

Haematological and spermatotoxic effects of ethylene glycol monomethyl ether in copper clad laminate factories

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Abstract

Objectives—To investigate the effects of ethylene glycol monomethyl ether (EGME) on haematology and reproduction in exposed workers.

Methods—53 Impregnation workers from two factories that make copper clad laminate with EGME as a solvent were recruited as the exposed group. Another group of 121 lamination workers with indirect exposure to EGME was recruited as the control group. Environmental monitoring of concentrations of EGME in air and biological monitoring of urinary methoxyacetic acid (MAA) concentrations were performed. Venous blood was collected for routine and biochemical analyses. Semen was collected from 14 workers exposed to EGME for sperm analysis and was compared with 13 control workers.

Results—Results of haematological examination showed that the haemoglobin, packed cell volume, and red blood cell count in the male workers exposed to EGME were significantly lower than in the controls. The frequency of anaemia in the exposed group (26.1%) was significantly higher than in the control group (3.2%). However, no differences were found between the female workers exposed and not exposed to EGME. After adjustment for sex, body mass index, and duration of employment, red blood cell count was significantly negatively associated with air concentrations of EGME, and haemoglobin, packed cell volume, and red blood cell count were significantly negatively associated with urinary concentrations of MAA. The pH of semen in the exposed workers was significantly lower than in the control workers, but there were no significant differences in the sperm count or sperm morphology between the exposed and control groups.

Conclusion—It can be concluded that EGME is a haematological toxin, which leads to anaemia in the exposed workers. However, the data from this study did not support the theory of a spermatotoxic effect of EGME.

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Keywords: ethylene glycol monomethyl ether; haematological disorders; spermatotoxicity

Ethylene glycol monomethyl ether (EGME, CH₃OCH₂CH₂OH, 2-methoxyethanol, 2-ME), one of many derivatives of ethylene glycol ethers (EGEs), is a volatile and almost odourless liquid. It has been commercially available for the past 4 decades and is used primarily as an industrial solvent for resins, lacquers, and paints.¹

The annual production rate for EGEs was estimated to be about 200 000 000 kg with 40 000 000 kg being EGME in the United States.¹ It was also estimated that at least 140 000 workers are exposed to it. As EGME is rarely used in consumer products, it can be assumed that most citizens are not exposed to EGME. About 2500-3000 tonnes of EGME are imported and used in Taiwan annually,² about 90% in the coating industry. Lack of recognition of the potentially toxic effects in Taiwanese workers was noted and the possibility of skin absorption was found to be common among EGME users.²

Overexposure to EGME has been shown to cause toxic effects. Subacute and chronic overexposure in humans through inhalation or percutaneous absorption has been associated with various haematological abnormalities, oligospermia, and toxic encephalopathy.¹ The objective of this study was to investigate the haematological and spermatotoxic effects among workers after exposure to EGME in two factories manufacturing copper clad laminate in Taiwan.

Materials and methods

MANUFACTURING PROCESSES

The usual operations in the manufacture of copper clad laminate include mixing, implantation, drying, cutting, lamination, trimming, and inspection. The main raw materials used in these plants include epoxy and phenolic resins, hardener (dicyanamide), catalyst (2-methylimidazole), antimony oxide, aluminum oxide, silica dioxide, titanium oxide, pigments, acetone, and EGME. The EGME and acetone are the only two volatile chemicals used in the workplace. The solvent of coating glue contains 70% EGME and 30% acetone.

STUDY POPULATION

Workers from two factories manufacturing copper clad laminate were selected as the study population. All participants gave informed consent. All 53 workers from the departments of mixing, implantation, drying, and cutting, with potentially direct exposure to EGME, were recruited as the exposed group. Another 121 lamination workers with indirect exposure to EGME were selected as controls.

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Table 1 The distribution of characteristics among the study population

Variables	Exposed (n=53)	Comparison (n=121)	p Value
Age (y, mean (SD))	29.89 (7.25)	29.58 (7.55)	0.86
Duration of employment (y, mean (SD))	2.60 (2.90)	3.58 (2.82)	0.03
Body mass index (kg/m ² , mean (SD))	23.81 (5.31)	22.45 (3.41)	0.04
Sex (male, n (%))	47 (88.7)	93 (76.0)	0.03
Marital status (single, n (%))	23 (43.4)	58 (47.9)	0.36
Smoking status (smoker, n(%))	32 (60.4)	80 (66.1)	0.50

DATA COLLECTION

Questionnaires were used to collect personal characteristics, personal habits—for example, alcohol drinking and smoking—reproductive history, detailed occupational history, and related symptoms.

