Semen quality in workers exposed to 2-ethoxyethanol

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ABSTRACT   To evaluate whether long term exposure to 2-ethoxyethanol (2EE) may affect semen quality, a cross sectional study was conducted among men exposed to 2EE used as a binder slurry in a metal castings process. Full shift breathing zone exposures to 2EE ranged from non-detectable to 24 ppm (geometric mean 6·6 ppm). Because of the potential for substantial absorption of 2EE through skin exposure, urine measurements of the metabolite of 2EE, 2-ethoxyacetic acid (2EAA) were conducted, showing levels of 2EAA ranging from non-detectable to 163 mg 2EAA/g creatinine. Only 37 exposed men (50% participation) and 39 non-exposed comparison (26% participation) from elsewhere in the plant provided a sperm sample. A questionnaire to determine personal habits, and medical and work histories, and a physical examination of the urogenital tract were also administered. The average sperm count per ejaculate among the workers exposed to 2EE was significantly lower than that of the unexposed group (113 *v* 154 million sperm per ejaculate respectively; $p = 0.05$) after consideration of abstinence, sample age, subjects' age, tobacco, alcohol and caffeine use, urogenital disorders, fever, and other illnesses. The mean sperm concentrations of the exposed and unexposed groups did not significantly differ from each other (44 and 53 million/ml respectively). No effect of exposure to 2EE on semen volume, sperm viability, motility, velocity, and normal morphology or testicular volume was detected, although some differences in the proportion of abnormal sperm shapes were observed. These data suggest that there may be an effect of 2EE on sperm count among these workers, although the possibility that other factors may be affecting the semen quality in both exposed and unexposed men in this population or that the results reflect bias introduced by the low participation rates cannot be excluded.

2-Ethoxyethanol (2EE; ethylene glycol monoethyl ether) is one of a family of glycol ethers used in a wide variety of industries as solvents, thinners, anti-icing additives, and other products.[1] The National Institute for Occupational Safety and Health (NIOSH) has estimated that at least 400 000 workers are potentially exposed to 2EE in the United States.[2] The current Occupational Safety and Health Administration (OSHA) eight hour time weighted average (TWA) permissible exposure limit (PEL) for 2EE is 200 ppm[3]; this is based primarily on the renal, hepatic, and haematological effects of 2EE observed in early animal experiments.[1] The American Conference of Governmental Industries Hygienists (ACGIH) recommends a threshold limit value (TLV) of 5 ppm as an eight hour TWA.[4] Both the OSHA PEL and the ACGIH TLV bear the "skin" notation indicating the potential for absorption of toxic amounts of 2EE through the intact skin. On the basis of recent animal data on the adverse

reproductive effects of 2EE in both female and male animals, NIOSH has recommended that exposure be reduced to the lowest extent feasible.[1] 2EE has also been reported to increase the rate of chromosome aberrations and sister chromatid exchanges in Chinese hamster ova in vitro,[5] but has not been shown to be mutagenic in the Ames test, according to a recent review by McGregor.[6] Currently, there are no data available on the potential carcinogenicity of 2EE in animals or man.

With respect to the male reproductive toxicity of 2EE, testicular atrophy and microscopic testicular changes (including degeneration of seminiferous tubules and damage to dividing spermatocytes and spermatids) have been reported in rats given 2EE by mouth[7-10] or subcutaneously[8] at various doses, in mice[11] and dogs[8] after oral administration, and in rabbits after exposure by inhalation.[12] Oudiz *et al* found partially reversible severe oligozoospermia or azoospermia among rats seven weeks after dosing with 1872 and 2808 mg/kg 2EE for five days, and a

significant increase in abnormal sperm morphology after treatment with 936 mg/kg 2EE.[13] Finally, Lamb *et al* found dose related decreases in sperm motility, an increase in the percentage of morphologically abnormal sperm, and decreases in testicular weight in mice given 1–2% 2EE in their drinking water for 14 weeks.[14] A significant reduction in fertility (number of live pups per litter) among untreated females mated with the males treated with 2% 2EE was also observed. There are no previous studies of the reproductive effects of 2EE in man.

