Urinary alkoxyacetic acids and renal effects of exposure to ethylene glycol ethers

J Laitinen, J Liesivuori, H Savolainen

Abstract

Objectives—Ethylene glycol ethers and their acetates are widely used in industry, because of their hydrophilic and simultaneously lipophilic properties. Ethylene glycol ethers and their acetates are mainly metabolised to alkoxyacetic acids, but there is also a minor pathway through ethylene glycol to oxalic acid. The main pathway of ethylene glycol ethers is associated with significant clinical or experimental health effects and the minor pathway is also interesting because formation of urinary stones depends principally upon the urinary concentration of oxalate and calcium.

Methods—Excretion of alkoxyacetic and oxalic acids was examined among silkscreen printers for an entire working week. The aim of the study was to evaluate alkoxyacetic acids as early indicators of exposure to glycol ethers and to evaluate their toxicity to kidneys. The load of alkoxyacetic and oxalic acids was compared with the excretion of calcium, chloride, ammonia, and glycosaminoglycans (GAG). Morning urine was chosen for the main analysis, as the overall metabolite, ethoxyacetic acid (EAA), has a long elimination time from the body.

Results—The excretion of calcium increased according to the urinary alkoxyacetic acid load. The excretion of ammonia and chloride was higher among the exposed workers than among the controls. The highest urinary alkoxyacetic acid load was also associated with increased excretion of GAG, which may reflect the toxicity of metabolites of ethylene glycol ether. The excretion of GAG correlated positively with that of calcium in the printers with highest exposure. The tendency to form urinary stones was 2.4-fold higher among silkscreen printers than among office workers.

Conclusion—On the basis of renal effects our study indicates the need for establishing a new biological exposure limit before a workshift that is clearly below 100 mmol ethoxyacetic acids per mol creatinine in morning urine of people occupationally exposed to ethylene glycol ethers.

Keywords: metabolic acidosis; oxalic acid; urinary stones

Ethylene glycol ethers and their acetates are widely used in industry, because of their hydrophilic and simultaneously lipophilic properties. Glycol ethers do not have special warning properties—for example, odour—and they rapidly enter the body unnoticed. Glycol ethers have low vapour pressure, but they are readily absorbed through the skin.

Ethylene glycol ethers and their acetates are mainly (60%-80%) metabolised through pathways to alkoxyacetic acids (fig 1). There is also a minor (10%-15%) pathway (fig 1) through ethylene glycol, glycolaldehyde, glycolic acid, and glyoxylic acid to oxalic acid.

The main pathway of ethylene glycol ethers through acetaldehyde to alkoxyacetic acids is associated with significant clinical or experimental health effects. Exposure to ethylene glycol ethers is associated with haematological problems, oligospermia, azoospermia, and reproductive problems. Animal studies indicate that they can induce germ cell toxicity, reproductive toxicity, and teratogenicity, and some of them are potential carcinogens.

The minor pathway is also interesting because formation of urinary stones depends principally upon the urinary concentration of oxalate and calcium, although the percentage of oxalate in stones is higher than that of calcium. On the other hand, calcium oxalate nephrolithiasis is associated with a defect in erythrocyte oxalate self exchange and an
abnormal rate of phosphorylation of erythrocyte membrane protein. The glycosaminoglycan (GAG) content of erythrocyte ghosts is found to be lower than normal in patients who form stones. The GAG content correlates inversely with oxalate self exchange and band 3 phosphorylation in erythrocytes. The erythrocyte oxalate is transported by way of band 3 protein, which shares the same functional similarities with anion exchangers, but it is not known whether non-erythroid anion exchangers are structurally related to it.\textsuperscript{22} Anyway, the role of glycosaminoglycans as inhibitors of calcium oxalate crystallisation is controversial, and reported actions range from negligible\textsuperscript{15} and inhibitory\textsuperscript{24} to promoting.\textsuperscript{16}

Karniski and Aronson have suggested that there are at least two separate anion exchangers mediating Cl\textsuperscript{-} transport on the luminal membrane of the rabbit proximal tubule cell. These exchangers may play important parts in mediating transstubular Cl\textsuperscript{-} and oxalate transport in this nephron segment.\textsuperscript{19} The proximal tubular cells also liberate ammonia from glutamine and the glutamate pool. It is possible that a higher oxalate concentration in these cells could also cause changes in the liberation of NH\textsubscript{3}\textsuperscript{11} in humans.\textsuperscript{16}