ENVIRONMENTAL MONITORING

The United States NIOSH 1403 and OSHA 79 sampling and analytical methods were adopted in this study. Briefly, the 8 hour time weighted averaged personal breathing zone air samples were collected by drawing air through standard size coconut shell charcoal tubes (SKC 226-01, PA, USA) with constant low flow personal pumps (Gilian, Model LFS-114, FL, USA). The charcoal was then desorbed in 1 ml mixed solvent of 95:5 (v/v) dichloromethane and methanol. After 40 minutes of shaking, the desorbed EGME was analysed with a gas chromatograph (Hewlett-Packard 5890 Series II, CA, USA) equipped with an HP 7673A autosampler and a flame ionisation detector (FID). The detailed analytical procedures have been described elsewhere.³

BIOLOGICAL MONITORING

Spot urine samples were collected from each worker for EGME metabolite measurement. Urinary methoxyacetic acid (MAA) was measured with a modified method previously reported by Sakai *et al.*⁴ This method was fully approved by the Institute of Occupational Safety and Hygiene (IOSH), Taiwan and two other reference laboratories before it was adopted in this study. The detailed procedures have been described elsewhere.⁵

MEDICAL EXAMINATION

About 10 ml blood was collected from each worker for haematological and biochemical examinations. Sperm was collected from 14

exposed and 13 control volunteers for sperm counts and morphological examinations. The motility of sperm was not used in this report due to the lag time between collection and examination being >1 hour.

STATISTICAL ANALYSIS

Student's *t* test and χ^2 test were used to compare the differences between the exposed and control groups. A test for equality of variance was performed for Student's *t* test and adjusted variance and degree of freedom were applied if the variances were not equal. Due to the small sample size, the comparative statistical analysis of sperm was performed with the non-parametric Mann-Whitney *U* test.

After identification of potential confounders, multiple linear regression was used to examine the net effects of exposure to EGME. Multiple linear regression was also used to evaluate the dose-response relation between concentrations of EGME in air or urinary concentrations of MAA and haematological outcomes.

Results

ENVIRONMENTAL AND BIOLOGICAL MONITORING DATA

The geometric mean air concentration of EGME in the impregnation area was 3.98 ppm (SD 2.88, n=55 personal samples), with a maximum of 30.07 ppm and minimum of 0.65 ppm in one factory. The air concentration of EGME in another factory was 4.27 ppm (SD 2.19, n=11 personal samples), with a maximum of 20.00 ppm and a minimum of 1.70 ppm. The range of EGME concentrations in the lamination area (control group, n=9) was between non-detectable and 0.28 ppm. Although the mean concentration was not over the permissible exposure limit of 5 ppm, some workers, especially impregnation and mixing workers, were exposed to high concentrations of EGME.

The geometric mean urinary concentration of MAA in workers from the impregnation area was 19.95 mg/g creatinine (SD 2.19, n=30) in one factory and 20.89 mg/g creatinine (SD 2.19, n=15) in another factory, ranging from non-detectable to 65.88 mg/g creatinine. The MAA concentration of lamination control workers (n=32) was 1.26 mg/g creatinine (SD

Table 2 Comparison of mean (SD) results of haematological tests among male workers exposed and not exposed to EGME

Items	Male			Female		
	Exposed group (n=46)	Comparison group (n=93)	p Value*	Exposed group (n=5)	Comparison group (n=28)	p Value*
Haemoglobin (g/dl)	14.43 (1.78)	15.50 (1.06)	<0.001	12.90 (2.60)	12.53 (1.16)	0.77
Packed cell volume (%)	43.33 (5.00)	47.81 (3.10)	<0.001	39.40 (7.16)	40.43 (3.05)	0.59
Red blood cells (10 ⁶ /μl)	4.73 (0.64)	5.37 (0.54)	<0.001	4.18 (0.78)	4.71 (0.56)	0.07
White blood cells (10 ³ /μl)	7.17 (2.11)	7.08 (1.80)	0.81	7.90 (5.03)	6.26 (1.53)	0.51
Neutrophil (10 ³ /μl)	4.36 (1.71)	4.46 (1.41)	0.75	5.60 (4.26)	3.96 (1.20)	0.44
Lymphocyte (10 ³ /μl)	2.45 (0.77)	2.24 (0.540)	0.09	1.95 (0.75)	1.95 (0.46)	0.99
Platelet (10 ³ /μl)	271.00 (66.66)	249.86 (58.88)	0.06	257.60 (65.92)	256.14 (71.33)	0.97
MCV (fl)	92.14 (6.02)	89.66 (7.45)	0.052	94.50 (4.61)	86.83 (10.37)	0.12
MCH (pg)	30.65 (2.05)	29.71 (6.58)	0.35	30.90 (2.21)	26.94 (3.75)	0.03
MCHC (g/μl)	33.27 (5.90)	33.10 (6.64)	0.86	32.67 (1.13)	30.96 (1.20)	0.006

