Effects of Exposure to Ethylene Glycol Ethers on Shipyard Painters: II. Male Reproduction

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To determine whether 2-ethoxyethanol (2-EE) and 2-methoxyethanol (2-ME) affected the reproductive potential of exposed men, we examined the semen of 73 painters and 40 controls who work in a large shipyard. An industrial hygiene survey was performed to characterize the work environment. The men supplied information on demographic characteristics, medical conditions, personal habits, and reproductive history; underwent a physical examination; and provided a semen sample. Semen samples were analyzed for pH, volume, turbidity, liquidity, viability by stain exclusion and hypo-osmotic stress, sperm density and count per ejaculate, motility using a videotape technique, morphology, and morphometry. Serum was analyzed for testosterone, FSH, and LH. The industrial hygiene survey revealed that the painters were exposed to 2-EE at a time-weighted average (TWA) of 0–80.5 mg/m³ with a mean of 9.9 mg/m³, and to 2-ME at a TWA of 0–17.7 mg/m³ with a mean of 2.6 mg/m³. Painters had an increased prevalence of oligospermia and azoospermia and an increased odds ratio for a lower sperm count per ejaculate, while smoking was controlled. This finding is consistent with prior animal studies and with one human study. Potential biases and confounding of the data are discussed.

Key words: infertility, oligospermia, azoospermia, 2-ethoxyethanol, 2-methoxyethanol, occupational disease

INTRODUCTION

Glycol ethers are useful solvents with widespread applications in industrial and commercial uses. They are used as: deicing additives to fuel; in coating, photographic applications, dyeing, and printing; in the electronics industry; and in cleaning solutions, among other uses. NIOSH has estimated that there are up to 2,000,000 workers exposed to eight glycol ethers and their acetates [NIOSH, 1983a]. The toxicity of the glycol ethers has been the subject of many reports over the past decades; 2-methoxyethanol (2-ME; CAS No. 109-86-4) and 2-ethoxyethanol (2-EE; CAS No. 110-80–

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5) are recognized hematologic and central nervous system toxins in humans [Zavon, 1963; Ohi and Wegman, 1978; Cullen et al., 1983; Cohen, 1984] and animals [Nagano et al., 1984; Miller et al., 1984; Doe et al., 1983; Nelson and Brightwell, 1984].

Animal experiments demonstrate that 2-ME, 2-EE, and their acetates are toxic to the reproductive system. Testicular atrophy, degeneration of the germinal epithelium, infertility, and abnormal sperm morphology are found in exposed male animals [Creasy and Foster, 1984; Oudiz et al., 1984; Miller, 1983], and embryotoxicity and teratogenicity are seen when female animals are exposed [Hardin, 1983; Sleet et al., 1985]. In animals, the earliest testicular effect was degeneration of pachytene spermatocytes; this was found 24 hours after a single dose of 100 mg/kg/day of 2-ME and 500 mg/kg/day of 2-EE [Foster et al., 1984].

There are few data on reproductive effects in humans. One study in a glycol ether production facility found decreased testicular size, but no increase in semen abnormalities in exposed men; the sample size was relatively small [Cook et al., 1982]. In a second study, men who used glycol ethers as part of the slurry in a foundry had decreased sperm concentrations when compared to controls [NIOSH, 1986].

Based on the existing literature, our hypothesis was that men exposed to 2-EE or 2-ME on a regular basis would be more likely to have an abnormality of semen parameters, decreased fertility, or abnormalities of hematologic function when compared to an unexposed population. Given this hypothesis, we attempted to: 1) identify a population of men known to be exposed to these glycol ethers and a similar group of men not so exposed; 2) characterize their exposure; 3) obtain information on other factors previously identified as affecting the outcomes of interest; and 4) analyze semen samples collected under standardized conditions.

Based on a report of airborne exposures to 2-EE above the OSHA standard in painting operations in a large shipyard, we undertook a cross-sectional study of the effects of combined exposure to 2-EE and 2-ME on reproductive and hematologic function. We report here a study of the effects of these glycol ethers on semen quality. Detailed descriptions of the concurrent investigations of the effects of the solvents on hematologic function [Welch and Cullen, 1988] and on male fertility [Welch and Plotkin, in preparation] are reported separately.

EVALUATION DESIGN AND METHODS
Environmental Factors

The men under study worked at a large shipbuilding facility in the Northeast United States. The site was chosen for study after evidence of exposure to glycol ethers was obtained in a health hazard evaluation performed by NIOSH [NIOSH, 1983b]. The hazard evaluation had been requested because of complaints of rashes, dizziness, fainting, and nausea at the shipyard. Sampling performed by the industrial hygiene office of the shipyard had also measured high levels of ethylene glycol ethers.

As part of this investigation, an industrial hygiene survey of the painting operations was performed; the methods are reported in detail elsewhere [Sparer et al., 1988]. In brief, a group of 30 painters volunteered to wear personal samplers while working. Samples were taken on all three shifts for three consecutive days, and all samples were split and analyzed for both 2-EE and 2-ME. Bulk sampling of the most widely used paints and cleaners was performed.

