分説明し、正しい理解を得て心理的な抵抗感を弱くするために、どういう場で誰がどのように説明するとより効果的であるかについては、今後明らかにされるべきであろう。事業所を通じて案内を出し、関心を示した人に再度詳しい内容を送付する方法、事業所の安全衛生委員会での承認を経て従業員への説明会を開く方法をこれまでに経験しているが、説明会当日に参加者側から質問が寄せられた経験は少ない。後日、匿名の電話で「精液採取には痛い器具を使いますか？」と尋ねられたことが複数回あり、なじみのない健康診断についての説明は、文章や話の中で言葉を尽くしたつもりでも内容が伝わりにくいこと、集団での説明会では質問があっても尋ねにくいことを実感した。この点で参考になりうる例として、保健師を含む研究チームが研究参加者の自宅を訪問し夫婦両方に対して説明を行った上で採精、採血を行った、スウェーデンの漁業従事者集団を対象とした研究(10)がある。しかし、自宅を訪問する場合は前述した生殖器の診察評価は事実上不可能で、また、自宅には来てもらいたくない人もいることを考慮する必要があろう。

上記の他に心理的抵抗感を弱め参加率を高めるための方法としては、調査研究への参加が個人の健康管理に還元されることを基本にしつつも、参加者の受診の手間や休業による減収分を補償する日当相当額の研究協力謝金を支払う場合があり(2, 23, 32)、これは国を問わずこの領域の研究者の共通の認識になっている。しかし、法定健康診断にあわせて事業所内で実施する場合は、謝金を支払うことは行いにくい。

また、調査を事業所の健康診断とは別個に事業所外で実施する場合は、会場や時間の設定において受診しやすさを考慮することも重要である。前述の不参加者へのアンケートでは、受診場所が不便である、忙しくて時間がとれないという回答があった。表面上は調査研究実施の了解が得られても、従業員が実際に参加するかしないかは調査実施期間内の仕事や勤務時間割の組まれ方に大きく左右され、特定の企業の参加希望者から「仕事の都合がつかず参加できない」と連絡が入り、結果的にその企業からの参加者がゼロだった経験がある。すなわち、この場合の事業主としての態度は、会社と無関係に実施される調査研究への従業員の参加は仕事に差し支えない限り自由であるが、参加しやすいような配慮は特にしない、ということであり、事業所に協力していただいた内容が個々の従業員への参加依頼の橋渡しのみである以上、それももっともなものとして理解できる。ちなみに、企業の研究者が著者に名を連ねている職域精液指

標調査研究の参加率は高く、その企業や業界が危機感や問題解決の必要性を感じていることが論文からうかがわれる(22)。そのような状況はまれであり、通常は研究者側が対象者の都合にあわせるしかない。筆者らの研究では、5 週間にわたり平日はもちろん土曜日の夜間、日曜日にも参加できるよう受診枠を設定し、参加者に都合の良い日時を選択してもらった結果、2 回実施した精液検査いずれかへの参加率は 33%（54 人中 18 人、各回の参加率は 26%と 28%）となり(7)、別の職域で事業所内定期健康診断にあわせ2回実施した際の参加率11%（70 人中 8 人、各回の参加率は 10%と 4%）を上回った。前者の研究では当初参加への関心を示した人が実際の参加者の２倍近くにのぼり、設定した健康診断会場が自動車で１時間近くかかる郊外の病院であったことを考慮すると、会場が職場からもっと近ければ参加率はさらに高くなった可能性があった。後者の研究との参加率の差が何によるのかは厳密には議論できないが、精液健康診断の会場は事業所の外に設定した方が心理的に参加しやすいのではと考えられる。