*Student's *t* test.

Abbreviation: MCV=mean corpuscular volume; MCH=mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration.

Table 3 The distribution of abnormal haematological test results among male workers exposed and not exposed to EGME

Items	Exposed n (%)	Not exposed n (%)	p Value*
Haemoglobin (g/dl)			0.0001
Abnormal (<13.5)	12 (26.1)	3 (3.2)	
Normal (\geq 13.5)	34 (73.9)	90 (96.8)	
Packed cell volume (%)			<0.0001
Abnormal (<40)	10 (21.7)	0 (0)	
Normal (\geq 40)	36 (78.3)	93 (100)	
Red blood cells ($10^6/\mu$ l)			<0.0001
Abnormal (<4.5)	13 (28.3)	3 (3.2)	
Normal (\geq 4.5)	33 (71.7)	90 (96.8)	
White blood cells ($10^3/\mu$ l)			0.85
Abnormal (<5.0)	7 (15.2)	13 (14.0)	
Normal (\geq 5.0)	39 (84.8)	80 (86.0)	
Platelets ($10^3/\mu$ l)			0.45
Abnormal (<150)	0 (0)	2 (2.2)	
Normal (\geq 150)	46 (100)	91 (97.8)	

* χ^2 test.

Table 4 The multiple regression model of the concentration of EGME in air on haematological effects (n=40)

Dependent variables	Independent variables	Coefficient	SD	p Value
Haemoglobin	Constant	7.43	—	—
	Sex	-0.79	0.81	0.34
	BMI	0.34	0.09	<0.001
	Work-year	-0.004	0.12	0.98
	Log (air EGME)	-0.14	0.39	0.71
Packed cell volume	Constant	25.75	—	—
	Sex	-1.02	2.39	0.67
	BMI	0.88	0.26	0.003
	Work-year	0.027	0.36	0.87
	Log (air EGME)	-1.59	1.15	0.18
Red blood cells	Constant	4.00	—	—
	Sex	-0.38	0.33	0.27
	BMI	0.062	0.04	0.10
	Work-year	0.011	0.05	0.82
	Log (air EGME)	-0.51	0.16	0.004
White blood cells	Constant	4.38	—	—
	Sex	-1.13	0.93	0.24
	BMI	0.16	0.10	0.12
	Work-year	-0.069	0.14	0.63
	Log (air EGME)	-0.16	0.45	0.72
Platelets	Constant	252.87	—	—
	Sex	-39.49	30.21	0.20
	BMI	3.51	3.29	0.30
	Work-year	-2.41	4.53	0.60
	Log (air EGME)	-15.42	14.56	0.30

Table 5 The multiple regression model of concentration of MAA in urine on haematological effects (n=62)

Dependent variables	Independent variables	Coefficient	SD	p Value
Haemoglobin	Constant	15.52	—	—
	Sex	-2.37	0.46	<0.001
	BMI	0.11	0.07	0.11
	Work-year	-0.041	0.07	0.57
	Log (MAA/Cr)	-0.93	0.30	0.003
Packed cell volume	Constant	47.50	—	—
	Sex	-5.79	1.26	<0.001
	BMI	0.27	0.18	0.14
	Work-year	-0.080	0.20	0.69
	Log (MAA/Cr)	-3.70	0.82	<0.001
Red blood cells	Constant	5.42	—	—
	Sex	-0.43	0.17	0.01
	BMI	0.015	0.02	0.54
	Work-year	0.016	0.03	0.54
	Log (MAA/Cr)	-0.57	0.11	<0.001
White blood cells	Constant	3.09	—	—
	Sex	0.44	0.58	0.45
	BMI	0.15	0.08	0.07
	Work-year	-0.14	0.09	0.14
	Log (MAA/Cr)	0.25	0.38	0.51
Platelets	Constant	207.29	—	—
	Sex	33.08	18.71	0.08
	BMI	0.42	2.69	0.88
	Work-year	-2.43	2.98	0.42
	Log (MAA/Cr)	14.07	12.21	0.25

MAA/Cr=MAA mg/g creatinine.