The painters were exposed to other substances that have been reported in the past to affect semen quality. We reviewed the work procedures and material safety data sheets; Table I presents all the substances identified as hazardous under the state's hazard communication act. Lead and epichlorohydrin were identified as the substances on this list previously reported to affect sperm counts in humans and animals [Lancranjan et al., 1975; John et al., 1983]. Biological monitoring for lead performed by the shipyard was reviewed, and industrial hygiene sampling was performed for epichlorohydrin.

Medical Factors

Population identification. The entire study population for the semen, hematologic, and fertility studies consisted of 94 painters and 55 nonexposed controls, whereas the population for the semen study consisted of 73 painters and 40 controls.

Exposed workers. The shipyard employed 900 painters, of whom approximately 600 were men. Men who lived within an approximate 20-mile radius of the clinical site were invited to participate with an explanatory letter and a subsequent phone call. This range was chosen to allow the men to deliver semen samples within 1 hour of collection. Of 159 men contacted, 128 agreed to participate.

Unexposed workers. Two job classifications were identified as ones in which the majority of jobs entailed no work aboard the ships: the clerks and the marine draftsmen. Other tradesmen who worked on the ships could have had bystander exposure to the glycol ethers because of the frequent work in closed, small spaces alongside painters.

The shipyard employed 190 clerks in jobs that did not entail going onto the ships and 440 draftsmen. As with the painters, we attempted to contact all the clerks

TABLE I. Potential Toxic Exposures of Shipyard Painters

Metals (and oxides)		
Aluminum	Chromium	Manganese
Barium	Copper	Nickel
Beryllium	Iron	Organotin
Cadmium	Lead	Titanium
Calcium	Magnesium	Zinc
Organic solvents		
Acetone	Freon	Naphtha
Aliphatic hydrocarbons	2-Heptanone	Naphthalene
(C6–C12)	Isobutyl acetate	n-Butyl acetate
Alkyl benzenes	Isopropyl alcohol	n-Butyl glycidyl ether
Benzene	Methanol	Perchlorethylene
2-Butoxyethanol	2-Methoxyethanol	Phenol
Butyl alcohol	1-Methoxy 2 propanol	Styrene
Cyclo hexanone	Methylene chloride	Toluene
Diacetone alcohol	Methyl amyl alcohol	1,1,1-Trichlorethane
Diglycidyl ether	Methyl butyl ketone	Turpentine
Diisobutyl ketone	Methyl ethyl ketone	Xylene
Dimethyl formamide	Methyl isobutyl ketone	
2-Ethoxyethanol	Methyl n-amyl ketone	
Ethyl amyl ketone	Mineral spirits	
Other chemicals		
Ammonia	Epichlorohydrin	Phosphoric acid
Carbon black	Ethyl silicate	Silica
Coal tar pitch volatiles	Formic acid	Toluene diisocyanate

by letter and a subsequent phone call; a random subset of draftsmen who lived within 1 hour's drive of the clinical site was also contacted.

Both painters and controls were informed prior to coming to the visit that the study included a semen sample. The controls were offered $50 for participation; the exposed were not paid for their time. Men with vasectomies were included in the study if they agreed to participate, for the study included assessment of hematologic effects and the use of a fertility analysis in addition to semen analysis.

Questionnaires, physical examination, and laboratory testing. Each participant was seen at the clinical site. At this visit we administered a questionnaire, performed a physical exam, collected samples of blood and urine, described the correct technique for semen collection, and scheduled a time for the participant to bring back a sample.

The questionnaire elicited basic demographic information and information about medical conditions and personal habits that have been reported to affect semen parameters. These factors include smoking [Kulikauskas et al., 1985; Vogt et al., 1986], alcohol consumption [Van Thiel, 1983], caffeine consumption, medications, radiotherapy or chemotherapy, recent febrile illness, past history of mumps, and genitourinary conditions [Hargreave, 1983]. Each participant was asked about his work history and hobbies. He was asked if he and his wife had ever had difficulty conceiving a child, whether he saw a physician for this problem, and the physician's diagnosis.

In addition, a questionnaire was sent to painters and controls who declined to participate in the study, to determine if they differed from the participants in age; years of employment; alcohol, cigarette, and caffeine consumption; and the history of a fertility problem in the past.

The physical exam included measurement of testicular size, examination for the presence of a varicocele, and examination of secondary sex characteristics. Testicular size was determined using a template orchiometer [Takihara et al., 1983).

Urine was collected for determination of ethoxyacetic acid and methoxyacetic acid, the principal metabolites of 2-EE and 2-ME, respectively. A sample of urine was obtained from each participant at the time of the interview and physical examination; a second sample was collected at the time that the participant brought his semen sample. Two aliquots of each urine sample were frozen in clean glass jars and transported to the NIOSH laboratories in Cincinnati for analysis. The analytic method used was that of Smallwood et al. [1988].

A sample of blood was obtained for a complete blood count, including differential, and for determination of serum follicular stimulating hormone (FSH), serum lutenizing hormone (LH), and serum testosterone.

Each participant was given a coded, clean glass jar and thermos with verbal and written instructions on the method for semen sample collection. Subjects were asked to collect a semen specimen at home by masturbating into the jar and to bring the sample in the thermos to the clinical site within 1 hour of collection. They were asked to abstain from sexual activity for minimum of 48 hours prior to collection of the sample. The date and time of ejaculation, abstinence period, and spillage (if any) was recorded by the subject on the jar label.

