ORIGINAL ARTICLE

Glycol ethers and semen quality: a cross-sectional study among male workers in the Paris Municipality

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Objectives: Apparent increases in human male reproductive disorders, including low sperm production, may have occurred because of increased chemical exposure. Various glycol ether-based solvents have pronounced adverse effects on sperm production and male fertility in laboratory animals. The authors investigated the effects of past and current exposure to glycol ether-containing products on semen quality and reproductive hormones among men employed by the Paris Municipality.

Methods: Between 2000 and 2001 the authors recruited 109 men who gave semen, blood and urine samples and underwent an andrological examination. Information on lifestyle, occupation, exposure and medical history was obtained by interview. According to their job and chemical products used during the period 1990–2000, men were classified as either occupationally exposed or non-exposed. Current exposure levels to glycol ethers at the time of the study were evaluated by biological monitoring of six urinary metabolites.

Results: Previous exposure to glycol ethers was associated with an increased risk for sperm concentration, for rapid progressive motility and for morphologically normal sperm below the World Health Organization semen reference values. No effect of previous glycol ether exposure on hormones levels was observed. By contrast, current glycol ether exposure levels were low and not correlated with either seminal quality or hormone levels.

Conclusions: This study suggests that most glycol ethers currently used do not impact on human semen characteristics. Those that were more prevalent from the 1960s until recently may have long lasting negative effects on human semen quality.

Male reproductive function has attracted increasing attention due to reports suggesting a recent decline in sperm production and quality in several areas of the world during recent decades.[1–3] These reports remain controversial and are currently the subject of intense research and debate. It is hypothesised that in utero exposure to environmental endocrine-disrupting chemicals at critical stages of testis differentiation affects subsequent sperm production.[4] However, there is clear evidence that exposure to some chemicals during adulthood could affect testicular and post-testicular functions and male fertility.[5] Studies on specific populations, exposed to well-identified chemicals in occupational circumstances, have provided convincing evidence that some of these chemicals have adverse effects on male fertility.[6–8]

The general population has been in frequent and regular contact with glycol ether solvents since the 1960s.[9] Their low acute toxicity and their highly miscibility in water and oils have favoured their inclusion in a range of products used professionally and domestically, including water-based paints, cleaning products, liquid soaps, cosmetics and even some pharmaceutical products. More than 30 different glycol ethers have been produced by the chemical industry, and not all have the same toxicity. Two glycol ether families can be distinguished according to the synthesis procedure: ethylene glycol ethers and propylene glycol ethers. Numerous toxicological reports have shown that various ethylene glycol ethers, particularly the short-chain ethylene glycol methyl ether (EGME) and ethylene glycol ethyl ether (EGEE), have pronounced adverse effects on sperm production and quality in the rat, mouse and rabbit.[9–11] The toxic effects of glycol ethers are thought to be mediated by their alkoxycarboxylic metabolites, which are rapidly eliminated by urines.[12 13] However, very few studies were conducted in human populations. At the end of the 1980s it was reported that shipyard painters and metal casting workers highly exposed to EGEE and EGME had low semen characteristics.[14 15] Recently, a study found no differences between the semen characteristics of 14 copper clad lamination workers exposed to EGME and 13 control workers, although the small size of this study population limited the conclusions.[16] A case-control study among infertility clinic consultants showed that urinary glycol ether metabolites were more frequently present in subjects with low semen characteristics.[17] Difficulties in conceiving children have been reported in men working in the semi-conductor industry and exposed to EGEE, EGME and perhaps other glycol ethers such as diethylene glycol dimethyl ether (DEGDME).[18]

Considering the potential detrimental effects of glycol ethers, their uses were progressively regulated in some Western countries during the mid-1990s.[19] Short-chain glycol ethers have been gradually replaced by long-chain ethylene glycol ethers or by propylene glycol ethers, which are considered less toxic. Glycol ethers currently classified in the European Union as harmful for reproduction (category 2) are EGEE, EGME, DEGDME, ethylene glycol dimethyl ether (EGDME), triethylene glycol dimethyl ether (TEGDME) and 1-propylene glycol 2-methyl ether (1PG2ME).

The objective of the present study was to determine whether past or current exposure to glycol ether-containing products is associated with a modification of testicular function and reproductive hormones. We performed a cross-sectional study to assess semen quality and hormones in men working for the Paris Municipality.