５．精液指標測定値に関連する要因

正確な精液指標調査を行うためには、測定における系統誤差や非系統誤差の原因となる因子について理解しておく必要がある。WHO は検査標準化のための留意点を詳細に述べたマニュアルを刊行(33)し、これが世界的に広く用いられているが、ここではマニュアルが触れていない点を含めて概説したい。精子濃度は同一人から精液を複数回採取した場合に 10 倍以上の変動を示すことも少なくないばらつきの大きい指標である(33)。精子濃度は禁欲期間が長くなると増加する一方、精子運動率、正常形態精子率は１週間程度までは大きな変化はなく、10 日を超えると低下する(34-38)。したがって、マニュアルでは禁欲期間は 48 時間以上７日以内が望ましいとされ(33)、参加者に対してはあらかじめこのことを告げておく必要があるが、この期間内でも濃度は経時的に変化し(34-37)、比較する群間で禁欲期間が異なるとその違いが指標の差となって現れる可能性がある。したがって、禁欲日数幅をさらに短く統一できれば望ましいが、研究者の指示通りにそれがきちんと行われるとは限らないので、解析時に調整できるよう精液試料提出時に禁欲時間を聴取する必要がある。

精液採取を複数の季節にわたり行う場合、精液質の季節変動も考慮の対象となる。最近は季節変動が乏しいとする研究が報告されている(35, 39)が、夏季

の精液質は一年のうちで最も低下するとする報告が多く(40-42)、異なる季節に採取した精液の指標をプールして解析するには慎重であった方がよい。

また、マスターベーションにより得られる精液の各指標については、射精前の性的興奮時間の長さが有意に影響することが報告されている(43)。職域集団を対象とする調査では不妊外来の採精室のように採精条件を同一にできるとは限らないが、射精に至るまでの採精時の環境はできれば調査を通して統一することが望ましい。採精場所を被験者の自宅とする場合は、採精環境の他に精液提出までの時間、温度条件も精子運動率に影響を及ぼすので、さらに注意が必要になる(2, 33)。

ヒトの精液は射精直後には粘性が高く、時間が経過すると粘性が低下（液化）する。液化に要する時間はサンプルによって異なるが、十分に液化しないと安定したピペッティング操作が困難になるなど、計測値が変動する原因となる。また、精子濃度が著しく低い場合、あるいは著しく高い場合は、分析サンプルの希釈倍率を変えて適切な濃度に調整することが重要であり、この点、WHO マニュアルが推奨する血球計算板とそれ以外の方法、すなわち、CASA（精子指標自動解析装置）や希釈しないで測定可能な Makler チャンバーを用いた精子濃度測定結果とが、必ずしも一致するとはいえない理由のひとつとなっている(33)。また、精子の運動性、形態の評価に関しては、精子濃度以上に測定者によるばらつき（測定バイアス）が生じやすい(44)。したがって、異なる施設間のデータを比較する場合、精液指標の測定法標準化に正面から取り組んだ研究(45-47)のようによくコントロールされている場合を除き、注意が必要である。将来的には、測定者によるバイアスがより小さい新たな指標を開発・導入し、既存の指標と併用することがめざされるべきである。当面この問題を回避するには、調査を通して同じ測定者が全被験者のサンプルを曝露に関する情報なしで測定し、WHO マニュアルの基準値を用いた正常、異常の分類を行わず、曝露・非曝露群間で測定値の比較をするのがよい。

ライフスタイル要因のうちでは、喫煙や飲酒が精液指標低下に関係があるかについて議論があり、影響するとの報告もある(48, 49)ので、群間の差が大きい場合には調整した方がよいと考えられる。

この他に考慮する必要があるのは、交絡因子として年齢(50-52)、精索静脈瘤(31)、放射線や抗ガン剤による治療歴、長期の服薬歴等があげられる。分裂細胞への影響が明らかな放射線や抗ガン剤が性腺細胞に作用することはよく知

られた事実であるが(53, 54)、ライディッヒ細胞のステロイド合成に影響を与える薬物も精子形成に悪影響を与え(54)、このような薬の内服で女性化乳房とともに乏精子症の生じた報告(55)がある。長期投与が行われる薬剤の中で女性化乳房の副作用がある薬物は少なくないが、副作用報告に明記されていなくても性腺機能に影響を及ぼす可能性に留意し、このような薬物を長期間服用している被験者は研究としての解析から除いた方がよいと考えられる。