Semen analysis methods. Semen analyses were conducted in two phases. Video recordings, viability measurements, sperm counts, volume and pH measurements, fixation of slides, and cryopreservation of seminal plasma were conducted at

the clinical site. Morphologic and morphometric analyses of slides and motility and velocity analyses of videotapes were conducted at the NIOSH laboratories. All samples were processed and analyzed in blind fashion by the investigators.

Sperm velocity and motility. Seven microliters of the semen samples were placed on a glass microscope slide and covered with glass coverslip. The samples were placed on a microscope stage warmed to 37°C by a heat curtain (Model #ASI 400, Nicholson Precision Instruments, Bethesda). Five to eight fields selected arbitrarily were video-recorded using a 25× phase objective. The video equipment consisted of a Panasonic NV 8200 video recorder, time-date generator, vertical enhancer, MTI black and white 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. Videotapes were analyzed using a Zeiss Videoplan semiautomatic image analysis system with video overlay and digitizing tablet. For sperm velocity measurements, 30 motile sperm from each sample were randomly selected and the path of each was digitized. The start and stop times to the nearest 0.01 second were recorded for each tracing.

Sperm velocity estimates involve two approaches: velocity along the actual sperm path and straight line (point-to-point) 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 and 1, where numbers closer to 1 indicate a more linear progressive path. The software of the microcomputer permitted calculations of path length and distance velocity and forward progression of each tracked sperm.

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

Semen pH and volume. The pH of the semen sample was determined using an Orion (Model 701) pH meter with a gel-filled plastic pH electrode (Fisher #13–639–252) The pH meter was calibrated (buffer pH 7.0; Fisher SO–B–107) and specimens were analyzed at room temperature. The 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 staining and analysis. These slides were stained in Papanicolaou stain according to the WHO semen analysis guidelines [Belsey et al., 1980]. Sperm morphology was scored according to Zanefeld and Polakoski [1977], with 200 cells read from each of two slides. The remaining two slides were used for objective analysis for sperm head shape (morphometry) using the Videoplan system [Jeyendran et al., 1986]. A 63× dry objective and HMI video camera with a 4× enlarger were used to evaluate 100 sperm on each of two slides. Individual sperm heads were outlined using the digitizing tablet, and the software allowed calculations of area, perimeter, length, width, width/length ratio, and 4pi (area)/perimeter^2 [Pi factor] as indices of sperm head shape.

Sperm viability. Viability by stain exclusion was determined by mixing 100 μl of semen with 100 μl of 0.5% (w/v) eosin y stain in Tyrode's buffer. Seven microliters of this suspension were placed on a microscope slide and 200 sperm were

counted and classified as unstained (viable) or stained (nonviable). Viability by hypo-osmotic swelling was analyzed by mixing 100 μl of semen with 0.1 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 phase 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 (Seif-Medical Instruments, Haifa, Israel), and the sperm were counted using DIC microscopy. Replicates were prepared and counted for each sample.

STATISTICAL ANALYSES

Linear modeling was used to determine if there was any contribution of smoking, alcohol consumption, age, or abstinence to the outcome for each variable. For the variables sperm density and count per ejaculate, smoking was a significant factor; for all other variables the factors were not significant and were not considered in further analysis.

The application of statistics that depend on a normal distribution of the parameter may be invalid when used for analysis of some semen parameters. It has been recommended that the data be transformed using a mathematical expression that will convert it to a normal curve [Snedecor and Cochran, 1972] and that this is needed for analysis of sperm density [Mortimer and Lenton, 1983]. Following this recommendation, data for each semen characteristic were tested for normality and appropriately transformed. A cube root transformation was used for sperm density and count per ejaculate. The difference between the groups for each transformed variable was compared using analysis of variance as described above.

The proportions of men in each group with oligospermia were compared using Fisher's exact test. For sperm density, we used a level of $\leqslant 20$ million sperm per milliliter of semen as a definition of oligospermia; for count per ejaculate, we used a level of $\leqslant 100$ million sperm per ejaculate [McLeod and Gold, 1951].

Finally, we calculated an odds ratio and confidence intervals using a Mantel-Haenszel chi-square analysis, stratifying the group for smoking.

RESULTS

Exposure Assessment

The shipyard employed 900 painters. They worked in four crews: shop, interior, exterior, and tank. The shop crew mixed the paints to formulation; the interior and exterior crews painted the inside and the outside of the boats, respectively; and the tank crew painted the interior of ballast tanks and other closed spaces. Men working on the tank crew primarily spray painted and wore air-supplied respirators. The interior crew members painted with both sprays and brushes and used epoxy paints, oil-based paints, and some water-based paints. Comfo II half-face cartridge respirators were available for these jobs, but actual respirator use was at the discretion of the painter. Cotton gloves were available for use.

The painters rotated from crew to crew in any one year. Many painters worked overtime, with interior painters performing tank painting operations on weekends.

Exterior work was done at the completion of a boat, and often all the painters were assigned to this job for a brief period of time. Exterior painters also sandblasted the boats prior to painting; air-suppled respirators were worn during this operation.