1.62), ranging from non-detectable to 4.22 mg/g creatinine.

DISTRIBUTION OF CHARACTERISTICS AMONG STUDY SUBJECTS

The distribution of characteristics among study subjects is listed in table 1. There were no differences between the exposed and control groups in the distribution of age, marital status, or smoking. However, the distribution of sex, body mass index (BMI), and duration of employment was significantly different between these two groups. The control group had longer duration of employment, lower BMI, and more female workers. As sex may be a confounder, the analysis in this study was stratified by sex.

HAEMATOLOGICAL EFFECTS

The result of haematological examinations stratified by sex is shown in table 2. There was a significantly lower count of haemoglobin, packed cell volume, and red blood cells in exposed male workers than in control male workers, however, there were no significant differences among the female workers. There were no haematological differences in the white blood cell or platelet counts.

The cut off points of male abnormal values for haemoglobin, packed cell volume, red blood cell count, white blood cell count, and platelets were set at 13.5 g/dl, 40%, 4.5 million/ μ l, 5000/ μ l, and 150 000/ μ l, respectively. The distribution of abnormal values in male workers is shown in table 3. The proportions of abnormal values in haemoglobin, packed cell volume, and red blood cell count in exposed male workers were significantly higher than in control male workers.

DOSE-RESPONSE RELATION BETWEEN EXPOSURE TO EGME AND HAEMATOLOGICAL EFFECTS

The multiple regression model of concentration of EGME in air on haematological variables is shown in table 4. Red blood cell count was significantly associated with concentration of EGME in air. The higher the concentration of EGME, the lower the red blood cell count. However, the negative coefficients of haemoglobin and packed cell volumes were not significantly associated with the concentration of EGME in air.

The multiple regression model of concentration of urinary MAA on haematological variables is shown in table 5. Haemoglobin, packed cell volume, and red blood cell count were significantly negatively associated with urinary MAA. Both environmental and biological monitoring data indicated a negative dose-response relation between exposure to EGME and red blood cell damage.

SPERMATOTOXIC EFFECTS

The result of sperm analyses is shown in table 6. Except for the pH value, there were no significant differences between the exposed and control workers. The mean pH value in the exposed workers was significantly lower than in the control workers.

Table 6 The results (mean (SD)) of sperm analysis among workers exposed and not exposed to EGME

Items	Exposed group n=14	Comparison group n=13	p Value*
Volume (ml)	3.68 (3.02)	3.65 (1.75)	0.98
pH	7.08 (0.29)	7.51 (0.43)	0.005
Sperm count (10 ⁶ /ml)	51.89 (3.14)	50.08 (2.40)	0.93
Morphology (normal, %)	87.14 (2.57)	86.15 (3.63)	0.42

*Mann-Whitney U test.

Discussion

Ethylene glycol ethers (including ethylene glycol ether esters (EGEs)) are a group of solvents with medium high boiling points and low evaporation rates. Ethylene glycol monomethyl ether, one of many derivatives of ethylene EGEs, is a volatile and almost odourless liquid. It has been commercially available for the past 4 decades and is used primarily as an industrial solvent for coating materials.¹ Data from a hazard survey showed that large amounts of E series EGEs are imported and used in Taiwan. Our estimates are: EGME 2500–3000 tonnes, ethylene glycol monoethyl ether (EGEE) 1200–1800 tonnes, ethylene glycol monoethyl ether acetate (EGEEA) 5000–8000 tonnes, and ethylene glycol monobutyl ether (EGBE) 8000–10000 tonnes annually. About 90% is used in the coating industries—that is, in the manufacture or use of paint, thinner, and ink, and EGME is used especially in the copper clad laminate manufacturing industry.²

The main route of entry into factory workers is inhalation, but skin absorption is often overlooked. Lack of recognition of the potential toxic effects of these chemicals was noted⁶ and the potentially toxic effects from skin absorption are common among the workers. Recently, low molecular weight E series glycol ethers (including EGME, EGMEA, EGEE, and EGEEA) have been found to have haematological, reproductive and teratogenic toxicity.¹ This study showed similar haematological toxicity (specifically erythropoietic), but no reproductive toxic effects among workers exposed to EGMEs.