Abbreviations: DEGDME, diethylene glycol dimethyl ether; EGEE, ethylene glycol ethyl ether: EGME, ethylene glycol methyl ether; FSH, follicle stimulating hormone; LH, luteinising hormone; WHO, World Health Organization

METHODS

Study population and design

The study population consisted of men employed in a permanent position at the Paris Municipality during the period 2000–1 and aged 20–55 years. Departments in charge of sporting equipment, maintenance, cleaning, data processing and communication agreed to participate in this study. Several briefings were organised in workplaces, during which the study was presented in detail to between 15 and 40 men. At the end of each briefing, participants received written information and a pre-stamped envelope to return by mail stating whether or not they agreed to take part in the study. If they accepted, they were asked to give their name and telephone number. If not, they were asked to provide anonymously some basic data, such as age, department of employment and current job title. An investigator contacted each person who had agreed to participate and proposed a first face-to-face interview to collect information about their occupational history and past and present exposures to glycol ethers and other agents with reproductive toxicity, such as n-hexane, carbon disulfide, 2-bromopropane, perchloroethylene, styrene, phtalates, pesticides, welding fumes, radiant heat and ionising radiation.[5] All participants then gave two urine samples, one month apart, at the end of two working weeks. Two to three months later, they were invited to visit the Reproductive Biology laboratory of Cochin Hospital to provide semen and blood samples, and to undergo physical and andrological examinations.

The directors of the Paris Municipality departments involved in the study allowed participants to attend these interviews and medical visits during working hours without a loss of wages. All subjects worked within 10 km of the laboratory. Each participant provided written informed consent and was offered standard compensation, equivalent to $60. This study was approved by the Ethics Committee of Bicêtre School of Medicine.

Exposure assessment

All chemical preparations available in the participating departments of the Paris Municipality were recorded (n = 758). Material safety data sheets and/or questioning of the manufacturers allowed us to determine the qualitative and quantitative chemical composition of 700 of them.[20] Preparations were classified by category of products and we determined the percentage of preparations in each category containing glycol ethers. Among these 700 preparations, 152 contained a glycol ether (21.7%). Ethylene and/or propylene glycol ethers were found in all waterproofing preparations, in 50% of paints, anti-graffiti, brake fluids and floor coating preparations, in 25% of cleaning agents, hardeners, inks, diluents, oil removers, antifreezes and varnishes and in 10% of photographic developers, pesticides, paint strippers, scale removers and disinfectants.

Participants were questioned about the category of products likely to contain glycol ethers they used in professional or domestic circumstances including: manual work at home, gardening or leisure activities. For each category of products, the number of years and frequency of use was recorded. We limited the timescale for inquiries to the last 10 years (1990–2000) as recall became poor for longer time periods. One industrial hygienist blindly reviewed job descriptions and validated the correspondence between job and the declared frequency and category of products used occupationally. Men who declared using at least one glycol ether-containing product during their job were classified as exposed. As the frequency of use and the number of different categories of glycol ether-containing products used varied widely according to individual, we calculated a continuous exposure index as follows. For each category of products we multiplied the number of times this category of products was used per week by the number of years for which this category of products was used, and then multiplied this result by the mean percentage of glycol ethers present in this category of products. To calculate the exposure index we totalled all the results from the individual categories. One unit of this index was defined as the use, at a frequency of once per week during one year, of one category of product containing a glycol ether in all preparations. For example, one individual reported using paints twice a week for six years and paint strippers once a week for five years. For this individual the exposure index was 2 (frequency per week) × 6 (years) × 0.5 (50% of paints containing glycol ethers) + 1 (frequency per week) × 5 (years) × 0.1 (10% of varnishes containing glycol ethers) = 6.5.

Biological monitoring of exposure to glycol ethers

Current glycol ether exposure was evaluated by measuring urinary alkoxycarboxylic acid metabolites at the time of the study. After collection, urine samples were stored at –20°C until testing. As previously described,[20] high-resolution gas chromatography with electron-capture detection was used to quantify six alkoxycarboxylic acid metabolites: five ethylene glycol derivatives, methoxyacetic acid (MAA, mainly derived from EGME), ethoxyacetic acid (EAA, mainly derived from EGEE,), butoxyacetic acid (BAA, mainly derived from ethylene glycol butyl ether or EGBE), n-propoxyacetic acid (PAA, derived from ethylene glycol n-propyl ether) and phenoxyacetic acid (PhAA, derived from ethylene glycol phenyl ether or EGPhE), and one propylene glycol derivative, 2-methoxypropionic acid (2-MPA, derived from the minor β isomer of propylene glycol methyl ether or PGME). The limits of detection were approximately 0.05 mg/l for each alkoxycarboxylic acid. Concentrations were expressed as mg/g creatinine.

Semen analysis

All semen samples were collected by masturbation after 3–5 days of sexual abstinence. The samples were incubated at 37°C for 30 min, and then analysed following the World Health Organization (WHO) recommendations.[21] Assessment of sperm morphology and the multiples anomalies index was performed as described.[22]

Hormone measurements

Blood samples were collected between 9:00 and 12:00 am and kept at room temperature until serum was separated. After centrifugation, the serum was kept at –20°C until use. Follicle stimulating hormone (FSH) and luteinising hormone (LH) were measured by an immunoradiometric assay (Immunotech, Marseille, France). The dose-response curve of FSH was calibrated against the international standard WHO 94/632, and the LH dose-response curve against the second international standard 80/552. Total testosterone was measured by radioimmunoassay (Immunotech) and dimeric inhibin B by enzyme-linked immunosorbent assay (ELISA, Oxford Innovation Ltd, Oxford, UK).