Owing to concern regarding the possible reproductive hazards to male workers of exposure to 2EE in a slurry mix used to prepare ceramic shells for casting metal parts, NIOSH researchers conducted an industrial hygiene survey and a cross sectional semen study at a metal casting plant in Portland, Oregon.[15] The present paper describes the findings from the semen study and presents a summary of the industrial hygiene surveys; the latter are described in detail elsewhere.[16]

Materials and methods

DESCRIPTION OF PROCESS AND WORKFORCE
The metal castings company is engaged in the manufacture of precision cast parts, ranging from compressors and turbochargers for aircraft to prosthetic limb joints, using the "lost wax" method of investment casting. Wax replicas of parts are repeatedly immersed in a slurry composed principally of ethanol and 2EE and dipped in a sand shower to build up a ceramic shell mould from which the wax is melted out and molten metal poured in for casting. Shells are dried on belts and racks in the investing rooms; the recirculation of air disperses vaporising 2EE evenly throughout these areas.

About 80 male workers are employed in the investing departments in three separate buildings at the plant; these include men who make up or who periodically check the slurry mix ("utility investors"), or both; hand dippers and grabber operators who dip the wax moulds in the slurry and sand; shell processors who handle the finished shells; supervisors; and engineers who regularly enter the investing rooms. All are potentially exposed to airborne levels of 2EE by inhalation. Airborne 2EE vapour may also be absorbed through the skin and some workers have potential skin exposure from splashes and spills of slurry. Some workers wore gloves but no other personal protective equipment or respirators were used at the time of our survey.

EXPOSURE MEASUREMENTS
Two industrial hygiene surveys were conducted, one about two months before and the second during the semen study. Air samples were collected and analysed for 2EE by gas chromatography according to NIOSH method 1403.[17] Full shift breathing zone samples were obtained from workers in each job category in the investing departments in each building on at least two separate days. No short term samples were taken since there were no identifiable sources of peak exposures. Area samples were also obtained at a higher flow rate than the personal samples, thereby permitting a lower limit of detection in the event that airborne levels were below 0·1 ppm. Blank and spiked field samples were analysed (blind) to check for contamination and analyte recovery rate.

To monitor urine excretion of the metabolite of 2EE, 2-ethoxyacetic acid (2EAA), 24 hour and spot urine samples were obtained from randomly selected volunteers in 200 ml plastic vials, transferred to 20 ml scintillation vials, and frozen for shipment to the laboratory. Samples were analysed by the method of Smallwood *et al.*[18] Blood samples were also obtained from nine exposed and four unexposed workers to determine whether the parent compound, 2EE, could be detected in blood as shown in animals,[18] but not previously in man. Five millilitres samples were obtained at the end of a shift, chilled, and analysed as described by Smallwood *et al.*[18] Five spiked samples containing 25 μg 2EE/g showed 100% recovery, suggesting that the method was adequate. None of the samples obtained contained detectable levels of 2EE. These limited data suggest that, if 2EE is present in the blood, it is at a level below the detection limit of 10 μg/g blood.

SELECTION OF PARTICIPANTS
In cooperation with the plants' industrial hygienist, all male workers who could be potentially exposed to 2EE (on a daily basis) were identified from personnel lists. Each man (a total of 79 available at the time of the study) was interviewed confidentially to describe the purpose and procedures of the study and to solicit participation. Six workers were ineligible owing to vasectomies or employment in a potentially exposed job category for less than one month. Fifty men (68% of the 73 eligible men) agreed to participate, of whom 37 provided semen samples (50% of the eligible men).