According to these findings, the excretion of large amounts of organic acids poses problems to renal tubular function, and the interference seems to change the function of the chloride exchanger in the kidney.\textsuperscript{10,21}

From a clinical point of view in occupational health care, the kidney effects are probably the most interesting as most of the glycol ether metabolites are excreted in the urine. Therefore, additional knowledge on the possibility of a link between exposure to ethylene glycol ether and changes in renal functions would be advantageous. More information on the minor pathway of metabolism would also be important, because small changes in oxalate excretion have a greater impact on the saturation of calcium oxalate in urine than changes in calcium excretion.\textsuperscript{22,23} The changes in the excretion of proteoglycans may be secondary, but they may reflect more chronic changes in the kidney. All variables are valuable with a view to validating the current biological exposure indices for ethylene glycol ethers and their congeners.

Methods
Eight silkscreen printers from four worksites participated in this study on a voluntary basis. Their ages varied from 20 to 40 years and they were all considered to be healthy. Urine samples were collected before and after the workshift in 56 instances and analysed for alkoxycetic and oxalic acids, ammonia, calcium, and pH. Urinary analysis before a workshift was completed with assay of GAGs and chloride (table 1). Twenty one office workers who were not exposed to ethylene glycol ethers were the control group. They were considered healthy and they gave urine samples before their workshift. Samples were not included in the study if the concentration of creatinine was below 2 mmol/l.

All of the collected urine samples were kept at \(-20^\circ\text{C}\) before the analysis, and those obtained for the oxalic acid analysis were not stored with preservatives. Methoxycetic acid (MAA), ethoxycetic acid (EAA), butoxycetic acid (BAA), and oxalic acid were analysed with the modified gas chromatographic method described by Sakai et al.\textsuperscript{24} The coefficients of variation within assays (\(n=5\)) for MAA, EAA, BAA, and OA were 9-2\%, 2-6\%, 1-4\%, and 3-4\%, respectively, at a concentration of 20 mg/l. Calcium was analysed with a spectrophotometer, ammonia with a reflectometer\textsuperscript{2a} and GAGs by the method of Savolainen.\textsuperscript{27}

The workers were divided into four groups according to the alkoxycetic acid burden. The purpose of the study was to evaluate the dose-response relation between the exposure and excretion. The first group (>100 mmol alkoxycetic acid/mol creatinine) and second group (100-50 mmol/mol creatinine) were formed with two international limit values for EAA, 110 mmol/mol creatinine (American Conference of Governmental Industrial Hygienists (ACGIH) 1994-5)\textsuperscript{28} and 55 mmol/mol creatinine (Deutsche Forschungsgemeinschaft (DFG) 1994).\textsuperscript{29} Limits for the third and fourth groups were chosen to be 50-20 mmol/mol and 20-0 mmol alkoxycetic acid per mol creatinine. The limit value for EAA was chosen, because the portion of MAA, EAA, and BAA of total excretion of alkoxycetic acids was on average 1-0\%, 93-6\%, and 5-4\%, respectively.

The significance of the difference in the excretion of oxalic acid, calcium, ammonia, chloride, pH, and GAG between the exposed and unexposed groups was tested by analysis of variance (ANOVA). The results for the exposed group were repeated observations from the same set of people and for the unexposed group they were single values. The statistical differences between exposed and control groups of the excretions and excretion pattern were tested separately each day. The

<table>
<thead>
<tr>
<th>Silkscreen printers</th>
<th>Controls</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA (mmol/mol creatinine)</td>
<td>ND</td>
<td>44 (18)</td>
<td>71 (30)</td>
<td>77 (22)</td>
<td>87 (27)</td>
<td>94 (35)</td>
<td>59 (23)</td>
<td>46 (17)</td>
</tr>
<tr>
<td>Oxalic acid (mmol/mol creatinine)</td>
<td>38 (3)</td>
<td>49 (11)</td>
<td>43 (5)</td>
<td>36 (4)</td>
<td>53 (8) (\dagger)</td>
<td>54 (15)</td>
<td>55 (12) (\dagger)</td>
<td>47 (6)</td>
</tr>
<tr>
<td>Calcium (mmol/mol creatinine)</td>
<td>243 (37)</td>
<td>211 (37)</td>
<td>217 (47)</td>
<td>176 (39)</td>
<td>193 (22)</td>
<td>261 (62)</td>
<td>320 (69)</td>
<td>233 (47)</td>
</tr>
<tr>
<td>Ammonia (mmol/mol creatinine)</td>
<td>3-9 (0-6)</td>
<td>3-6 (0-5)</td>
<td>3-2 (0-5)</td>
<td>4-0 (0-7)</td>
<td>4-0 (0-9)</td>
<td>3-7 (0-3)</td>
<td>4-0 (0-3)</td>
<td>3-3 (0-3)</td>
</tr>
<tr>
<td>pH</td>
<td>6-4 (0-3)</td>
<td>6-0 (0-3)</td>
<td>6-3 (0-2)</td>
<td>5-8 (0-2)</td>
<td>5-8 (0-2) (\dagger)</td>
<td>6-1 (0-2)</td>
<td>5-7 (0-2) (\dagger)</td>
<td>5-9 (0-2)</td>
</tr>
<tr>
<td>F value (mmol)</td>
<td>0-2505</td>
<td>0-9389</td>
<td>0-0864 (\dagger)</td>
<td>0-0357 (\dagger)</td>
<td>0-2787</td>
<td>0-0899 (\dagger)</td>
<td>0-1912</td>
<td></td>
</tr>
</tbody>
</table>

