Exposure	72.42	<0.001
	Age of subject	371.92	<0.001
	Ethnicity	42.27	<0.001
Macrocephalic heads	Exposure	4.45	0.035
	Age of subject	1.27	0.260
	Ethnicity	0.89	0.347
Microcephalic heads	Exposure	0.68	0.411
	Age of subject	19.45	<0.001
	Ethnicity	0.10	0.753
Double heads	Exposure	0.38	0.539
	Age of subject	2.90	0.088
	Ethnicity	0.45	0.503
Immature forms	Exposure	0.83	0.363
	Age of subject	14.82	<0.001
	Ethnicity	32.09	<0.001
Amorphous heads	Exposure	1.57	0.211
	Age of subject	6.71	0.010
	Ethnicity	0.01	0.940
ALL ABNORMAL FORMS	Exposure	130.74	<0.001
	Age of subject	403.31	<0.001
	Ethnicity	88.02	<0.001

1: Caucasians v. non-caucasians.

TABLE 14

Effect of exposure on sperm morphometry after correction for potentially confounding variables and effect modifiers

Characteristic	Variables in final model	P	F	r ²	Adjusted means	
					Unexposed	Exposed
Area of head(um ²) ¹	Exposure	0.065	4.38	0.134	9.09	8.65
	Age of subject	0.018				
	Ethnicity ²	0.010				
Length of head (um)	Exposure	0.697	1.22	0.041	4.44	4.41
	Age of subject	0.273				
	Ethnicity	0.108				
Width of head (um) ¹	Exposure	0.037	3.60	0.113	2.64	2.53
	Age	0.031				
	Ethnicity	0.035				

MANOVA test of significance for hypothesis of no overall effect of independent variables on groups of dependent variables (i.e. area, length and width of head.

Exposure	p=0.098
Age	p=0.119
Ethnicity	p=0.054

1: Using log_etransformation

2: Caucasians v. non-caucasians.

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