The industrial hygiene survey demonstrated that exposure to 2-EE as an 8-hour time weighted average (TWA) ranged from 0–80.5 mg/m^3 (0–21.5 ppm), with a mean of 9.9 mg/m^3 (2.6 ppm) and a median of 4.4 mg/m^3 (1.2 ppm). 2-ME exposure ranged from 0–17.7 mg/m^3 (0–5.6 ppm) (8-hour TWA), with a mean of 2.6 mg/m^3 (0.8 ppm) and a median of 1.4 mg/m^3 (0.44 ppm). Sampling was performed only for the interior crew work, for there were no tanks being painted at the time of the study. The sampling may underestimate usual or prior exposure, both for the members of the interior crew and other crews. This is discussed in more detail in the companion paper [Sparer et al., 1988].

Most of the exposure measured was to 2-EE; exposure to 2-ME was less frequent. 2-ME is a more potent reproductive toxin in animal experiments. Since the painters frequently moved from job to job, we cannot separate the effect of these two compounds in this study.

The possibility of exposure to the other known reproductive toxins in the work environment—lead and epichlorohydrin—was also evaluated. Exposure to lead was limited to blasting operations; air samples taken by NIOSH in 1978 [NIOSH, 1983a] showed that lead was not detectable above 10 μl/m^3 during brushing or cleaning operations. There were significant air lead levels during blasting, up to 11 mg/m^3, but the painters wore air-supplied respirators during this operation. Forty-five of the 94 participants were considered lead exposed and have been monitored for lead during their work as painters. Blood lead levels were reviewed. Only nine of the 45 men had average lead levels over 15 μg%, and only two had averages over 20 μg%. The highest average was 30 μg%, and the individual had only one value as high as 40 μg%. Epichlorohydrin was not detected in the air sampling.

The workers' exposure to glycol ethers was verified by measuring urinary metabolites; results of the analysis will be reported separately. In brief, this analysis confirmed that the study subjects had exposure to ethylene glycol ethers.

The controls did not report any opportunities for exposure or potential exposure to ethylene glycol ethers at the time of the study. Some had held other jobs in the shipyard prior to being employed as a clerk or draftsman, and so might have had prior exposure to glycol ethers; these jobs had all been at least 10 years prior to this study. None of these men had held the job of painter. No control had metabolites of glycol ethers detected in his urine.

Participation

One hundred and fifty-nine painters were invited to participate in the study, and 128 scheduled an appointment. Ninety-four of these kept the appointment, and from these participants we received 73 sperm samples. Nine of the 94 participants had had a vasectomy. This is a 50% participation rate among the eligible men (after adjusting for a 10% vasectomy rate).

For the controls, 33% of those invited to participate made and kept an appointment. Twenty-two percent of the participants had had vasectomies, and 98% of the remaining men brought back a semen sample. The overall participation rate from initial invitation to return of a semen sample was 32% among the controls (corrected for a 22% vasectomy rate).

Studies including semen analysis have often had a low rate of participation, and bias may be introduced from the effect of volunteers. To determine if this was the case in this study, we performed a follow-up survey of a subset of men from both exposed and control groups who had declined to participate in this study. Bias could be introduced if the men were aware of a problem with fertility and either participated because of it or chose not to participate on that basis. Theoretically, the participation could be in different directions in control and exposed groups.

We asked the 28 painters who had declined to participate in the study to fill out a simple questionnaire about their demographic characteristics and their fertility history; 10 of them (35%) returned the questionnaire. The men who had declined to participate were slightly older, with an average age of 41 as compared to 38 for the participants. They had the same educational background, and the same proportion of them smoked and drank alcohol as among the study participants. Of the ever-married subset of nonparticipants, 40% reported that they had seen a physician for a fertility problem; this rate was only 12.5% in the study group.

In addition, we performed an analysis of potential participation bias among the controls. We sent the same questionnaire to the 39 clerks who had declined to participate and received 15 replies, for a 38% rate of return of the forms. The nonparticipants were somewhat older (average age of 55 years as compared to 48 years for the participants), had the same average duration of employment at the shipyard, and had the same rate of cigarette smoking. Only two (12.5%) of the nonparticipants reported drinking once a day or more, compared to 34% of the study population. None of the nonparticipants reported having had a vasectomy. Seven of them (47%) reported fertility problems, as compared to 27% in the study group.

Although these results are based on a small number of individuals, we can conclude from this survey that the men who agreed to participate in the study did not differ systematically from the nonparticipants, except in age. Age has not been associated with decreased sperm count if abstinence is controlled. In addition, we concluded that men with a history of fertility problems did not accept an invitation to participate in the study more frequently than men without such a history.

Medical Studies

Characteristics of the groups. Tables II–IV describe the results of the questionnaire for the exposed and unexposed groups. The controls were older, more frequently consumed alcohol, and had worked longer in the shipyard. The painters had a higher proportion of current smokers. These differences were statistically significant. The goups did not differ in the reported history of mumps, genitourinary tract infections, recent febrile illness, or intake of medications. Mean abstinence was the same for both groups.

There were no significant differences in age, rate of smoking, alcohol consumption, or history of medical conditions between the painters who did and did not return a semen sample, excluding those men who had had a vasectomy.

