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Biological monitoring of occupational exposure to 1-methoxy-2-propanol

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Abstract

At the end of a workweek 23 silkscreen printers gave a urine sample for capillary gas chromatographic analysis for 1,2-propanediol. The mean concentration was 2.52 (S.D. 2.01) mmol mol creatinine^{-1} (median=1.76, n=23). The urinary excretion of 1,2-propanediol was linearly dependent on the preceding 1-methoxy-2-propanol exposure measured in the worker's breathing zone [$y=0.99+0.28x$, $n=23$, $r=0.67$, where y is the urinary 1,2-propanediol concentration, in mmol mol creatinine^{-1} and x is the concentration, in cm^3 m^{-3}, of 1-methoxy-2-propanol (90.2%), 1-ethoxy-2-propyl acetate (5.8%), 1-methoxy-2-propyl acetate (2.1%) and 1-ethoxy-2-propanol (1.9%) in the air].

Keywords: 1-Methoxy-2-propanol; 1,2-Propanediol

1. Introduction

The use of glycol ethers as a replacement for more classical hydrocarbon solvents has been on the increase throughout the industrialized world. Advantages of their use include their very mild odour and miscibility in water. Thus many paints, inks and solvent systems for other coatings may contain 10–15% by weight of various glycols [1]. According to the Finnish customs service, over 9 million litres of 1,2-propanediol were imported into Finland in 1993.

Concern has been raised, however, as to the safety of ethylene glycol based ethers (for a review, see Ref. [2]). Possible chronic risks include reproductive problems and hematological malignancies [2]. The effects may be related to the hypothetical toxic metabolites that eventually give rise to the formation of various alkoxyacetic acids [2]. This biochemical metabolic hazard can be avoided if 1,2-propanediol based ethers are used, as one of the isomers, (i.e., 1-alkoxy-2-propanol cannot be dehydrogenated to an eventual acid metabolite).

The commonest congener in use is 1-methoxy-2-propanol. It has been evaluated for its potential toxicity in animal experiments [2], and specifically, its metabolism has been studied in a rodent model [3]. The latter work shows that 1,2-propanediol is liberated and that it is excreted or incorporated into metabolism as a lactic acid precursor with a short biological half-life [3].

Even if 1,2-propanediol may be relatively harmless, high doses may cause acidosis and great osmolar gaps [4–6]. In addition the metabolically liberated methyl fragment can cause excess formation of formic acid. Formic acid is a natural component of human urine [7] so that its analysis in

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1-methoxy-2-propanol exposure would be more difficult to interpret. In this paper, we show a linear relationship between urinary 1,2-propanediol concentration and preceding a 8-h occupational 1-methoxy-2-propanol exposure.

2. Experimental

2.1. Chemicals

Standard compounds were purchased from the following sources: ethanediol (EG) from Merck (Darmstadt, Germany), and 1,2-propanediol (1,2-PG) and the internal standard 1,3-propanediol (1,3-PG) from Fluka (Buchs, Switzerland). Di(ethylene glycol) (DEG) was purchased from Aldrich (Steinheim, Germany). The derivatization agent 2,3,4,5,6-pentafluorobenzoylchloride was purchased from Fluka. Sodium hydroxide and *n*-hexane were purchased from Merck.

2.2. Standard solutions

Stock solutions (0.5 g l^{-1}) of EG, 1,2-PG, DEG and 1,3-PG were prepared in deionized water. The working standard solutions in water were obtained by adding 100 μl, 200 μl, 300 μl, 400 μl and 500 μl stock solutions to the 25-ml vials and filling them with deionized water. Standards corresponded to EG, 1,2-PG and DEG concentrations of 2, 4, 6, 8 and 10 mg l^{-1}.

The internal standard (1,3-PG) was prepared by adding stock solutions (100 μl) to 50-ml vials and filling them with 2.5 *M* sodium hydroxide.

2.3. Instrumentation and chromatographic conditions

The determinations were carried out using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA, USA) equipped with an electron capture detector. The detector output was connected to an integrator equipped with the Chemstation program (Avondale, PA, USA). The column was a high-performance cross-linked methyl silicone gum capillary column (HP-1), 50 m×0.32 mm I.D., film thickness 0.52 μm. The instrumental conditions for the calibrations and assays were as follows: helium flow-rate 1.02 ml min^{-1}, injector port temperature 250°C and detector temperature 330°C. The column was temperature-programmed from 185°C to 255°C as follows: the initial temperature 185°C was held for 26 min, then increased to 200°C at 15°C/min (held 10 min) from 200°C to 215°C at 15°C/min (held 10 min) and from 215°C to 255°C at 40°C/min (held 2 min).