Randomly selected workers from elsewhere in the plants, including workers in the wax mould preparation and metal casting departments, *x* ray and finishing departments, quality controllers, and process engineers, were interviewed to solicit participation. Fifty of about 150 unexposed men who were interviewed agreed to participate, of whom 39 (26%) provided semen samples. Men who had previously worked in the investing department (with previous potential exposure to 2EE) were excluded.

QUESTIONNAIRE, PHYSICAL EXAMINATION, AND SEMEN COLLECTION

Each participant was given a questionnaire by personal interview to provide data on demographic characteristics, personal habits (including tobacco, alcohol, and caffeine consumption), medical history, work history, and history of current and previous exposures to chemical and physical hazards. Participants underwent a brief physical examination concentrating on the urogenital tract. Testicular volume was measured using a Prader orchidometer. Subjects were asked to provide a semen specimen at home by masturbation into the glass jar provided, after a minimum of two days of sexual abstinence, and to bring the sample in the thermos provided to the medical department within an hour of collection, as described previously.[19]

SEMEN ANALYSIS METHODS

Semen analysis methods are described in detail elsewhere,[20] but are briefly outlined below. The methods of analysis for semen pH and volume, sperm concentration, viability, motility, velocity, morphology, and morphometry have been described in detail previously.[19] Sperm viability was measured by stain exclusion, modified from Eliasson and Treichl,[21] and by hyposomotic swelling according to Jeyendran et al.[22] Sperm motility and velocity were measured using videotapes and an image analysis system; both actual path and straight line ("distance") velocity were recorded.[22-25] Sperm morphology was scored according to Zanefield and Polakoski[26] and morphometric analysis of sperm head shapes analysed from air dried slides as described in Schrader et al.[27] Video recordings of samples, viability assessments, sperm counts, volume and pH measurements, and preparation of air dried smears were conducted on fresh samples at the field site. Analysis of video tapes for motility and velocity measurements and morphological analysis of slides were conducted in the laboratory. All samples were processed and analysed in blind fashion by the investigators.

STATISTICAL ANALYSIS

Data for each semen characteristic were analysed as described previously.[19] Transformations were performed for data that were not normally distributed after testing for normality using the Shapiro-Wilks statistic[28]; for proportions—for example, the percentage of motile sperm—logistic transformations were used, and a square root transformation was found to approximate most closely to a normal distribution for sperm concentration and count per ejaculate. Data for other characteristics did not significantly depart from normal. Variables were analysed using two sided t tests and least squares stepwise linear regression analysis. In the case of abnormal sperm morphology classifications data were

analysed using the CATMOD (formerly FUNCAT) procedure[29] which permits multivariate modelling of categorical frequency data by performing a simultaneous analysis of the frequency of responses in each category of abnormal sperm (by comparison with the proportion of normal sperm) in relation to exposure and other independent variables. Approximate χ^2 statistics are produced. Tobacco smoking (cigarettes, pipes, cigars a day), alcohol consumption (drinks a week), caffeine consumption (cups of coffee or tea, or both, a day), prescription medication use in the past year, history of fever within the past three months, history of urogenital disorders or an abnormal urogenital examination (including the presence of varicocele) and prior exposure history, the subject's age, sample age at analysis, and sexual abstinence time were considered a priori for inclusion in regression models as potential confounders. Variables were excluded from the model if the p value of the coefficient was less than 0·1 and the variable had a negligible effect on the exposure variable.

Results

EXTENT OF EXPOSURE
Air samples
Full shift TWA area air samples collected in survey I, about two months before the semen study (survey II),

Table 1   *2EE exposure by job category and location (8 hour TWA breathing zone and area samples; parts per million*)*