Physical examination and medical interview

The physical examination was carried out by an andrologist unaware of the subject's exposure status. The men were measured and weighed. A detailed examination of their external genitalia and a staging of the secondary sexual development were performed according to WHO guidelines to detect abnormalities relevant to fertility.[23] The andrologist used a standardised questionnaire to obtain information about medical, surgical, urogenital and reproductive history and lifestyle. In addition, a question concerning the time elapsed

Table 1 Characteristics of study participants

Variables	All (n = 98)	Non-exposed (n = 50)	Exposed (n = 48)
Age (years)*	40.9 (8.4)	40.3 (8.1)	41.6 (8.7)
Body mass index (kg/m^2)*	24.9 (3.4)	25.2 (3.6)	24.6 (3.4)
At least one living child at birth†	56 (57.1)	27 (54.0)	29 (60.4)
Time to conception >12 months for the last child+	4 (4.1)	1 (3.6)	3 (10.3)
Current smokers†	41 (42.3)	23 (48.9)	18 (36.0)
Current drinkers†	59 (60.8)	26 (55.3)	33 (66.0)
Right testicular volume (ml)*	23.1 (3.2)	23.4 (3.0)	22.8 (3.6)
Left testicular volume (ml)*	22.9 (3.6)	23.4 (2.8)	22.5 (4.3)
At least one episode of urogenital infection†	15 (15.5)	9 (19.1)	6 (12.0)
Sexual abstinence before semen analysis (days)*	4.2 (1.5)	4.4 (1.6)	4.1 (1.3)
Season of semen analysis:			
Spring or summer†	70 (71.4)	37 (74.0)	33 (68.8)
Autumn or winter†	28 (28.6)	13 (26.0)	15 (31.2)

*Mean (SD).
†Number of men (%).

since the previous ejaculation allowed real sexual abstinence to be calculated in hours for further adjustment of the semen values (see data analysis).

Data analysis

From the physical examination, and the medical and fertility history, we collected the following information: age, weight, height, time to pregnancy with unprotected intercourse for last child, duration of ejaculation abstinence before semen analysis and testicular volume. All were continuous variables. Body mass index was calculated as weight/height^2 (kg/m^2). We also recorded the following variables: conceived at least one live child (yes vs no); season of sperm analysis (spring or summer vs autumn or winter); smoking (past or current smoker vs non-smoker); alcohol consumption (current drinker vs non-drinker); recent illness (yes vs no); fever or medical treatment during the past three months (yes vs no). Continuous outcomes were pH and volume of semen (ml), sperm concentration (millions/ml), total sperm count (millions, the product of semen volume and sperm concentration), ''a'' rapid progressive motility (the percentage of the only rapid progressive sperm), ''a + b'' total progressive motility (the percentage of rapid plus slow progressive sperm), sperm morphology (the percentage of morphologically normal sperm), sperm viability (the percentage of live sperm), multiples anomalies index (mean number of anomalies per abnormal sperm) and concentration of reproductive hormones (ng/ml for testosterone, pg/ml for inhibin B, IU/l for FSH and LH). Seminal characteristics were categorised as dichotomous variables. We compared the exposure association below and above the WHO reference values for semen.[21]

We used the χ^2 test or Fisher's exact test for categorised variables and the unpaired t test (equal variances), analysis of variance/covariance, and linear regression analysis for continuous variables. We normalised the distributions by applying a square (sperm morphology), a square root (sperm concentration, total sperm count), or a log 10 transformation (seminal volume, serum hormones, urine metabolites) and then used the Kolmogorov-Smirnov test to ensure normality. Urinary metabolite values below the detection limits were considered to be half of the detection limits. To study the effect of exposure on the categorised outcomes, we used logistic regression analysis to produce odds ratios (OR) and 95% confidence intervals (95% CI) for the association between seminal characteristics and exposure variables, adjusted for confounding factors. Logistic

Table 2 Glycol ethers metabolites in urine of currently exposed and unexposed workers