We asked each participant if he had seen a doctor in the past for evaluation of infertility. Controls were more likely to have reported a prior problem. For the entire group, including those who did not return a semen specimen, a fertility problem was reported for 27% of the controls and 10% of the painters. Among the subset for whom we have a semen analysis, 29% of the controls reported a problem achieving a pregnancy, compared to 12.5% of the painters.

TABLE II. Characteristics of all Men Exposed to Ethylene Glycol Ethers and All Unexposed Men*

Characteristic	Exposed (n = 94) % (n)	Unexposed (n = 55) % (n)
Age of subject (years)		
Mean	38	48[b]
Range	19–62	28–63
Cigarette smoking		
Current	51 (48)	20 (11)[b]
Never smoked	47 (44)	75 (41)[b]
Exsmoker	2 (2)	5 (3)
Alcohol consumption		
Less than twice a month	28 (27)	13 (7)
Less than once a day	78 (73)	60 (33)[b]
At least once a day	22 (21)	40 (22)
Duration of employment (years)		
Mean	8	22**
Range	0.5–33	7–42
History of fever in pre-		
ceding 3 months	(6)	13 (7)
History of mumps	61 (57)	67 (37)
Presence of		
varicocele[a]	16(14)	22 (11)
Testicular size[a]		
15–20 ml	63 (55)	75 (38)
10–15ml	35 (31)	25 (12)
< 10 ml	2 (2)	0
Vasectomy	9.5 (9)	20 (12)
Any prescribed medication	33 (31)	42 (23)

*Includes participants both with and without semen analysis.
[a]88 exposed and 50 unexposed men had a physical examination. All men with a semen analysis had a physical examination.
[b]$p < 0.05$.

Hormone analysis. FSH, LH, and testosterone levels were measured for all participants. Two exposed individuals with azoospermia had elevated levels of FSH(21 and 25 mU/ml). Five individuals with oligospermia had LH levels that were below the normal range; all of these men had normal FSH levels. It is unlikely that hypothalamic or pituitary dysfunction was the cause of azoospermia or oligospermia in any of the participants. Table V presents the results of the hormone analyses.

Semen analysis. Tables V–VIII present the means and standard deviations of pH, volume, sperm concentration, total sperm count, viability, motility, morphology, and morphometry variables.

The exposed group had a significantly higher pH. Although statistically significant, the biological importance of such a small difference is uncertain. No significant differences were found in measures of sperm motility, either percent motile or velocity, viability, morphology, or morphometry.

Controls who smoked were significantly more likely to have a decreased sperm count; this association was not present among the painters. There were only eight controls who smoked. There was no significant association between age, coffee consumption, or alcohol consumption with either average count or count per ejaculate.

TABLE III. Characteristics of Those Men Exposed to Ethylene Glycol Ethers and Unexposed Men Who Underwent Semen Analysis

Characteristic	Exposed (n = 73) % (n)	Unexposed (n = 40) % (n)
Age of subject (years)		
Mean	37.5	47.9[a]
Range	19–62	28–64
Cigarette smoking		
Current	54 (39)	20 (8)
Never smoked	44 (33)	75 (30)
Exsmoker	1.5 (1)	5 (2)
Alcohol consumption		
Less than twice a month	30 (22)	15 (5)
Less than once a day	79 (58)	60 (24)[a]
At least once a day	21 (15)	40 (16)[a]
Duration of employment (years)		
Mean	7.9	22.5[a]
Range	0.5–33	7–42
History of fever in preceding 3 months	8.3 (6)	10 (4)
History of mumps	61 (44)	67 (27)
Presence of varicocele	14 (10)	17 (7)
Testicular size		
15–20 cm	57 (42)	77.5 (31)
10–15 cm	40 (29)	22.5 (9)
< 10 cm	3 (2)	0
Abstinence mean (days)	6.8	6.9
Any prescribed medication	33 (23)	45 (18)

[a]$p < 0.05$.

TABLE IV. Characteristics of Participants With Vasectomies

Characteristic	Exposed (n = 9) % (n)	Unexposed (n = 12) % (n)
Age of subject (years)		
Mean	45	48
Range	28–58	32–60
Alcohol consumption of at least one drink per day	11 (1)	8 (1)
Smoking status		
Current	45 (4)	16 (2)
Never smoked	55 (5)	58 (7)
Exsmoker	0	25 (3)
Any prescribed medication	45 (4)	25 (3)

TABLE V. Semen Characteristics and Hormone Levels of Exposed and Unexposed Men*

Characteristic	Exposed (n = 73)	Unexposed (n = 40)
Semen pH	7.94 ± 0.15	7.88 ± 0.16[a]
Semen volume (ml)	2.52 ± 1.2	2.88 ± 1.3
Sperm concentration (millions/cc)	66.5 ± 40.3	78.6 ± 53.9
Total sperm count (millions/ejaculate)	158 ± 108	211 ± 140
LH (mIU/ml)	4.7 ± 2.7	5.1 ± 2.2
FSH (mIU/ml)	5.3 ± 4.8	5.5 ± 3.9
Testosterone (ng/ml)	4.6 ± 1.8	4.1 ± 1.5

*All measurements are mean ± S.D.
[a]$p < 0.05$.