Job category/location	Survey I† gm gsd	No	Survey II† gm gsd	No
Building A:				
Area samples:				
Investment room	16·9(1·1)	[3]	3·0(6·8)	[3]
2EE mixing room	4·8(2·0)	[2]	NA	
Breathing zone samples:				
Hand dipper	14·5(1·2)	[5]	NA	
Hand shell processor	3·0(4·7)	[3]	NA	
Utility investor	8·5(1·6)	[3]	NA	
Building B:				
Area samples:				
Investment room	10·7(1·3)	[3]	14·9(1·1)	[4]
2EE mixing room	6·6(1·4)	[4]	NA	
Breathing zone samples:				
Grabber operator	6·5(1·1)	[2]	10·0(2·9)	[7]
Forklift shell processor	NA		8·5(2·4)	[6]
Supervisor	6·0(1·1)	[2]	5·0(1·1)	[1]
Building C:				
Area samples:				
Investment room (A)	NA		2·4(5·5)	[5]
Breathing zone samples:				
Grabber operator	NA		5·7(2·5)	[2]
Forklift shell processor	NA		1·6(2·2)	[3]

*Limit of detection for 2EE is 0·03 ppm (area samples) and 0·15 ppm (breathing zone samples).
†Survey I conducted two months before semen study; survey II conducted during the semen study. 2EE use discontinued in buildings A and C at the time of survey II.
gm = Geometric mean.
gsd = Geometric standard deviation.
No = Number of samples.
NA = Not applicable or not sampled.

Table 2 *Urine excretion of 2EAA (mg 2EAA/g creatinine*) by job category*

Job category	Mean	SD	No
Hand dipper	35·5	(10·9)	10
Grabber operator	84·9	(31·3)	16
Shell processor	73·0	(11·9)	5
Shell processor (2 days off work)	ND		1
Supervisor	29·9	(6·4)	5
Supervisor (1 day off work)	17·3	(2·3)	3
Controls	ND		9

No = Number of individual urine voids.
*Limit of quantitation = 10 mg/l (before creatinine adjustment).

indicated levels of 2EE ranging from 4·8 ppm to 16·9 ppm (geometric mean 6·5 ppm) at two of the three buildings (A and B) (table 1). Exposures in the investing room in both buildings were generally higher than chemical storage and mixing areas. About two to three weeks before survey II, the use of 2EE was suspended in buildings A and C; although some 2EE was still in the process stream, the concentrations in building A (and presumably in building C) appeared to have declined on average in survey II (table 1). In building B, where the use of 2EE was not discontinued,

Table 3 *Characteristics of unexposed and exposed workers*

	Unexposed (n = 38)	Exposed (n = 37)
Duration of employment (y) (mean ± SD)	5·2 ± 5·0	7·3 ± 5·5
Duration of employment in 2EE exposed jobs (y) (mean ± SD)	—	4·9 ± 4·1
Age of subject (mean ± SD)	30·3 ± 7·5	30·1 ± 7·0
Cigarette smokers (%):		
Current	44·7	43·2
Ex	18·4	18·9
Non	36·9	37·9
Alcohol consumption (drinks/week) (mean ± SD)	7·2 ± 10·0	12·5 ± 14·6
Caffeine consumption (cups tea/ tea/coffee/day) (mean ± SD)	2·5 ± 2·8	2·7 ± 3·2
Use of prescribed medication in previous year (%)	26·3	16·2
History of fever in preceding 3 months (%)	7·9	13·5
History of urogenital disorders (%)*	23·7	29·7
Presence of varicocele (%)	15·8	'32·4‡
Other abnormal medical history (%)†	8·1	8·1
Testicular vol (ml):		
Right (mean ± SD)	22·1 ± 4·0	21·5 ± 4·7‡
Left	21·1 ± 3·8	20·7 ± 5·0‡
For semen analysis:		
Duration of sexual abstinence (days)	3·7 ± 4·9	2·8 ± 2·2
Age of semen sample at analysis (mins)(mean ± SD)	54·2 ± 32·1	55·8 ± 33·2

*Urinary tract infection, venereal disease, or testicular trauma.
†History of diabetes, hepatitis, rheumatic fever.
‡n = 34.
All differences between exposed and unexposed groups not significant (p > 0·05).

air levels were comparable in both surveys.