Metabolites (mg/g creatinine)	All (n = 98)	Currently non-exposed (n = 53)	Currently exposed (n = 45)
Methoxyacetic acid			
Median	<DL	<DL	<DL
Range	<DL –0.27	<DL –0.24	<DL –0.27
n>DL	n = 33	n = 13	n = 23
Ethoxyacetic acid			
Median	<DL	<DL	<DL
Range	<DL –0.45	<DL –0.38	<DL –0.45
n>DL	n = 8	n = 3	n = 5
n-Propoxyacetic acid			
Median	<DL	<DL	<DL
Range	<DL –0.11	<DL –0.10	<DL –0.11
n>DL	n = 5	n = 3	n = 2
Butoxyacetic acid			
Median	0.09	0.05	0.12
Range	<DL –0.61	<DL –0.40	<DL –0.61
n>DL	N = 66	n = 27	n = 39
Phenoxyacetic acid			
Median	<DL	<DL	<DL
Range	<DL –2.66	<DL –2.03	<DL –2.66
n>DL	n = 42	n = 22	n = 20
2-Methoxypropionic acid			
Median	1.16	1.12	1.21
Range	<DL –5.14	<DL –2.5	<DL –5.14
n>DL	n = 98	n = 53	n = 45

DL, detection limits.

Table 3 Relation between glycol ether metabolite levels in urine and semen and hormonal parameters

Parameters	Butoxyacetic acid (mg/g creatinine) R* (95% CI)	2–Methoxypropionic acid (mg/g creatinine) R* (95% CI)
Seminal volume (ml)	−0.19 (−0.37 to 0.02)	0.05 (−0.15 to 0.25)
Sperm concentration (millions/ml)	−0.08 (−0.27 to 0.13)	0.03 (−0.18 to 0.23)
Total sperm count (millions)	−0.17 (−0.35 to 0.03)	0.06 (−0.14 to 0.26)
''a'' rapid progressive sperm motility (%)	0.05 (−0.15 to 0.25)	−0.03 (−0.23 to 0.18)
''a'' + ''b'' total progressive sperm motility (%)	0.08 (−0.12 to 0.28)	0.01 (−0.19 to 0.21)
Sperm morphology (%)	−0.08 (−0.29 to 0.13)	−0.03 (−0.24 to 0.18)
Testosterone (ng/ml)	−0.04 (−0.24 to 0.16)	−0.21 (−0.40 to 0.01)
FSH (IU/l)	0.07 (−0.13 to 0.27)	−0.12 (−0.31 to 0.08)
LH (IU/l)	0.01 (−0.19 to 0.21)	−0.01 (−0.21 to 0.20)
Inhibin B (pg/ml)	0.02 (−0.18 to 0.22)	0.07 (−0.13 to 0.27)

FSH, follicle stimulating hormone; LH, luteinising hormone.
*Standardised coefficient of linear regression.

regression analysis was used to evaluate potential confounding factors. Factors were considered confounding if their inclusion in the model modified the odds ratio of the outcome by more than 10%.[24] Variables evaluated as confounders were age, body mass index, alcohol and tobacco consumption, duration of ejaculation abstinence, season of sperm analysis and history of genital infections. Age and length of abstinence were always included as confounding factors in seminal analysis. All analyses were carried out using the Statview software package (SAS Institute Inc, Cary, NC, USA). All p values were two-sided, and considered to be significant if $p<0.05$.

RESULTS

Among the 420 men who attended the information meetings, 109 (22%) agreed to participate. One hundred and thirty one additional men who did not wish to participate agreed to give information on their age and job. There was no difference, either in age or in the proportion of manual workers and office workers between these 131 men and the 109 men who agreed to participate (data not shown). Eleven men who agreed to participate were excluded for one of the following reasons: failure to collect a semen sample, sexual abstinence of less than 48 h, history of cryptorchidism, or occupation involving reproductive toxicant agents exposure other than glycol ethers.

Based on their occupational exposure to glycol ethers during the period 1990–2000, we classified 50 men as non-exposed (ie, never exposed) and 48 men as exposed (ie, having been exposed at some time during this period). The median years of use in occupational circumstances of at least one glycol ether-containing product was six years. The exposed group was composed of manual workers (maintenance, mechanics, building painters, chassis painters and woodworkers) whereas the non-exposed group was composed of office workers, computer operators and library clerks. Four men from the exposed group and five from the non-exposed group reported occasional use (once a week during short periods for less than two months per year) of glycol ether-containing products in non-occupational circumstances (mainly manual work at home) during the period 1990–2000.

Table 1 shows the general characteristics of the 98 participants included in the final analysis as well as comparisons between exposure groups. There were no characteristic differences between the exposed and the non-exposed men.