For the verification of peaks a VG Trio-2 GC–MS system (Manchester, UK) was used. The gas chromatograph was a Hewlett-Packard Model 5890. All derivatives were analysed in the electron impact mode using scanning ion recording. Ionization was performed at 70 eV.

2.4. Extraction procedure

2.4.1. Calibration graph

Standards (100 μl) were pipetted into a 10-ml screw-capped tube and 1 ml of internal standard was added. Then the derivatization step was taken by adding 3 ml of 2,4,5,6-pentafluorobenzoylchloride (3.33% in *n*-hexane). Closed tubes were shaken for 30 min and the water-phase was removed. The *n*-hexane phase was cleaned-up twice with 5 ml of deionized water by shaking tubes for 30 s on a Vortex-type mixer. The *n*-hexane phase was removed and incubated at 65°C for 1.5 h. Thereafter the *n*-hexane phase was concentrated to 300 μl in a nitrogen stream, and 4 μl of the concentrate was injected into the gas chromatograph.

2.4.2. Samples

Urine samples (1 ml) were shaken for 30 s on a Vortex-type mixer and centrifuged at 1083 *g* for 2 min to remove the protein precipitate. A 100-μl aliquot of the supernatant was transferred to 10-ml screw-capped tubes and analysed as in Section 2.4.1.

2.4.3. Recovery and precision

The calibration graphs were obtained by linear regression of the peak-area ratios of the compounds as compared with the internal standard concentration. The linearity was assessed between 0 and 10 mg l^{-1}. The recovery was calculated by comparing the measured values for spiked urine samples with those of standard aqueous solutions at three concentrations

(about 2.5, 5.0 and 10.0 mg l^{-1}). Background concentrations of urine were taken into account in the calculations. The intra-assay precision was done at three concentrations (2.5, 5.0, 10,0 mg l^{-1}) in triplicate.

2.4.4. Biological samples

Urine samples were collected immediately after the workshift at the end of the workweek. Samples were stored at −21°C, until analysis. The urinary creatinine concentration was determined with the alkaline picric acid method.

2.5. Air samples

Air samples were analysed according to the Finnish standard method [8] and air sampling was performed using SKC charcoal tubes (Eighty Four, PA, USA). The extraction of hydrophilic 1-methoxy-2-propanol from the charcoal tubes was improved with 5% isopropanol in carbon disulfide.

2.6. Occupational application

The study was conducted at the end of a workweek. Silkscreen printers ($n=23$) inhalation exposure to 1-methoxy-2-propanol, 1-ethoxy-2-propanol and their acetates was measured from the breathing zone during an 8-h workshift. Healthy clerks ($n=14$) and fire fighter cadets ($n=27$) served as unexposed controls, and they also gave urine samples on Friday afternoon at the end of the workshift. None of the controls had a health problem. The statistical differences in the excretion between the controls and the exposed subjects were evaluated with the Student *t*-test.

3. Results and discussion

The method used in this study is based on the work of Göen et al. [9] but modifications made for clean-up of the samples, derivation technique, temperature program and type of column (see Fig. 1 for gas chromatographic profiles and Table 1 for the recovery and precision of the method). The clean-up of the samples was performed with deionized water to avoid unnecessary use of hazardous solvents. After adding of derivatization agent the degree of derivation was increased with sufficient warming of samples. The warming also aided in the clean-up of the samples and a major interfering peak near the peak of 1,2-ethanediol disappeared. This step improved enormously precision of the ethanediol analysis. The resolution of ethane- and 1,2-propanediol was increased by lowering temperature used in the method of Göen et al. [9]. On the other hand the temperature was increased for 1,3-propanediol and diethyleneglycol to achieve shorter retention times. The new type of column chosen was actively used for years in our laboratory where it tolerated high temperatures and extensive urinalysis. The method developed in this study is sensitive enough for the occupational monitoring of 1,2-propanediol and 1-alkoxy-2-propanols and it also provides a possibility for ethanediol and diethylene glycol to be monitored simultaneously. The method was tested with real field samples, which proved that it was suitable also when all confounding factors were available in urine. Previously reported methods have only been developed for clinical purposes e.g., diagnosis of glycol poisoning [10–12]. The use of 1,2-propanediol as urinary indicator of exposure and comparison of excretion to inhalatory exposure of 1-methoxy-2-propanol were not reported before. In addition large collected data of unexposed persons are useful for the background exposure evaluation.