TABLE VI. Sperm Viability and Motility in Exposed and Unexposed Workers*

Characteristic	Exposed (n = 69)[a]	Unexposed (n = 40)
Viability by stain exclusion (%)	67.9 ± 13.6	62.3 ± 16.3
Viability by hypo-osmotic swelling (%)	66.9 ± 9.8	63.6 ± 10.8
% Motility	34.5 ± 15.2	33.4 ± 16.1
Velocity (path length in μm/sec)	62.7 ± 11.7	59.8 ± 15.2
Velocity (distance in μm/sec)	46.7 ± 9.26	45.8 ± 11.4
Ratio of length to distance velocity	0.76 ± 0.09	0.77 ± 0.09

*All measurements are mean ± S.D.
[a]Three of the 73 men were azoospermic, and a fourth was essentially azoospermic with a sperm concentration of 0.2 million/cc. The characteristics in this table could not be calculated for these four men.

TABLE VII. Sperm Morphology in Exposed and Unexposed Workers*

Characteristic	Exposed (n = 69)[a]	Unexposed (n = 40)
Oval	150.4 ± 18.9	149.8 ± 17.3
Macrocephalic	1.67 ± 1.48	1.63 ± 1.48
Microcephalic	1.03 ± 1.32	1.41 ± 1.55
Absent heads	7.62 ± 5.33	6.81 ± 4.28
Tapered heads	3.1 ± 3.5	2.4 ± 2.1
Double heads	2.09 ± 2.69	2.01 ± 2.28
Amorphous heads	2.99 ± 2.99	2.70 ± 3.35
Abnormal tails	8.92 ± 8.89	13.2 ± 10.2
Immature forms	22.0 ± 12.9	19.8 ± 12.8

*Two hundred sperm were analyzed for each semen sample. All measurements are mean ± S.D.
[a]Three of the 73 men were azoospermic, and a fourth was essentially azoospermic with a sperm concentration of 0.2 million/cc. The characteristics in this table could not be calculated for these four men.

TABLE VIII. Sperm Morphometry in Unexposed and Exposed Workers*

Characteristic	Exposed (n = 69)	Unexposed (n = 40)
Area (μm^2)	8.10 ± 0.8	8.10 ± 0.8
Length (μm)	4.25 ± 0.36	4.20 ± 0.3
Width (μm)	2.48 ± 0.16	2.49 ± 0.18
ELL[a]	0.597 ± 0.06	0.61 ± 0.05
PI[a]	0.85 ± 0.04	0.85 ± 0.04

*All measurements are mean ±S.D. Two hundred were analyzed for each semen sample.
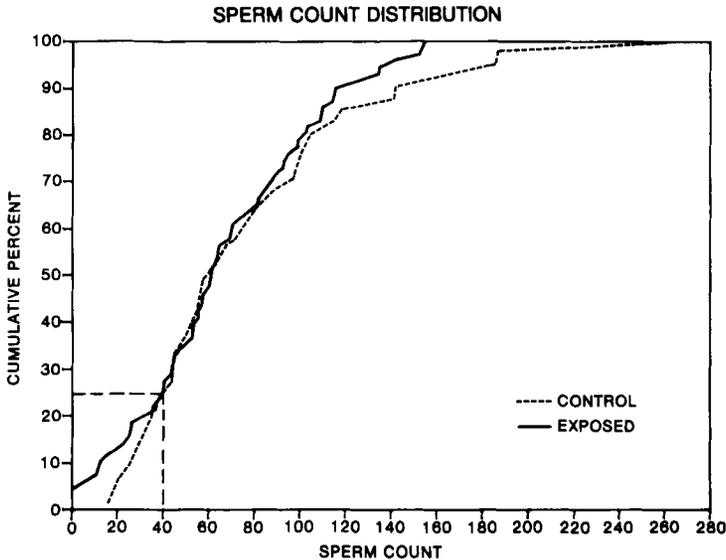[a]ELL, width/length ratio; PI, 4 pi (area)/perimeter^2.

Fig. 1. Sperm count in million sperm/ml (sperm density) for control and exposed groups. The exposed group has fewer individuals with sperm densities below 40 million/ml and above 120 million/ml.

Using analysis of variance, we compared mean sperm density and count per ejaculate between painters and controls while controlling for smoking, age, and abstinence; for this analysis, the measures of sperm count were lower in the painters, with p = 0.10 for density and p = 0.11 for total count.

The proportion of men with a sperm density ⩽20 million/cc was higher in the exposed group than in the unexposed group, 13.5% vs. 5% (p = 0.12). There were ten painters and two controls with sperm counts less than 20 million/cc. Three of these painters were azoospermic, one was essentially azoospermic with a count of 0.2 million/cc, and the remainder were oligospermic. Neither of the two controls were azoospermic. Figures 1 and 2 illustrates the distribution of sperm density for each group.

We compared the proportion of each group with oligospermia defined as a count per ejaculate ⩽ 100 million. Eight of the controls and 24 of the painters had counts less than or equal to 100 million per ejaculate, representing 20% and 33%, respectively (p = 0.20) (Table IX).