We measured glycol ether metabolites in urine samples collected at the time of the study. Paired analysis revealed no differences in the mean concentrations of any of the six urine metabolites between the two urine samples obtained one month apart from each individual. Thus, for subsequent

Table 4 Semen and hormone values according to past glycol ether exposure status

	Non-exposed n = 50	Exposed n = 48		
	Mean	Mean	Mean difference (CI 95%)	Adjusted p
Semen characteristics				
Seminal volume (ml)	3.7	4.1	−0.3 (−1.0 to 0.4)	0.52*
Seminal pH (units)	7.8	7.8	0.03 (−0.14 to 0.19)	0.19*
Sperm concentration (millions/ml)	119.1	74.0	45.0 (21.0 to 69.1)	<0.001*
Total sperm count (millions)	416.3	277.4	138.8 (37.7 to 240.0)	<0.001*
''a'' rapid progressive motility (%)	18.4	12.8	5.5 (2.4 to 8.6)	<0.001*
''a'' + ''b'' total progressive sperm motility (%)	39.6	36.3	3.3 (−1.0 to 7.6)	0.61*
Normal sperm morphology (%)	54.2	47.1	7.1 (0.8 to 13.4)	0.005*
Multiple Anomaly Index (units)	1.7	1.8	−0.08 (−0.16 to 0.01)	0.08*
Sperm viability (%)	72.4	71.3	1.2 (−3.8 to 6.1)	0.65*
Serum hormones				
Testosterone (ng/ml)	6.2	6.3	0.1 (−1.35 to 1.16)	0.97†
FSH (IU/l)	3.9	5.5	−1.6 (−3.3 to 0.01)	0.05†
LH (IU/l)	3.5	3.5	−0.01 (−0.74 to 0.73)	0.86†
Inhibin B (pg/ml)	219.0	215.0	−3.8 (−27.2 to 34.0)	0.71†

FSH, follicle stimulating hormone; LH, luteinising hormone.
*Calculated using analysis of variance/covariance to adjust for age, sexual abstinence, and season of semen analysis.
†Calculated using analysis of variance/covariance to adjust for age and body mass index.

Table 5 Association between semen characteristics and past glycol ether exposure status

	Seminal volume (<2 ml)		Sperm concentration (<20.10^6/ml)		Total sperm count (<40.10^6)		"a" Rapid progressive sperm motility (<25%)		"a" + "b" Total progressive sperm motility (<50%)		Sperm morphology (<30%)	
Exposure status	**n**	**OR (95% CI)**	**n**	**OR (95% CI)**	**n**	**OR (95% CI)**	**n**	**OR (95% CI)**	**n**	**OR (95% CI)**	**n**	**OR (95% CI)**
Non-exposed (n = 50)	7	1.0	3	1.0	0	1.0	35	1.0	37	1.0	5	1.0
Moderately exposed (n = 23)*	3	1.1 (0.3 to 5.0)	4	3.2 (0.6 to 16.1)	3	NC	19	2.1 (0.6 to 7.5)	17	0.8 (0.3 to 2.7)	4	2.3 (0.5 to 9.7)
Highly exposed (n = 25)*	4	1.0 (1.0 to 1.1)	4	3.0 (0.6 to 14.6)	3	NC	24	10.2 (1.3 to 82.5)	23	4.3 (0.9 to 20.9)	6	3.2 (0.9 to 11.8)

NC, not calculable.
*Adjusted for age, sexual abstinence and, season of semen analysis.

analyses, we used the mean values of the two urine analyses. Table 2 shows the values for acid metabolites in the study population. Three men from the exposed group had stopped using glycol ether-containing products at the time of urine analysis. Exposure groups were then constituted according to current occupational use of glycol ether products. We limited the comparison analysis to BAA and MPA metabolites, which were detected in 67% and 100% of samples, respectively. Only BAA levels were higher in the currently exposed group than in the currently non-exposed group ($p = 0.01$).

We analysed the relation between the level of urinary glycol ether metabolites and semen and hormone values. We found no correlation between BAA or MPA concentrations and semen characteristics or serum hormone concentrations (table 3). When dichotomised semen characteristics were used, no difference was observed in glycol ether metabolite levels below or above the WHO reference limits for semen (data not shown).

We compared semen values and hormone levels of exposed and non-exposed men (table 4). Sperm concentration, total sperm count, percentage of rapid progressive sperm, and percentage of morphologically normal sperm were lower in the exposed group than in the non-exposed group. No differences were observed for semen pH or sperm viability. Serum testosterone, LH and inhibin B concentrations were similar in both groups, whereas FSH concentration was increased, but not significantly, in the exposed group compared to the non-exposed group.

From WHO semen reference values, we found that glycol ether exposure was associated with an increased OR for low sperm concentration (3.1, 95% CI 0.8 to 12.5), low percentage of rapid progressive sperm motility (4.5, 95% CI 1.3 to 15.0), and low percentage of morphologically normal sperm (3.6, 95% CI 1.3 to 9.7), whereas no association was observed for low seminal volume (1.1, 95% CI 0.4 to 3.6) or low total progressive sperm motility (1.5, 95% CI 0.6 to 4.2). These associations were similar for sperm concentration but higher for sperm motility and sperm morphology in men who were highly exposed compared to men who were moderately exposed (table 5). Odds ratios were not calculated for total sperm count as six of the exposed men had a total sperm count below the WHO threshold value compared with none of the non-exposed men.