\(\dagger\) \(P<0.05\); \(\ddagger\) \(P<0.1\).

ND = not detected; AAA = alkoxycetic acids; mva = multivariate analysis.
Table 2  Mean (SEM) excretions of silkscreen printers before shifts compared with those of controls

<table>
<thead>
<tr>
<th>Silkscreen printers</th>
<th>Controls</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA (mmol/mol creatinine)</td>
<td>ND</td>
<td>30 (15)</td>
<td>27 (9)</td>
<td>70 (31)</td>
<td>88 (39)</td>
<td>82 (26)</td>
<td>87 (36)</td>
<td>45 (20)</td>
</tr>
<tr>
<td>Oxalic acid (mmol/mol creatinine)</td>
<td>38 (5)</td>
<td>36 (3)</td>
<td>32 (4)</td>
<td>42 (4)</td>
<td>42 (4)</td>
<td>41 (6)</td>
<td>34 (3)</td>
<td>35 (7)</td>
</tr>
<tr>
<td>Calcium (mmol/mol creatinine)</td>
<td>243 (30)</td>
<td>247 (87)*</td>
<td>332 (73)</td>
<td>373 (77)†</td>
<td>452 (105)*</td>
<td>377 (51)</td>
<td>291 (30)</td>
<td>166 (35)</td>
</tr>
<tr>
<td>Ammonia (mmol/mol creatinine)</td>
<td>2.8-0.6</td>
<td>4.0 (0.5)†</td>
<td>4.7 (0.8)†</td>
<td>4.7 (0.8)†</td>
<td>4.9 (0.5)*</td>
<td>4.4 (0.4)†</td>
<td>3.5 (0.3)</td>
<td>3.8 (0.3)</td>
</tr>
<tr>
<td>pH</td>
<td>6.4 (0.3)</td>
<td>5.9 (0.3)</td>
<td>5.7 (0.1)†</td>
<td>6.0 (0.2)†</td>
<td>6.0 (0.2)†</td>
<td>5.7 (0.2)†</td>
<td>5.7 (0.2)†</td>
<td>5.5 (0.1)*</td>
</tr>
<tr>
<td>P value (mva)</td>
<td>—</td>
<td>0.2312</td>
<td>0.1993</td>
<td>0.3135</td>
<td>0.0609†</td>
<td>0.1916</td>
<td>0.4658</td>
<td>0.0129*</td>
</tr>
<tr>
<td>GAG (g/mol creatinine)</td>
<td>4.7 (0.5)</td>
<td>4.4 (1.1)</td>
<td>4.5 (1.0)</td>
<td>4.3 (0.7)</td>
<td>7.0 (1.5)</td>
<td>4.5 (0.8)</td>
<td>4.8 (1.4)</td>
<td>2.9 (0.8)†</td>
</tr>
<tr>
<td>Chloride (mol/mol creatinine)</td>
<td>10.9 (1.3)</td>
<td>17.3 (4.1)†</td>
<td>13.8 (4.6)</td>
<td>13.3 (2.2)</td>
<td>19.3 (6.5)</td>
<td>16.7 (3.2)†</td>
<td>20.0 (9.0)</td>
<td>9.2 (1.7)</td>
</tr>
</tbody>
</table>

*P < 0.05; †P < 0.1
ND = not detected; GAG = glycosaminoglycans; AAA = alkoxyacetic acids; mva = multivariate analysis.