6．結果の解釈と被験者への結果返却の問題

研究的な性格が強くとも健康診断として調査を行った場合、その結果は被験者に返さなければならないが、精液指標をはじめとする生殖機能測定結果の解釈には慎重な姿勢と配慮が求められる。精液指標は同一個人でも測定ごとの変動幅が大きく、また、前章で触れたように年齢の影響を受ける。そして、WHOの参照値を職域健康診断の結果判定に用いる際には、特に乏精子症に該当する場合の判定の表現には注意を払うべきで、不用意に「乏精子症の人は子供ができにくい」と伝えることは慎まなくてはならない。筆者は常に、検査結果はその時により大きく変動すること、一度の精液検査結果で妊孕性に関して判断することはできないことをよく説明すると同時に、生殖機能の健康に不安がある場合は専門医が相談に応じることのできる体制を用意している。

しかし、調査研究への参加者に無精子症などの深刻な機能低下所見が最終的に確認された場合、医療面でのケアとは別に労災問題が思いもかけない形で発生する可能性があるので注意が必要である。業務起因性の有無の判断にあたっては、他の業務上疾病（職業病）の場合と同様に、曝露している物質の種類、曝露量、曝露期間、鑑別診断、検査結果の推移及び曝露との時間的関係、生殖歴を含む既往歴、職業歴、同様に曝露している他の人の検査結果等を含め総合的に判断することになる。しかし、曝露前後の情報のない１時点での検査結果をもって曝露と精液指標低下との因果関係を証明することは困難で、不妊などの生殖機能低下との因果関係の言及にはさらに慎重であるべきである。

7．まとめ

以上をまとめると、職域における精液指標に関する調査研究では、健康診断結果にもとづく事業所としての対応の問題、参加者のプライバシーの確保、心理的抵抗を低くし参加率を高めるための工夫の余地の大きさ、偶然測定誤差を

小さくする観点からみて、現状では事業所外に健康診断会場を設定し、対象者には事業所とは無関係な形で参加してもらう方法が比較的スムーズに実施可能であると考えられる。１回の横断研究、特に対象者数の少ない調査から得た結果の結論づけには慎重な姿勢が必要であるが、その結果は、将来ヒトと実験動物との種差が定量的に解明され、毒性機序に基づくリスク評価手法が発展した後でも、最も基本的な情報になると考えられる。職域集団を対象とした生殖機能のリスク評価が我が国でももっと積極的に行われるようになることを期待し、結びの言葉としたい。

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表1　職域で実施された精液採取を伴う調査研究への被験者参加状況（1999年以降に英文で発表された論文）