DISCUSSION

This study aimed to evaluate the effects of exposure to glycol ether-containing products on male fertility as estimated from semen characteristics and reproductive hormone levels. However, semen samples are difficult to obtain in the general population, explaining the low participation rate, which is usually less than 20%. Also, a retrospective estimation by questionnaire of exposure to widely used solvents is obviously imprecise. Thus, conclusions derived from such studies should be interpreted with caution.

It is unclear if the men who provided semen samples truly reflect the parent population or whether a selection bias resulted in biased risk estimates.[25] Ideally, to identify a selection bias, basic information should be collected from the entire study population and a non-responder analysis undertaken.[26] Unfortunately, in our study we managed only to obtain basic data from 34% of men who did not want to participate. Men who have experienced an infertile period or medical problems that may impair fertility are more likely to participate in semen studies than other men.[27] To prevent such bias, we performed a full medical and andrological examination and we excluded all subjects with identified infertility risk factors.

Knowledge of exposure status may also influence participation. People exposed to chemicals in general are more likely to participate than non-exposed people. However, in the particular case of glycol ethers, most people are not aware that they are exposed to such chemicals because glycol ethers are not the active ingredients that usually characterise the identity of chemical preparations. Furthermore, glycol ethers are frequent co-solvents in water-based products and thus are popularly considered to be safe.

As glycol ether-containing products are widespread and may be employed in a large variety of circumstances, misclassification of exposure should be considered. Here we evaluated glycol ether exposure by inquiry of use and frequency of use of glycol ether-containing products. This allowed us to identify an occupationally exposed group that has been continuously exposed during several years. However, some men reported using glycol ether-containing products in non-occupational circumstances. The intensity, frequency and duration of such non-occupational exposure was lower than that observed in occupational settings. Moreover, the non-exposed men reported using glycol ether-containing products in non-occupational circumstances with the same frequency as exposed men. Finally, we cannot take into account the exposure through daily use of common domestic products at home, although we assume that such exposure should be quite low and the same for both groups. Consequently, retrospective evaluation of exposure may lead to misclassification, but the effect on the estimates should tend towards the null.

We also estimated current levels of glycol ether exposure by measuring urinary alkoxycarboxylic acids. This has the advantage of integrating all routes of exposure whatever the circumstances of use. It also specifically detects glycol ethers that generate acidic metabolites, which are thought to be the active toxicants. However, the presence of glycol ether metabolites in urine reflects only very recent exposure as their half-lives are between several hours and 77 h depending on the glycol ether.[28 29] Therefore, measurement of urine metabolites at the end of a working week will only give us an estimation of exposure during the last days or previous week.

The maximum urinary concentrations of glycol ether metabolites found in the present study were more than one hundred times lower than those previously reported for between 1988 and 1993 in a French occupationally-exposed population.[30 31] Such a large difference corresponds with the

Main messages

- Short-chain glycol ethers that were more prevalent from the 1960s until recently may have long-lasting negative effects on human semen quality.
- Most glycol ethers used at current exposure levels do not impact on human semen quality.

Policy implications

- Replacement of toxic short-chain glycol ethers by long-chain ethylene glycol ethers or by propylene glycol ethers should be encouraged in the workplace.

replacement in recent years of ethylene glycol ethers by propylene glycol ethers. According to industry reports, the production and use of the most toxic glycol ethers (EGME, EGEE) has dramatically diminished in recent years, at least in Western countries. In Europe, the use of EGME and EGEE was regulated in 1994 by the 14th amendment of the Restrictions on Marketing and Use directive (94/60/EC). We confirmed this trend, as we found toxic short-chain glycol ethers in only 2% of the professional chemical products currently available to our study population.[20]

The only alkoxycarboxylic acid detected in all subjects, and present at the highest levels, was 2-MPA. This metabolite is derived from the minor β isomer (usually present in less than 5%) of the propylene glycol ether derivative PGME. When we compared metabolite levels in urine according to exposure status we found that exposed men had higher current levels of 2-MPA (not significantly) and BAA (significantly) than non-exposed men. PGME, and EGBE, from which BAA is derived, are the most frequent glycol ethers currently used in occupational circumstances.[19] PhAA was found at relatively high levels in both exposure groups. This is not surprising because EGPhE, from which PhAA is generated, is rarely found in professional products but is commonly used as a preservative agent in cosmetics preparations.

We found no significant relation between glycol ether exposure levels at the time of the study, as measured by the presence of metabolites in urine, and semen quality or hormone values. This may be explained by low levels of current exposure. By contrast, past exposure to glycol ethers during the period 1990–2000 was found to be associated with poor semen characteristics. Therefore, our results suggest that the observed seminal effects may be due to exposure before the mid-1990s, after which glycol ether regulations were put in place in Europe. Many studies of laboratory animals have clearly shown that spermatogenesis recovers progressively after exposure to glycol ethers has stopped.[9–12 32] The human male has a relatively low fertility and thus may be at a greater risk from reproductive toxins than males from common laboratory animal model species.[33] The continued observation of seminal effects several years after exposure to toxic short-chain glycol ethers had stopped may be interpreted as an incomplete restoration of testicular function and suggests that recovery may depend on the intensity and length of exposure. A comparison of men exposed before 1995 and those exposed after 1995 could verify this. However, we were unable to do this in our population study because most men were exposed both before and after 1995.