The significance of the difference in the excretion patterns between exposed and control groups was tested with multivariate analysis, which included oxalic acid, calcium, ammonia, and pH, because they had same control group.

The tendency (T) for subjects to form stones was estimated by calculating the shortest distance (D) of calcium (= xi) and oxalic acid (= yj) values from the supersaturation curve {x1, (3655907,93a–0.9)(x–0.9)} by a computer program (Mable V, Waterloo Maple Software, Waterloo, Ontario, Canada) for symbolic mathematics. For each subject a saturation curve was corrected according to the excretion of creatinine (a). Distances were marked negative for subjects who exceeded the saturation point (x_i > x). The T was calculated by the equation T = 1/D(mean).30

Results

The results were separated into urine analyses before and after the shift. The analysis after the shift did not show any dose-response relation between the alkoxyacetic acid burden and the excretion of calcium, ammonium, or the pH. The urinary excretion of alkoxyacetic acids increased during the working week and the highest levels of excretion occurred on Friday evening. The highest peak of oxalic acid was

![Figure 2](http://oem.bmj.com)  Relation between the excretion of alkoxyacetic acids and that of calcium, GAG, ammonia and chloride. The results are given as the means (SEM).
also found at the end of the week. The excretion of oxalic acid and pH differed moderately from controls on Thursday and Saturday. The excretion patterns of exposed workers differed moderately from those of the control group on Wednesday and Saturday and significantly on Thursday evening (table 1).

Urinary ammonia, calcium, or pH before the shift differed significantly from those of controls on Monday, Thursday, and Sunday mornings (table 2), and the excretion of calcium seemed to follow the alkoxyacetic acid burden (fig 2). The excretion of chloride was higher among the exposed workers than among the controls (fig 2). The excretion patterns of exposed workers differed moderately from those of controls on Thursday and significantly on Sunday morning (table 2).

The mean excretion of GAG seemed to decrease from the control group to the second most highly exposed group but the mean excretion in the most highly exposed group was by far the greatest (fig 2). The excretion of glycosaminoglycans (y) correlated positively (y = 120·74x + 48·34, r = 0·75) with that of calcium (x) in this group (fig 3).

The calculated distance (mean (SEM)) from the supersaturation curve were among unexposed workers, silkscreen printers before the shift, and silkscreen printer after the shift (149 (20), 62 (9), and 104 (18) respectively). The mean tendencies to form urinary stones were 0·006, 0·016, and 0·010, respectively.