対象物質	調査集団	総被験者数（参加率）	対照群の設定	調査実施国	著者名（出版年）（文献番号）
殺虫剤（カルバリル）	曝露工場	48人（参加率記載なし）	あり	中国	Xia et al. (2005) (5)
殺虫剤（DDT）	散布作業者	65人(74%)	なし（血清DDT濃度との関連を検討）	南アフリカ	Dalvie et al. (2004) (6)
殺虫剤（有機リン系、ピレスロイド系）	散布作業者	36人（曝露群33%）	あり	日本	Kamijima et al.(2004) (7)
殺虫剤（フェンバレレート）	製造工場従業員	42人（参加率記載なし）	あり	中国	Xia et al. (2004) (8)
有機リン系殺虫剤	農業労働者	227人（参加率記載なし）	なし	メキシコ	Sanchez-Pena et al.(2004) (9)
ヘキサクロロビフェニル(CB-153)、p,p'-DDE	漁業従事者	195人（3.8%）	曝露量による2群の比較または回帰分析	スウェーデン	Rignell-Hydbom et al. (2004) (10)
溶接作業	溶接工	17人（参加率記載なし）	曝露期間による2群の比較	インド	Kumar et al. (2003) (11)
有機溶剤（トリクロロエチレン）	不妊相談者	8人（参加率記載なし）	なし	カナダ	Forkert et al. (2003) (12)
鉛	バッテリー工場等	503人（曝露群18%）	あり	英伊ベルギー	Bonde et al (2002) (13)
有機リン系殺虫剤	農業労働者	9人（参加率記載なし）	なし	メキシコ	Recio et al. (2001) (14)
有機溶剤他	石油化学工場従業員	198人（曝露群無作為抽出、参加率記載なし）	あり	中国	Wang et al. (2001) (15)
有機溶剤	ゴム工場従業員	90人（参加率記載なし）	あり	メキシコ	Celis et al. (2000) (16)
殺虫剤	農業労働者	122人（61%）	曝露量による3群の比較	デンマーク	Abell et al. (2000) (17)
防カビ剤	農業労働者	30人（参加率記載なし）	同一人の季節間の比較	フィンランド	Harkonen et al. (1999) (18)
農薬	農業労働者	248人（31.4%）	同一人の散布前後の比較	デンマーク	Larsen et al. (1999) (19)
農薬	農業労働者	256人（32.4%）	有機農法野菜摂取量間の比較	デンマーク	Juhler et al. (1999) (20)
有機溶剤（スチレン）	プラスチック製造工場従業員	37人（30%）	曝露量による4群の比較	デンマーク	Kolstad et al. (1999) (21)
除草剤（モリネート）	製造工場従業員	272人(67%)	曝露量との回帰分析	米国	Tomenson et al. (1999) (22)

除草剤（2,4-ジクロロフェノキシ酢酸）	農業労働者	97人（22%）	なし	カナダ	Arbuckle et al.（1999）（23）
殺虫剤（メチルパラチオン、エチルパラチオン、メタミドフォス）	製造工場従業員	43人（曝露群62.5%）	あり	中国	Padungtod et al.（1999, 2000）（24, 25）
有機溶剤（2-ブロモプロパン）	製造工場従業員	8人（73%）	なし	中国	Ichihara et al.（1999）（26）
殺虫剤（DBCP）	農業労働者	精液検査参加率不明	なし	フィリピン他	Slutsky et al.（1999）（27）

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Short Communication

Health Effects of Exposure to Ethylene Glycol Monoethyl Ether in Female Workers

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Abstract: Ethylene glycol monoethyl ether (EGEE) is a solvent commonly used in industry. To find the health effect of the solvent exposure in women, we did an investigation on 32 female workers exposed to EGEE in factories manufacturing photopolymer sensitization plate, and 20 subjects working in the same companies without potential exposure to the solvent. The mean age was 35.0 and 33.9 yr in the two groups, respectively. The mean concentration of the urinary metabolite (ethoxyacetic acid) was 120.87 mg/g creatinine (geometric mean) in the exposed group, and 2.71 mg/g creatinine in the control group. Average RBC count and hemoglobin levels were normal in both groups. However, there were 2 subjects in the exposed group with an RBC count and hemoglobin concentration slightly lower than the standard. Out of 20 controls, 5 subjects reported irregular menstruation, and in comparison, 4 out of 32 exposed females had the same complaint. The most common health complaints were dizziness and swelling of the legs, with the same frequencies seen in both groups. Overall, our study suggests that although female workers were exposed to high concentrations of EGEE, subsequent health problems possibly due to such exposure were not significant.

Key words: Ethylene glycol monoethyl ether, Occupational exposure, Health effect, Women

Ethylene glycol monoethyl ether (EGEE) is one of a family of glycol ethers widely used as an organic solvent for resins, paints and dyes, and as a thinner in industry. It is miscible with both water and other organic solvents, and can be easily absorbed through the skin as well as via inhalation[1,2]. The compound possesses a low order of acute toxicity as shown in animal experiments[3,4]. However, repeated exposure to EGEE can induce disorders in the haematopoietic system and pathological changes in the testes[5–7]. Studies with laboratory animals also showed that in utero exposure to EGEE induces malformation among offspring, suggesting that the solvent is a developmental toxin[8]. However, despite extensive exposure to the solvent, investigations regarding its effect on humans are very limited. In males exposed occupationally to EGEE, reproductive effects and anemia have previously been reported[9–11]. However, little is known about its health effects among women. In the present study, we report the effects of EGEE exposure on the health of female workers in China.