The seminal changes we observed are in agreement with what is known about the toxicology and target cells of glycol ethers. Testicular spermatocytes undergoing the pachytene stage of meiosis during spermatogenesis constitute the major site of testicular damage.[34] It has been shown that MAA, the most active testicular toxicant generated by glycol ethers, interferes with the synthesis of DNA,[35 36] and reduces synthesis of the mRNA encoding for the subunit of cytochrome C oxidase, the terminal enzyme in the mitochondrial electron transport chain.[37] Both mechanisms may explain the alteration in production, morphology or motility of sperm cells. We did not observe any significant changes in serum reproductive hormone concentrations, in agreement with experimental studies.[9 10 12] The slight increase in FSH in the exposed group may be interpreted as an hypothalamic response to a modest alteration of spermatogenic function.[38]

Other chemical or physical factors may influence semen quality. We were careful not to include subjects occupationally exposed to solvents such as n-hexane, carbon disulfide, 2-bromopropane, perchloroethylene, and styrene or other agents such as pesticides, phtalates, metal fumes, radiant heat or ionising radiation, as they are know to impair semen quality.[5 39] Although we cannot exclude other unidentified chemicals, we believe that the seminal effects observed in this study were related to glycol ether exposure.

Although restricted by the inherent limitation of cross-sectional sperm studies and the size of the study, our results suggest that exposure to toxic glycol ethers may be detrimental to male fertility. Glycol ethers have been widely used since the 1960s by a large proportion of the adult population. The increased use of chemicals, due to human activities, has been linked with a secular decrease in seminal quality. Even though recent laws have resulted in a major decrease in the use of the most toxic glycol ethers, we question their historical impact on secular changes in semen quality.

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REFERENCES

1 **Carlsen E**, Giwercman A, Keiding N, *et al*. Evidence for decreasing quality of semen during the past 50 years. *BMJ* 1992;**305**:609–13.
2 **Auger J**, Kunstmann J, Czyglik F, *et al*. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 1995;**332**:281–5.
3 **Swan SH**, Elkin EP, Fenster L. The question of declining sperm density revisited: an analysis of 101 studies published 1934–1996. *Environ Health Perspect* 2000;**108**:961–6.
4 **Sharpe RN**, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 1993;**343**:1392–5.
5 **Bonde JP**. Environmental Factors. In: Comhaire FH, eds. *Male infertility*. London: Chapman & Hall Medical, 1996:267–4.
6 **Taylor JR**, Selhorst JB, Houff SA, *et al*. Chlordecone intoxication in man. I. Clinical observations. *Neurology* 1978;**28**:626–30.
7 **Ratcliffe JM**, Schrader SM, Steenland K, *et al*. Semen quality in papaya workers with long term exposure to ethylene dibromide. *Br J Ind Med* 1987;**44**:317–26.
8 **Slutsky M**, Levin J, Levy B. Azoospermia and oligospermia among a large cohort of DBCP applicators in 12 countries. *Int J Occup Environ Health* 1999;**5**:116–22.