Eight hour TWA breathing zone exposures were generally similar to those indicated by area samples, with an overall geometric mean of 6·6 ppm but varied by job (table 1). Men who had close contact with the slurry (hand dippers, utility investors, and grabber operators) had, as expected, higher breathing zone exposures (ranging from a mean of 5·7 to 14·5 ppm) than shell processors and supervisors (ranging from a mean of 1·6 to 8·5 ppm). The recovery of analyte from spiked samples was less than 100% and as low as 60%.[16] The spiking data were not used to correct the actual exposure but measurements suggest that airborne exposures may be higher than indicated by these data.

URINE SAMPLES

All but two urine samples showed detectable excretion of 2EAA among workers exposed to 2EE (table 2), ranging from 16 to 163 mg 2EAA/g creatinine for individual voids, indicating absorption of 2EE. There were insufficient data statistically to evaluate differences between job categories; however, grabber operators and shell processors had higher levels of 2EAA excretion (with means 485 and 73 mg/g creatinine respectively) than hand dippers (mean 36 mg/g creatinine) in this limited series, despite apparently less opportunity for airborne and skin exposure. In some cases urine samples were collected sequentially from individuals over a workshift, 24 hours, or over several days. There was some evidence that excretion of 2EAA in individuals increased during the workday (data not shown: see ref 16) and decreased on days off work. It was not possible, however, to determine adequately whether 2EAA excretion increased during the workweek due to sampling variability.

CHARACTERISTICS OF THE GROUPS

There were no statistically significant differences between the exposed and unexposed groups for characteristics identified by questionnaire and physical examination (table 3). All the participants were white (except one Hispanic unexposed worker) and of similar age (mean 30) and duration of employment. The average duration of employment in jobs exposed to 2EE was less than the total employment time for exposed men due to prior periods spent in other, unexposed, job categories by some workers. One exposed subject was found to be severely oligozoospermic; on physical examination, the cause was determined to be small testicular size which preceded employment in this occupation. This subject was eliminated from further analysis. Three exposed men declined the physical examination. A higher proportion of exposed men had evidence of a

Table 4 *Semen volume, sperm concentration, and count in unexposed and exposed workers*

Characteristic	Unexposed (n = 38)		Exposed (n = 37)		
	Mean	SD	Mean	SD	p*
Semen pH	8·08	0·18	8·03	0·17	
Adjusted mean‡	8·09		8·01		NS
Semen volume (ml)	3·1	1·4	2·8	1·3	
Adjusted mean§	3·0		2·9		NS
Sperm concentration (millions/ml)	60·2	37·0	48·5	30·2	
Adjusted means	53·3		45·0		NS
Sperm count (millions/ejaculate)	178·6	118·0	123·4	81·7	
Adjusted mean	153·9		112·7		0·05
%Subjects with sperm concentration ⩽ 20 million/ml	10·5		16·2		NS

NS = Not significant (p > 0·05 (two sided)).
*Two sided p value for the effect of exposure adjusted for other factors in the model or †two sided Fisher's exact test.
‡Adjusted for abstinence (p = 0·03) and presence of varicocele (p = 0·04).
§Adjusted for abstinence (p = 0·02) and subject's age (p = 0·04).
‖Adjusted for use of prescription medication in past year (p < 0·01) and presence of varicocele (p < 0·01).

varicocele on physical examination (32% *v* 16% in controls), although this difference was not statistically different. The mean testicular volumes of exposed and unexposed subjects were 20·7/21·5 and 21·1/22·1 ml (left/right) respectively and did not differ significantly between the two groups.