A total of 32 female workers exposed to EGEE in two factories in suburban Beijing were included in the study. The factories were manufacturing photopolymer sensitization plates and EGEE was used as a paint thinner on the plates. Twenty female subjects in the same workplaces who did not use the solvent were enrolled as controls. Control subjects were matched to the exposed group in terms of age range, mean age, percentages of drinking and smoking habits. All subjects were interviewed by qualified occupational

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Table 1. General characteristics of study subjects

		Control (n=20)	EGEE Exposure (n=32)
Mean age ± S.D. (Min–Max) (yr)		33.85 ± 8.54 (20–49)	35.00 ± 8.86 (18–50)
Age group (No. and %)	-24	4 (20)	6 (19)
	25–34	6 (30)	9 (28)
	35–44	7 (35)	12 (38)
	45–	3 (15)	5 (16)
Mean work duration ± S.D. (Min–Max) (yr)		2.13 ± 1.62 (0.5–8.0)	2.23 ± 1.55 (0.5–7.0)
Work duration	< 1 yr	3 (15)	5 (16)
	1–2 yr	12 (60)	16 (50)
	3–4 yr	4 (20)	9 (28)
	5 yr–	1 (5)	2 (6)

physicians on a one-to-one basis, to ascertain basic demographic items, work history, health complaints and history of childbirth. A total of 32 items relating to health complaints were included, such as symptoms of the central nervous system, mucous membrane irritation, digestive system, urinary and reproductive system, perception organs (eye, nose, ear and fingers), general symptoms (fatigue, change in body weight, etc) and menstruation conditions. Blood samples were taken for examination of blood cells, sex-related hormones and plasma aminotransferases. To monitor total exposure through the skin and via inhalation, spot urinary samples were collected at the end of 8 h work-shifts. Quantification of the solvent and its metabolite ethoxyacetic acid (EAA) was achieved using gas chromatography with a mass detector, and the metabolite was corrected for concentrations of urinary creatinine. Organic gas sampling badges (3M) were fixed on the chest pocket position of some workers (4 and 23 in control and exposure group, respectively) to monitor exposure to ambient EGEE and any other solvents during work hours. The sampling time was 6 to 8 h.

As subjects in the two factories were engaged in similar work and exposed to the same materials, combined data from the two factories was analyzed. Age and work duration are shown in Table 1. Most of the workers had worked for less than 5 yr. There were no subjects who smoked tobacco and only a few who drank alcoholic beverages occasionally. The average work (exposure) period was little over 2 yr in both groups, with the shortest exposure being 6 months. The results of monitoring badges showed that control subjects were exposed to very low concentrations of EGEE (0.56 ppm), whereas EGEE exposure was 6.44 ppm in the exposed group. Traces of acetone and ethylene glycol monomethyl ether were still detected in a few badges, although concentrations of these latter chemicals were very low (lower than 0.1 ppm), indicating that EGEE was virtually the only organic gas in the workplace. Although these subjects were clearly exposed to EGEE (due to ambient EGEE and contact of their hands with materials containing the solvent), protective masks or gloves were not being used at the time of our survey.

Table 2. EAA level in urine of study subjects by work duration*

	Control (n=20)	EGEE Exposure (n=32)
Overall	2.71 (0.32–9.46)	120.87 (10.66–3670.99)
<1 yr	3.12 (1.26–6.49)	109.63 (28.19–635.62)
1–2 yr	3.85 (1.02–9.46)**	173.02 (10.66–3670.99)
3–4 yr	1.10 (0.33–2.00)	91.19 (13.59–1271.30)
5 yr–	n.d.	63.75 (31.44–129.27)

*: Geometric mean and range. **: EAA was only detected in 9 of 12 subjects. n.d.: not detected.