**Figure 3** Relation between the excretion of GAG and calcium in the most exposed group (y = 120·74x + 48·34, r = 0·75).
Discussion
The urinary analysis after the shift did not show any significant differences between the
exposed and unexposed workers (table 1). This lack of correlation may have been caused by
the long biological half life of the metabolites in the main metabolic pathway. The half
life of alkoxyacetic acids decreases as the length of the alkyl chain increases. The half
lives for MAA, EAA, and BAA are 71, 42, and six hours, respectively.2133 This could also
cause a metabolic adaptation to the generation of the organic acid metabolites in kidneys.
Most of MAA, EAA, and BAA is not typically conjugated with glycine. Therefore, a signifi-
cant reabsorption probably occurs also in the tubular section of the nephron. The liberation
of ethylene glycol represents the minor meta-
A formate and chloride exchanger protein
has been isolated from the kidney membrane
fraction. It may also accept anions other than
formate or oxalate in the exchange system, as
in our study the urinary chloride concentration
followed that of acids (fig 2). The exchanger
was inhibited by furosemide, and one wonders
what effects its use would have on the kinetics
of alkoxyacetic acids. In one case of intoxica-
tion, a dose of furosemide helped the victim to
survive a very high dose of formic acid.34
It is interesting to note that the urinary
ammonia increased with increasing urinary
alkoxyacetic acid (fig 2). It is possible that
alkoxyacetic acids change the renal ammonia-
genesis at high concentrations as does formic
acid.35 As ammonia occurs in the urine pri-
marily as NH4+, its excretion would require
the excretion of an equivalent number of anions
such as chloride or phosphate. This
link was also found in our study (fig 2). Free
ammonia is very toxic and it cannot be trans-
ported through the blood; therefore it is con-
verted into an amide group of glutamine by
glutamine synthetase.
The highest urinary acid load is also associ-
ated with increased proteoglycan excretion,
which may also reflect the toxicity of the glycol
ether metabolites (fig 2). The excretion of
GAG seems to decrease with the load of
alkoxyacetic acids in moderate exposure as
was found to be the case in our previous study
among car mechanics.37 Baggio et al also found
a lower GAG content in erythrocyte ghosts in
patients who form stones than in normal peo-
ple.11
Calculi could also qualify as a renal effect
indicator because of the associated risk of
nephrolithiasis. It is interesting to note that the
proteoglycan excretion peak coincided with the
highest rate of calcium excretion. It has
been maintained that urinary proteoglycans
prevent the crystallisation of urinary calcium
salts.38 The excretion of calcium increased
with increasing alkoxyacetic acid. This occur-
rence in turn is positively correlated with that
of GAG (fig 3). The increase in urinary GAG
may explain the finding that none of our cases
had a history of urinary stone disease despite
the fact that the urinary calcium and oxalate
concentrations were at or above the supersatu-
ration line in many cases.
Formation of urinary stones starts rapidly
when the products of the reaction of oxalic acid
with calcium exceeds the supersaturation
level. The tendency to form crystals in urine
samples can be estimated roughly by hyper-
local formation25 (fig 4). Urine samples taken
before the shift showed a higher tendency to
form urinary stones than did samples taken
after the shift. The calculated results indicated
a 2-4-fold higher tendency for urinary stones
among silkscreen printers than among office
workers in samples taken before a shift (fig 4).
Dietary oxalate is thought to contribute
10% to 20% to urinary oxalate, 40% to 50%
to endogenous metabolism, and the remaining
40% to 50% to the breakdown of ascorbic acid
in the body.39 It is well known that hormonal
fluctuation may contribute more to the vari-
ability in oxalate excretion than for example
the dietary intake of protein.40 A good example
of a hormonal effect is the fact that women
produce more oxalate relative to creatinine than
men. The prevention of urolithiasis through a
decreased dietary intake of calcium and oxalic
acid is controversial because a decreased
intake of calcium and oxalic acid increases the
intestinal absorption and urinary concentra-
tion of calcium and oxalic acid.41 The role of
exposure has been found to have a causal rela-
tion to urolithiasis among railway shopmen,42
and also in occupational groups with low
physical activity. A similar relation has also
been found with exposure to high tempera-
tures and an increased fluid loss.43
In conclusion, chronic occupational expo-
sure to ethylene glycol ethers and their con-
geners is associated with important alkoxyl
and oxalic acids loads which alter renal function.
The risk of urinary stones seems to be higher
among silkscreen printers than among con-
trols, and some of the changes are typical of
chronic metabolic acidosis. It is very impor-
tant to emphasise that ethylene glycol ethers
are well absorbed through the skin and that
analyses for their vapours mostly underesti-
mate the exposure. Metabolites of ethylene
glycol ethers also accumulate during the work-
ing week, so that biological monitoring is the
only means of obtaining an evaluation of total
exposure. Our study also indicates the need,
the basis of renal effects, to establish a new
biological exposure limit clearly below 100
mmol ethoxyacetic acids per mol creatinine,
in the morning urine before a workshift, of peo-
ple occupationally exposed to ethylene glycol
ehers.

1 Angerer J, Lichterbeck E, Begerow J, Jekel S, Lehnert G
Occupational chronic exposure to organic solvents. Int
2 Johnson G. Toxicokinetics of 2-butoxyethanol: uptake, distribution, metabolism and excretion in man and laboratory animals. Arboc och Halsa 1988;3
4 Carney BW. An integrated perspective on the developmental toxicity of ethylene glycol. Reprod Toxicol 1994;2(suppl 8):99-113
16 Williams HE, Wandelzlak TR. Oxalate synthesis, transport and hyperoxaluric syndromes. J Urol 1989;141:748-7
18 Hayaishi Y, Kaplan RA, Par CYC. Effect of sodium cellulose phosphate (thermal) on crystallization of calcium oxalate in urine. Metabolism 1975;24:1273

22 Robertson WG, Peacock M. The cause of idiopathic calcium stone disease: hypercalcua or hyperoxaluria? Nephron 1980;26:105-10
23 Sakai T, Araki T, Masayama Y. Determination of urinary alkoxyacetic acids by a rapid and simple method for biological monitoring of workers exposed to ethylene glycol ethers and their acetates. Int Arch Occup Environ Health 1985;59:253-60
29 Duffey J, Savolainen H. Metabolism of ethylene glycol ethers: a review. Toxicologist 1992;66:522-4