SEMEN ANALYSIS
The mean semen volume and sperm concentration among the exposed group were lower than among unexposed workers, but the differences were not statistically significant before or after correction for the effect of significant confounders (table 4). The sperm count per ejaculate, however (calculated as sperm concentration (millions/ml) multiplied by semen volume (ml)), was marginally significantly lower among men exposed to 2EE (p = 0·05) after consideration of the potentially confounding factors listed in the methods section. Only the rise of prescription medication and the presence of varicocele were significant confounders (table 4). The proportion of men with oligozoospermia (a sperm concentration of 20 million/ml or less) was higher in the exposed group

than in the unexposed group (16·2% *v* 10·5%) but this difference was not significant (table 4). There were no azoospermic men. No significant differences between exposed and unexposed men were found with respect to measures of sperm pH (table 4), sperm viability, percentage motility or velocity (table 5), the overall proportion of sperm with normal morphology (oval heads and normal tails) (table 6), or morphometry (table 7) after adjustment for significant confounding variables where specified in the tables. (In the case of sperm velocity, morphology, and morphometry none of the independent variables included in the regression models was a significant confounder.)

In the case of abnormal sperm forms (table 6) the exposed men had a significantly lower proportion of double headed sperm and a significantly higher proportion of immature forms (NB p ⩽ 0·001 was taken as the level of significance for the χ^2 test used in the CATMOD procedure to avoid small differences, given the large number of observations (200 cells per subject) being detected as statistically significant.

The possibility that duration of exposure due, for example, to a cumulative effect of exposure on

Table 5 *Sperm viability, motility, and velocity in unexposed and exposed workers*

Characteristics	Unexposed (n = 38)		Exposed (n = 36)*		
	Mean	SD	Mean	SD	p†
Viability by stain exclusion (%)	71·2	9·1	71·5	10·1	
Adjusted mean‡	72·1		72·3		NS
Viability by hyposomotic swelling (%)	66·8	10·2	68·6	7·6	
Adjusted mean§	67·5		69·4		NS
Motility (%)	40·4	12·3	43·9	10·5	
Adjusted mean‖	39·8		43·6		NS
Velocity (path length) (μm/sec)	65·2	14·2	65·6	13·3	NS
Velocity (distance)(μm/sec)	39·8	8·8	40·2	7·7	NS
Ratio of length/distance velocity	0·7	0·1	0·7	0·1	NS

NS = Not significant (p > 0·05 (2 sided)).
*One sample insufficient for viability, motility, and velocity determination due to low sperm count.
†Two sided p value for the effect of exposure adjusted for other factors in the model.
‡Adjusted for abstinence (p = <0·01).
§Adjusted for presence of varicocele (p = 0·01).
‖Adjusted for presence of varicocele (p = 0·03).

Table 6 *Sperm morphology classifications* in unexposed and exposed workers*

Category	Unexposed (n = 38)		Exposed (n = 37)		p†
	Mean	SD	Mean	SD	
Oval (normal) heads (%)	79·42 ± 10·59		78·02 ± 9·47		NS p‡
Macrocephalic (large heads (%)	1·40 ± 2·39		0·95 ± 0·70		NS
Microcephalic (small) heads (%)	0·40 ± 0·56		0·36 ± 0·37		NS
Absent heads (%)	1·78 ± 1·46		1·97 ± 1·70		NS
Tapered heads (%)	3·58 ± 4·24		3·65 ± 3·55		NS
Double heads (%)	1·65 ± 3·16		0·95 ± 1·11		<0·001
Amorphous heads (%)	1·00 ± 1·92		1·18 ± 1·01		NS
Abnormal tails (%)	3·38 ± 3·15		4·05 + 3·89		NS
Immature forms (%)	7·40 ± 6·22		8·82 ± 6·71		0·001

NS = Not significant (p > 0·05 (2 sided)).
*Mean % of two slides: 200 cells read/slide.
†p Value of 2 sided *t* test (normal forms).
‡p Value of χ^2 test for simultaneous analysis of ratio of each abnormal form in comparison with normal forms for exposed and unexposed groups (CATMOD procedure). Note that this analysis does not compare the difference between means for each abnormal category as in the *t* test.