Solvent exposure as represented by urinary EAA concentration and related to work duration, is shown in Table 2. Urinary EAA content in the exposed group was over 40 times higher than in the control subjects (although with a wide range), suggesting that female workers were exposed to high concentrations of EGEE. There was no EGEE detected in any urinary samples from the controls. However, out of 32 samples from exposed subjects, 10 samples were positive for the solvent (geometric mean: 1.96 mg/g creatinine). Average RBC and WBC counts and hemoglobin levels were normal in both groups, as shown in Fig. 1. However, there were 2 subjects in the exposed group with RBC counts and hemoglobin concentrations slightly lower than the standard range. Out of 20 controls, 5 subjects reported irregular menstruation, and in comparison, 4 out of 28 exposed workers had the same complaint (Table 3). The mean concentrations of blood prolactin were within the normal range for both groups (9.11 and 10.83 ng/ml, in the

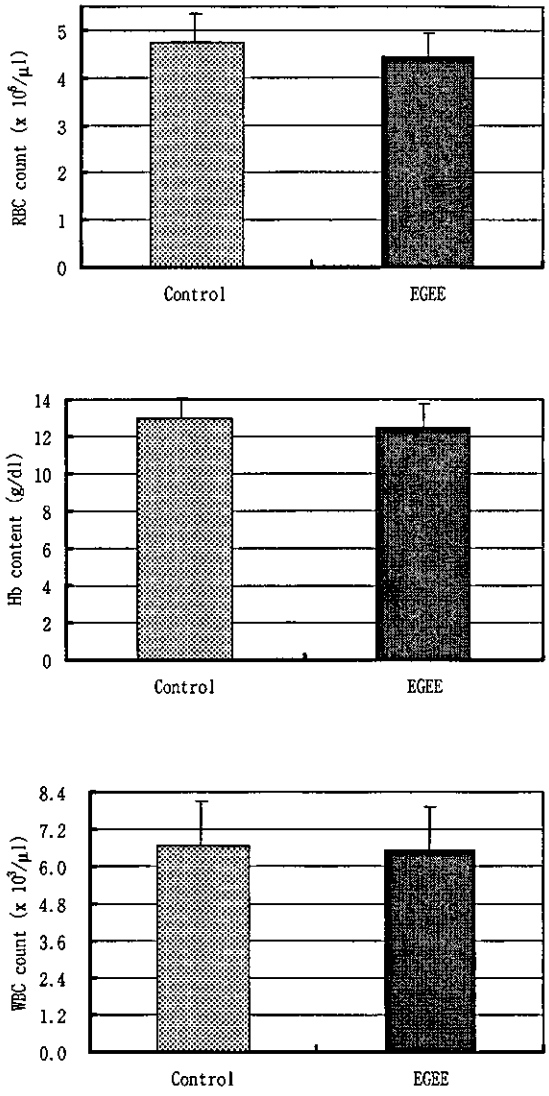

Fig. 1. Hematological results in female workers.
Each bar represents the mean + SD for 20 and 32 subjects in control and EGEE groups, respectively.

control and exposed groups, respectively), but there were respectively 3 and 5 women who exhibited higher levels of this hormone in the two groups.

Most of the female workers in this study had been married and given birth before they were engaged in EGEE work. No more than two children were reported from each woman due to China's stringent family planning policy (Table 4). Therefore, it is not known if exposure to this solvent affects childbirth. The most common health complaints were dizziness, headache or swelling of the legs, with similar frequencies seen in both groups (Table 3). Other complaints included fatigue, reduced appetite, eye irritation, low back pain and so on, but they were only from one or two subjects.