Since the association between sperm count and smoking was present among the controls, and there was a difference between the groups in the rate of smoking, the

rate of oligospermia was analyzed separately for smokers and non-smokers. Among the non-smokers, the exposed group had a higher rate of oligospermia (p = 0.05) (Table IX). In addition, a Mantel-Haenszel chi-square analysis was conducted controlling for smoking. This revealed an odds ratio of 1.85% for a decreased count per ejaculate among the painters, with a 95% confidence interval of 0.6–5.6, indicating (Table X).

DISCUSSION

The painters in this study had exposure to ethylene glycol ethers through aerosol and skin contact. Most of them used respiratory protection while spray painting and
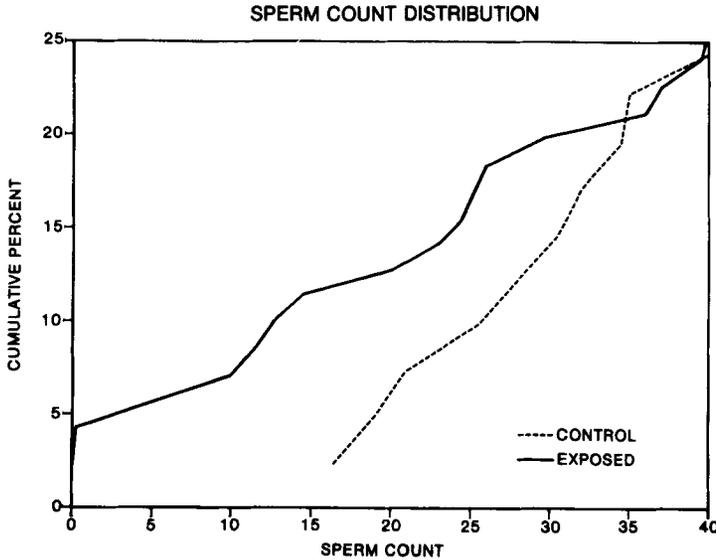

Fig. 2. Sperm count in million sperm/ml (sperm density) for control and exposed groups, for the range of 0-40 million/ml.

TABLE IX. Fischer's Exact Test for Sperm Density and Total Sperm Count, With Oligospermia Defined as a Count/cc of ≤20 Million and a Count/Ejaculate of ≤100 Million

	Exposed	Control	Total	
Count per cc				
≤20 million	10	2	12	
>20 million	63	38	101	p = 0.13
Total	73	40	113	
Count per ejaculate				
≤100 million	24	8	32	
>100 million	49	32	81	p = 0.2
Total	73	40	113	
Count per ejaculate: non-smokers only				
≤100 million	12	5	17	p = 0.05
>100 million	21	27	48	
Total	33	32	65	
Count per ejaculate: smokers only				
≤100 million	12	3	15	p = 0.49
>100 million	28	5	33	
Total	40	8	48	

TABLE X. Mantel-Haenszel Chi-Square Analysis for Total Sperm Count, With Oligospermia Defined as a Count/Ejaculate of $\leqslant 100$ Million*

	Count[a]			Count[b]		
	Low	Normal		Low	Normal	
Exposed	12	28	40	12	21	33
Unexposed	3	5	8	5	27	32
Total	15	33	48	17	48	65

*The analysis was stratified for smoking, with ex-smokers classified as non-smokers.
[a]Smokers, OR = .89.
[b]Non-smokers, OR = 2.83.
Combined odds ratio = 1.86, with a 95% confidence interval: 6.62 = 5.6.
Chi square for homogeneity = 1.22, p = 0.27.

intermittently used cartridge respirators while brush painting. Our industrial hygiene analysis demonstrated that there was significant air exposure during these operations and that skin contact was possible. In addition, the industrial hygiene analysis showed that painters not engaged directly in the painting were exposed from the work of painters nearby; this means that a painter performing clean-up or preparation work could have inhalation exposure even if not using the products himself. Finally, analysis of metabolites in the urine documented that this group of painters was exposed to ethylene glycol ethers.

Because of the nature of painting in this shipyard, the painters could not be classified into dose groups. The painters rotated from one job to another on a regular basis. Exposure from 2 to 6 months prior to semen analysis is likely to produce an effect at the time of study, and it was not possible to determine where each man worked during this time. Since these men rotate from job to job, it is reasonable to presume that they all come in contact with the high-exposure areas on a regular basis. An effect on sperm has been demonstrated in animals secondary to acute exposure to glycol ethers [Foster et al., 1984]; an exposure at a critical point could have caused a decreased count at the time of the study. For these reasons, we concluded that all the painters were exposed to a mean level of 9.9 mg/m^3 2-EE on a 8-hour TWA day, with peak exposures up to 80.5 mg/m^3 in some operations. Painter exposure was at a mean level of 2.6 mg/m^3 of 2-ME for an 8-hour TWA, with a peak exposure of 17.7 mg/m^3.

As discussed above, the controls did not have any exposure to ethylene glycol ethers at the time of the study. Some of them may have had incidental exposure when they held other jobs in the shipyard; any such exposures were at least 10 years prior to the study, and none of the controls had ever worked as a painter.