spermatogonial cells or the hypothalamopituitary testicular axis, was examined. Duration of exposure was calculated as the cumulative total of months of potential exposure to 2EE based on time in different jobs since the start of employment reported by the worker. No effect on the various semen characteristics was observed when a test for linear trend was performed. It should be noted, however, that several currently exposed workers had not had continuous potential exposure to 2EE since the start of employment due to time spent in other departments or away from work. Groups considered to have potentially higher exposure to 2EE through air and skin contact (based on industrial hygiene data and observation of work practices)—that is, hand dippers, grabber operators, and utility investors (n = 10)—were also compared separately with workers with potentially lower exposure and unexposed men in the regression analyses. No differences in semen characteristics due to potential intensity of current exposure were observed; the number of workers in each exposure

Table 7 *Sperm morphometry in unexposed and exposed workers*

Category	Unexposed (n = 38)		Exposed (n = 37)		p*
	Mean	SD	Mean	SD	
Area (μm^2)	9·0	1·0	9·0	0·8	NS
Perimeter (μm)	11·4	0·6	11·5	0·6	NS
Length (μm)	4·4	0·3	4·5	0·3	NS
Width (μm)	2·6	0·2	2·6	0·1	NS

NS = Not significant [p > 0·05 (2 sided *t* test or > 0·001 (χ^2 test); see text for explanation). *p Value of 2 sided *t* test.

group may, however, be too small to detect an effect. (Insufficient numbers of men contributed urine samples to make a comparison with their semen characteristics valid.)

Discussion

The data presented here show a statistically significant decrease in the mean sperm count per ejaculate (due principally to a lower sperm concentration and reflected in the higher proportion of oligozoospermic men in the exposed group) among workers exposed to 2EE compared with unexposed workers from the same plants. A conservative approach to the statistical interpretation of data where multiple tests are compared is to use a Bonferroni correction factor to obtain the acceptable level of significance for each test.[30] In such a case the effect of exposure to 2EE on sperm count could be considered to be of borderline or no significance (depending on whether a one or two sided p value is considered). Nevertheless, the effect of exposure on sperm count is in the direction hypothesised and is consistent with evidence of degeneration of seminiferous tubules from several animal species[7–12] and of oligozoospermia in rats[13] exposed to 2EE.

No statistically significant differences in semen volume, sperm concentration, semen pH, viability, motility, velocity, and normal morphology or testicular volume were observed between exposed and unexposed men. These results did not change when the potentially confounding effects of sexual abstinence, sample age, caffeine, alcohol, and tobacco consumption, urogenital and other medical disorders, and other factors were considered. The two groups differed with respect to the proportion of certain categories of abnormal sperm shapes, exposed men having a significantly higher proportion of immature forms and a lower proportion of double headed forms. In view of the small absolute differences in these proportions and the lack of consistency of an exposure effect, however, we consider that there is insufficient evidence from these data to conclude that 2EE adversely affects sperm morphology in this group, although 2EE has been shown to affect sperm morphology in rats and mice.[13 14] No effect of duration of exposure to 2EE on semen quality was observed.

Possibly the sperm concentrations of both groups in this study are adversely affected by occupational or other factors that could not be studied directly, so that it may not be possible to distinguish a specific effect due to 2EE alone given the limited sample size and interpersonal variation in this characteristic. Most workers in the control group and some of the group exposed to 2EE reported exposure to one or other agent, such as metal fumes and dusts, solvents (perchlorethylene for example) or heat and vibration; whereas none of these could be quantified, one or a

combination of them may also affect semen quality. It is also possible that control subjects may be exposed to 2EE—for example, during occasional visits to the investing departments or through low level contamination of general worksite air—although none of the nine urine samples (from three controls) contained detectable levels of 2EAA. To the extent to which other exposures were reported more frequently by control subjects than by men exposed to 2EE (particularly heat, since the investing rooms tended to be somewhat cooler than certain other areas of the plant), and to which exposure to 2EE among controls is possible, the results of the study would tend to be biased against finding a significant adverse effect of exposure to 2EE rather than the reverse.