9 **Multigner L**, Catala M, Cordier S, *et al*. The INSERM expert review on glycol ethers: findings and recommendations. *Toxicol Lett* 2005;**156**:29–37.
10 **Boatman RJ**. Glycol ethers: ethers of propylene, butylenes glycols and other glycol derivatives. In: Bingham E, Cohrssen B, Powell CA, eds. *Patty's toxicology*. John Wiley & Sons, 2001:271–95.
11 **Boatman RJ**, Knaak JB. Ethers of ethylene glycol and derivatives. In: Bingham E, Cohrssen B, Powell CA, eds. *Patty's toxicology*. John Wiley & Sons, 2001:73–270.
12 **Miller RR**, Hermann EA, Langvardt PW, *et al*. Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. *Toxicol Appl Pharmacol* 1983;**67**:229–37.
13 **Foster PM**, Lloyd SC, Blackburn DM. Comparison of the in vivo and in vitro testicular effects produced by methoxy-, ethoxy- and N-butoxyacetic acids in the rat. *Toxicology* 1987;**43**:17–30.
14 **Welch LS**, Schrader SM, Turner TW, *et al*. Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. *Am J Ind Med* 1988;**14**:509–26.
15 **Ratcliffe JM**, Schrader SM, Clapp DE, *et al*. Semen quality in workers exposed to 2-ethoxyethanol. *Br J Ind Med* 1989;**46**:399–406.
16 **Shih TS**, Hsieh AT, Liao GD, *et al*. Haematological and spermatotoxic effects of ethylene glycol monomethyl ether in copper clad laminate factories. *Occup Environ Med* 2000;**57**:348–52.
17 **Veulemans H**, Steeno O, Masschelein R, *et al*. Exposure to ethylene glycol ethers and spermatogenic disorders in man: a case-control study. *Br J Ind Med* 1993;**50**:71–8.
18 **Correa A**, Gray RH, Cohen R, *et al*. Ethylene glycol ethers and risks of spontaneous abortion and subfertility. *Am J Epidemiol* 1996;**143**:707–17.
19 **de Kettenis P**. The historic and current use of glycol ethers: a picture of change. *Toxicol Lett* 2005;**156**:5–11.
20 **Ben-Brik E**, Jerome L, Arnaud I, *et al*. Exposure to glycol ethers in a population of French men evaluated by measurement of urinary alkoxycarboxylic acids. *Int Arch Occup Environ Health* 2004;**77**:368–72.
21 **World Health Organization**. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction*. Cambridge: University Press, 1999.
22 **Auger J**, Eustache F, Andersen AG, *et al*. Sperm morphological defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities. *Hum Reprod* 2001;**16**:2710–17.
23 **Rowe PJ**, Comhaire FH, Hargreave TB, *et al*. *WHO manual for the standardised investigation and diagnosis of the infertile couple*. Cambridge: University Press, 1993.
24 **Grenland S**, Rothman KJ. Introduction to stratified analysis. In: Rothman KJ, Greenland S, eds. *Modern epidemiology*. Second edition. Philadelphia: Lippincot-Raven, 1998:253–79.
25 **Larsen SB**, Abell A, Bonde JP. Selection bias in occupational sperm studies. *Am J Epidemiol* 1998;**147**:681–85.
26 **Cohn BA**, Overstreet JW, Fogel RJ, *et al*. Epidemiologic studies of human semen quality: considerations for study design. *Am J Epidemiol* 2002;**155**:664–71.
27 **Eustache F**, Auger J, Cabrol D, *et al*. Are volunteers delivering semen samples in fertility studies a biased population? *Hum Reprod* 2004;**19**:2831–37.
28 **Groeseneken D**, Veulemans H, Masschelein R, *et al*. Comparative urinary excretion of ethoxyacetic acid in man and rat after a single low doses of ethylene glycol monoethyl ether. *Toxicol Lett* 1988;**41**:57–68.
29 **Groeseneken D**, Veulemans H, Masschelein R, *et al*. Experimental human exposure to ethylene glycol monomethyl ether. *Int Arch Occup Environ Health* 1989;**61**:243–47.
30 **Vincent R**, Poirot P, Subra I, *et al*. Occupational exposure to organic solvents during paint stripping and painting operations in the aeronautical industry. *Int Arch Occup Environ Health* 1994;**65**:377–80.
31 **Vincent R**, Rieger B, Poirot P, *et al*. Exposure assessments to glycol ethers by atmosphere and biological monitoring. *Occup Hyg* 1996;**2**:69–80.
32 **Foster PM**, Creasy DM, Foster JR, *et al*. Testicular toxicity of ethylene glycol monomethyl and monoethyl ethers in the rat. *Toxicol Appl Pharmacol* 1983;**69**:385–99.
33 **Working PK**. Male Reproductive Toxicology: Comparison of the Human to Animal Models. *Environ Health Perspect* 1988;**77**:37–44.
34 **Miller RR**, Carreon RE, Young JT, *et al*. Toxicity of methoxyacetic acid in rats. *Fundam Appl Toxicol* 1982;**2**:158–60.
35 **Mebus CA**, Welsch F, Working PK. Attenuation of 2-methoxyethanol-induced testicular toxicity in the rat by simple physiological compounds. *Toxicol Appl Pharmacol* 1989;**99**:110–21.
36 **Stedman DB**, Welsch F. Inhibition of DNA synthesis in mouse whole embryo culture by 2-methoxyacetic acid and attenuation of the effects by simple physiological compounds. *Toxicol Lett* 1989;**45**:111–17.
37 **Saunders PT**, Millar MR, West AP, *et al*. Mitochondrial cytochrome C oxidase II messenger ribonucleic acid is expressed in pachytene spermatocytes at high levels and in a stage-dependent manner during spermatogenesis in the rat. *Biol Reprod* 1993;**48**:57–67.
38 **Simoni M**, Weinbauer GF, Gromoll J, *et al*. Role of FSH in male gonadal function. *Ann Endocrinol (Paris)* 1999;**60**:102–6.
39 **Sundaram K**, Witorsch R. Toxic effects on the testes. In: Witorsch R, eds. *Reproductive toxicology*. New York: Raven Press, 1995:99–122.