As well as encephalopathy and neurological disorders,⁷ haematological disorders were the most commonly reported effects. The haematological toxicity of EGME includes leukopenia, lymphocytosis, pancytopenia, marrow depression, and decreased red blood cell count, haemoglobin, and platelet count.^{6–11} Parsons and Parsons⁷ reported two patients with EGME intoxication in “fused collar” workers. Both patients presented with severe anaemia with leukopenia and lymphocytosis. Groetschel and Schuermann⁸ reported one patient with EGME intoxication in a small printing shop, who presented with anaemia and lymphopaenia. Zovon⁹ reported five workers in the printing department of a plastic plant who had low red blood cell counts and low haemoglobin values, but white blood cell counts ranged from low to high. Cohen¹⁰ reported a worker in a microfilm production factory with 9 months of exposure to EGME who developed macrocytic anaemia and mild leukopenia. Larese *et al*¹¹ reported a frame worker who presented with macrocytic anaemia and leukopenia after

exposure to EGME. However, the patient's abnormalities returned to normal after EGME exposure stopped.

As well as the case reports of haematological effects of EGME intoxication, several epidemiological investigations were conducted to elaborate the haematological toxicity. Greenburg *et al* found that 19 workers exposed to high concentrations of EGME during the manufacture of fused shirt collars had abnormal haematology results.¹² Nine of the workers' blood tests showed disturbed erythropoiesis including six subnormal red blood cell counts. The anaemias were thought to be caused by a bone marrow toxin rather than haemolysis or peripheral toxicity. Cook *et al* investigated the haematological effects among 65 workers in EGME manufacturing and packing with very low exposures, but no significant abnormalities were found.¹³ A cross sectional study of shipyard painters exposed to a mean (SD) EGME concentration of 0.8 (1.0) ppm, and a mean EGEE concentration of 2.6 (4.2) ppm, found a higher proportion of anaemia (haemoglobin <14 g/dl) in the painters than in the non-exposed workers, but the mean values of haemoglobin and other haematological indicators were not different between exposed and non-exposed workers.¹⁴ Our study also indicated a higher proportion of anaemia and lower mean haemoglobin, packed cell volume, and red blood cell counts in the group exposed to EGME than in the control group. However, the white blood cell and platelet counts were not lower in the exposed group. These findings are consistent with previous studies that show that high exposure of EGME will induce haematological defects. Haematological disorders induced by exposure to EGME have also been confirmed in animal studies. The haematological toxicity in animals included decreased concentrations of haemoglobin and packed cell volume, decreased white and red blood cell counts, reduced bone marrow cellularity, and reduced erythropoiesis and haemolysis.¹

The toxic effects on animals also included male reproduction. The animals exposed to EGME showed testicular atrophy, abnormal sperm morphology, degeneration of late spermatocytes and spermatids, decreased numbers of sperm, and increased numbers of immature germ cells, decreased sperm motility, peritubular membrane damage, and germinal epithelial distortion of the seminiferous epithelium.^{15–17} There are few published studies on the effects on human reproduction.^{13, 18} Cook *et al* studied six workers exposed to EGME and nine controls.¹³ The size of the testes was smaller in the workers exposed to EGME than in the control workers, but the sperm count was not different between these two groups. Welch *et al* studied shipyard painters and found the proportions of oligospermia and azoospermia were non-significantly higher in the painters than in the non-exposed workers.¹⁸ Also, the semen pH was significantly lower in the exposed group, but the sperm morphology and motility were not significantly different. Our study also found that there were no differences

in sperm count or morphology, except for lower sperm pH in the exposed group.

Based on the potential toxicity of EGEs, the American Conference of Governmental Industrial Hygienists adopted a new EGME threshold limit value of 5 ppm in 1984; Germany, Japan, and Sweden also lowered their exposure standards in 1986, 1990, and 1991, respectively. As a result, most of the manufacturers of EGEs have also taken action to stop the production and sale of these potentially hazardous products. Thus, occupational health education, modification of current legislation, and provision for substitutes are urgently needed.

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