Several other methodological and biological considerations should be taken into account in interpreting these results. Possibly 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 the unexposed group or vice versa. The possibility of selection bias is increased when the participation rate is low, particularly in the case of the controls. All potentially exposed men were interviewed in the same manner to solicit participation and volunteers for the comparison group were recruited randomly from the pool of unexposed workers, which minimised the possibility of systematic bias at the sample selection stage. There were no clear differences between participants and non-participants in the exposed group for demographic characteristics for which information was available to the investigators. (Inadequate information on unexposed non-participants was available.) Further, the reasons given for not participating were generally unrelated to factors that may potentially affect semen quality. Whereas selection bias cannot be ruled out, we do not consider it likely that it could explain our results, albeit, with a participation rate of only 50% for eligible exposed and 26% for eligible non-exposed, bias must be considered another explanation for the observed results.

The number of subjects studied may limit the ability to detect small changes in certain semen characteristics due to exposure even if underlying differences are present. Our sample of 37 exposed and 38 unexposed men was sufficient to have had an 80% chance of detecting an approximately 39% or greater difference in mean sperm concentration compared with the adjusted mean for the unexposed group at a two sided alpha level of 0·05. For semen volume, our sample size permitted an 80% chance of detecting a 20% or greater decrease in volume compared with the unexposed group at alpha = 0·05. Similarly, for sperm motility and velocity, an approximately 26%

and 10% difference (respectively) could have been detected; for the proportion of normally shaped sperm, an approximately 15% difference could be detected in exposed workers. Thus the possibility that smaller true differences in semen characteristics exist between the groups cannot be determined with confidence in this study population.

The question then arises as to whether a possible effect of 2EE on certain semen characteristics may not have been observable at the time of the study due to recent withdrawal of the use of 2EE in two of the three buildings. As the data in table 2 indicate, the airborne levels of 2EE were lower in building A at the time of the semen study than two months earlier (approximately 3 ppm *v* 17 ppm), and were comparable with those in building C, where the use of 2EE was also suspended. By contrast, air levels in building B, where the use of 2EE had not yet been discontinued, were similar at both sampling dates (about 11 ppm and 15 ppm respectively). Since, however, this reduction in potential exposure occurred within the average duration of a spermatogenic cycle (of about 70 days), an effect on semen quality would probably still be observable at the time of study, even assuming complete reversibility of a putative effect.

In this population the presence of a varicocele was associated with a decrease in semen volume, concentration, count, viability, and motility. This finding is supported by data from fertility clinic studies,[31] although not all authors have found such an association.[32 33] The taking of prescribed medication in the past year was also associated with a decrease in sperm concentration and count; in this case, qualitative examination of the data indicated that men taking long term medication were more likely to have sperm concentrations below 20 million per ml than those not taking medication. Clearly, it is not possible to distinguish a putative effect of a drug from a direct effect of the condition under treatment, particularly where no condition or medication in common was reported. We did not find an association of the subjects' age or alcohol use on semen characteristics as in our previous study[19]; this may be due to the smaller range of age and alcohol use in this population. Abstinence was associated with a lower semen volume as found previously by us[19] and by other authors,[34] but not, as might be expected, significantly with sperm concentration, even when forced into the regression model. This may be due to the similarity of, or possible errors in, reporting abstinence times in this population.

In conclusion, our findings suggest a possible effect of exposure to 2EE on sperm counts in these workers at levels considerably lower than the current OSHA PEL of 200 ppm but no significant differences in other parameters of sperm morphology or function. Other factors may possibly be affecting the semen quality of

both groups or the results may reflect a bias introduced by the low participation rates. Given the toxicological evidence which clearly shows the testicular toxicity of 2EE in several species, the results of this semen study would suggest limiting exposure and that follow up studies, in other populations exposed to 2EE, should be conducted to confirm this interpretation.

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