The results of the semen analysis suggest that there was an effect of ethylene glycol ethers on sperm count. Although mean values did not differ significantly between the groups, biologically important differences were seen when the proportion of men with oligospermia was examined. The proportion of exposed men with a sperm count less than or equal to 20 million/cc was 13%, with 5% expected based on other population surveys [McLeod and Gold, 1951; Rehan et al., 1975]. The proportion of painters with azoospermia was 5%, with only 1% expected based on other population surveys. Non-smoking painters were more likely to have oligospermia defined as a sperm count per ejaculate of less than or equal to 100 million. The odds ratio for oligospermia among the painters is increased to 2.8 among the non-smokers.

This result is consistent with the effect seen in animal studies. Studies in several species show that these glycol ethers cause loss of germinal epithelium and testicular atrophy [Foster et al., 1984; Zenick et al., 1984]. Cellular studies show that this effect occurs by inhibition of cell division in the early pachytene stage of spermatogenesis, an effect that would be expected to result in a decreased count rather than an effect on motility or morphology.

In addition, a recent study of men exposed to 2-EE in a foundry demonstrated a decreased number of sperm per ejaculate. The exposure levels measured in that plant were somewhat higher than the ones demostrated here, and significant skin contact was observed [NIOSH, 1986].

It is possible that the difference in the prevalence of oligospermia in the two groups is due to a factor other than exposure to ethylene glycol ethers. The other factors present in this study that might affect the conclusions include exposure of the painters to another toxin that affects sperm count or a systematic bias in the participation of men with preexisting fertility problems.

The groups did differ in factors that may affect semen analysis. The controls as a group were older, drank alcohol more frequently, and were more likely than the exposed group to have reported a fertility problem, whereas the painters smoked more frequently. However, these factors are unlikely to explain the results, since the difference in alcohol consumption, age, and any preexisting fertility problems would tend to decrease counts in the control group.

Smoking cigarettes has been associated with decreased sperm count and with an increased proportion of morphologically abnormal sperm [Vogt et al., 1986; Kulikauskas et al., 1985]. In this data set, smoking was associated with decreased sperm density among the control population, but not in the exposed group or the study group as a whole. We examined sperm count, controlling for smoking, and the analysis indicated an association between exposure and oligospermia.

Since the participation rate was less than 100%, a bias could be introduced if painters with oligospermia were more likely to participate in the study. The same proportion of all painters and painters with a total sperm count ≤ 100 million reported a prior problem with fertility, 9/74 vs. 13/23, whereas the rate among the controls was 15/40. Many sources report that the rate of infertility in this country is currently 10–15%; the painters reported a rate of 13%.

Twenty-seven percent of the controls reported a fertility problem at some time. Since the controls were older and thus had had more years in which to have a problem, we could expect a higher rate in this group. Even though the groups cannot be directly compared because of the difference in age, the higher rate of infertility reported by the unexposed men lends support to the conclusion that the difference in sperm count between the painters and controls was not due to participation bias.

We reviewed the questionnaires of the exposed men with azoospermia and oligospermia to see if they were aware of a prior problem. Of the four painters with azoospermia, two were young, unmarried, and did not report any infertility. The third reported a period in the mid 1970s when he had fertility difficulties, but had two children before employment in the shipyard. The fourth was aware of infertility but had thought it was due to a problem with his wife. One other man with a total count ≤ 100 million reported that his former wife had difficulty conceiving, but he had two children with his current wife.

The follow-up survey of the nonparticipants also demonstrated that the men who participated were not more likely to have a fertility problem than those who declined

to participate. Based on 1) the rates of reported problems with conception in the exposed and unexposed groups, 2) the fact that most of the painters with low sperm counts were not aware of a prior problem, 3) the rate of men who were aware of a problem is similar to that predicted from other population sources, and 4) the higher rate of reported problems in the nonparticipants, we can conclude that the results are not due to a differential participation of men with oligospermia in the exposed group.

Lead is known to cause a depression of sperm count [Lancranjan et al., 1975]. As discussed above, the mean lead levels of the 45 men who had been monitored for lead were mostly below 20 μg%, and the highest single level in any individual was 40 μg%. This level of lead exposure has not been documented to cause depressed count. Epichlorohydrin was not detected in the air sampling during the study.

The presence of a dose-response relationship would strengthen the conclusions drawn from this data. We measured metabolites of ethylene glycol ethers in the urine of each participant during the week of the study. However, the level of alkoxyacetic acids could not be used as an indicator of the exposure of that individual in the preceding months, for our industrial hygiene survey demonstrated that painters moved frequently from one exposure area to another, and the metabolites reflect only the exposure of the day of the sample. To be able to create a dose-response model for an acute effect of glycol ethers, we would have had to be able to describe the exposure of each individual 3 to 6 months prior to the semen analysis. Neither the air sampling nor the urine metabolites gave us a useful way to characterize individual exposure.

CONCLUSIONS

We conclude that exposure to the ethylene glycol ethers 2-ethoxyethanol and 2-methoxyethanol lowered sperm count in this group of painters. The overall result that ethylene glycol ethers affect reproductive function is reinforced by prior experimental data and epidemiologic studies. This study adds weight to the evidence that ethylene glycol ethers cause disturbances of male fertility.

ACKNOWLEDGMENTS

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