Table 3. Frequency of health complaints among study subjects

Group	Control	EGEE
Abnormal menstruation	5/20 (25.0%)	4/28 (14.3%)*
Dropsy in legs	2/20 (10.0%)	3/32 (9.4%)
Dizziness/Headache	5/20 (25.0%)	5/32 (15.6%)

*: Except for the abnormal menstruation, four women with their ages over 46 yr in EGEE group had menopause.

Table 4. Children birth history of the married female workers

Group	Control (n=18)	EGEE (n=28)
Women with kids		
No. (%)	14 (77.8)	27 (96.4)
Marriage year	14.1 ± 5.5	14.3 ± 6.0
Work year	2.2 ± 1.9	2.4 ± 1.6
Women without kids		
No. (%)	4 (22.2)	1 (3.6)
Marriage year	2.0 ± 1.4	2.0
Work year	1.9 ± 0.9	3.0

Blood AST, ALT and LDH activities were not significantly altered by solvent exposure (data not shown).

In the workplaces we investigated, EGEE by itself, rather than a mixture of solvents, was the only solvent to which the workers were exposed. This made it possible to more clearly distinguish the effects due to EGEE on women's health. Female workers in the exposed group were exposed to EGEE concentrations over 5 ppm, which is the TLV for EGEE in China. A high urinary EAA content in the exposed group also indicates that workers were exposed to high concentrations of EGEE via inhalation and through the skin. On the other hand, low levels of air EGEE and urinary EAA were detected among some control subjects. All control subjects were working in offices separated from, but in reasonable proximity to, the high EGEE concentration workplaces. Although this appears to have included a small EGEE exposure for the control subjects, their overall burden was clearly much lower than in the exposure group. Regarding effects of the solvent on the haematopoietic system, only 2 females out of 32 showed slightly lower RBC counts and Hg content than the lower limit of the standard range. Average values for subjects were almost the same as those in controls. It is important to note that lifestyle factors, such as weight loss, diet and nutrition can affect the results of blood counts in women[12, 13]. It appears therefore, that the effect of EGEE exposure on the haematopoietic system was equivocal among females within this study.

Although EGEE may cause a reduction of sperm count in males[14], we did not find any obvious effects of EGEE on the menstrual cycle of exposed females, suggesting that it may not influence the process of ovulation. Age was also taken into consideration with regard to abnormal menstruation, but no clear relationships were found. Possibly, this was due to the small number of subjects in our study. Some sex-related hormones, such as estradiol, luteinizing hormone and follicle-stimulating hormone, cannot be used for the evaluation of reproductive function in females due to wide fluctuations in blood levels during the ovarian cycle. Therefore, we determined the blood prolactin concentration, a hormone with some relationship to reproductive function, among other physiological effects[15]. No significant changes in this hormone were found when compared to controls. On the other hand, most female workers had been married and given birth before they were exposed to EGEE. Therefore it was difficult to ascertain whether the solvent affects pregnancy or child birth during this study. Further research will be needed to test such a hypothesis.

Aside from common complaints such as headache, dizziness, fatigue and so on, a unique symptom (dropsy in lower legs) was reported by individuals in both the exposed and control groups. EGEE has previously been reported to cause kidney damage[16] and butoxyethanol caused acidosis[17] during acute intoxication. Whether the dropsy witnessed in our study was related to such effects is not known.

EGEE is metabolized *in vivo* to ethoxyacetaldehyde by alcohol dehydrogenase and cytochrome P450 2E1 and further to EAA by aldehyde dehydrogenase[18, 19]. In this study we found that a small proportion is also excreted as a transformation-free form in urine, suggesting the possibility of urinary EGEE as an alternative of exposure biomarker. It is generally believed that the metabolite EAA plays a significant role in the solvent's toxicity[20]. On the other hand, enzymes involved in the metabolism of EGEE are known to be polymorphic[21], and whether such polymorphisms exert any effect on the metabolism and/or toxicity of EGEE in female individuals, will need to be clarified.

Overall, this study suggests that although female workers were clearly exposed to high concentrations of EGEE, subsequent health problems possibly due to this exposure were difficult to identify. More detailed research is currently underway to help clarify this result.

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