The excretion of 1,2-propanediol by the exposed printers was 2.52 (S.D. 2.01), mmol mol creatinine^{-1} (median=1.76, $n=23$). This level was due mainly (90.2%) to the 8-h exposure to 1-methoxy-2-propanol, which averaged 4.92 (S.D. 4.87) cm^3 m^{-3} (median=2.31, $n=23$). A minor part of the inhalation exposure consisted of exposure to 1-ethoxy-2-propyl acetate 0.49 (S.D. 0.59) cm^3 m^{-3} (median=0.18, $n=15$), 1-methoxy-2-propyl acetate 0.66 (S.D. 0.30) cm^3 m^{-3} (median=0.72, $n=4$) and 1-ethoxy-2-propanol 0.30 (S.D. 0.12) cm^3 m^{-3} (median=0.29, $n=8$). A correlation ($r=0.67$) between the inhalation exposure to 1-methoxy-2-propanol and 1,2-propanediol excretion was found (Fig. 2). Control urine contained 1,2-propanediol concentration of 1.18 (S.D. 0.89) mmol mol creatinine^{-1} (median=0.98, $n=41$, $p<0.01$ from the exposed). This may have been due by the 1,2-propanediol content of consumer products [13].

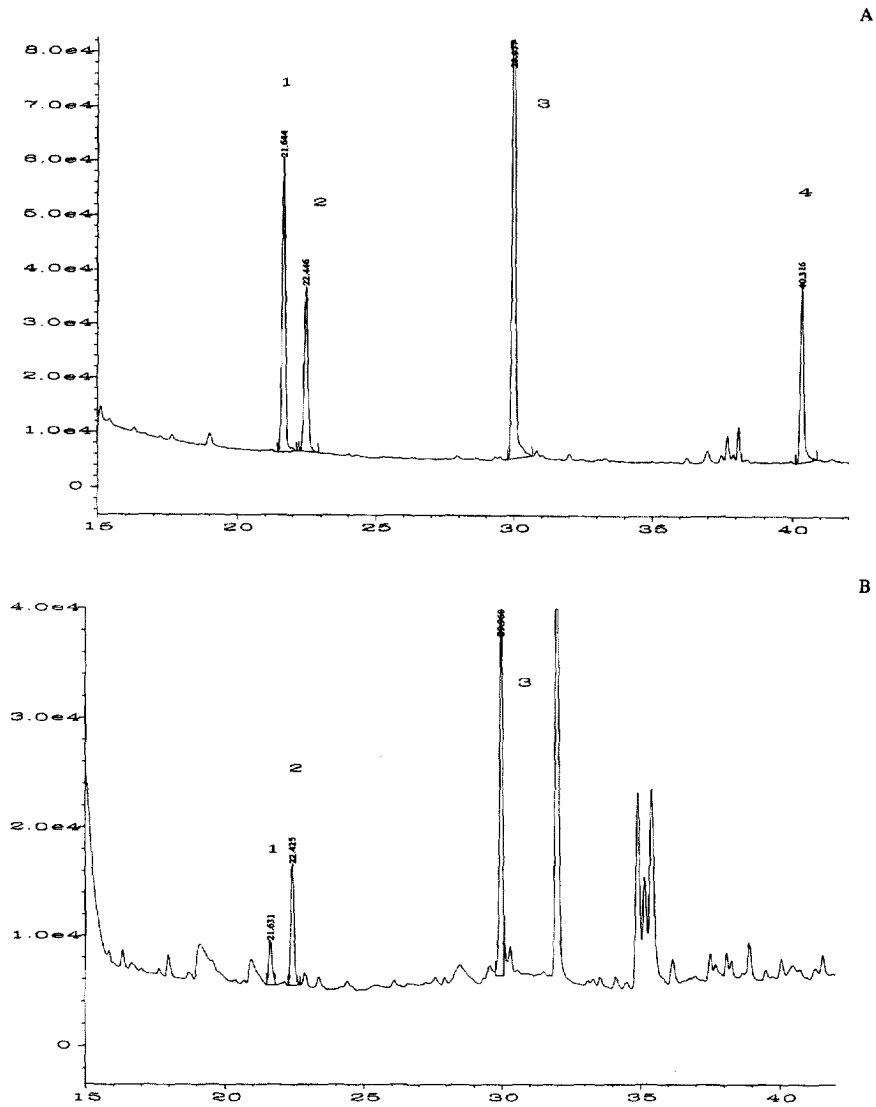

Fig. 1. Gas chromatographic profiles of (A) a spiked extracted water standard (containing 2.5 mg l^{-1} of each compound) for ethanediol (EG), 1,2-propanediol (1,2-PG) and di(ethylene glycol) (DEG) with internal standard 1,3-propanediol (1,3-PG) and (B) exposed worker's urine containing EG (0.45 mg l^{-1}) and 1,2-PG (2.05 mg l^{-1}) with internal standard (I.S.). Peak 1=EG, 2=1,2-PG, 3=1,3-PG, 4=DEG.

Table 1
Recovery and precision of the gas chromatographic method for ethanediol (EG), 1,2-Propanediol (1,2-PG) and diethylene glycol (DEG)

Concentration (mg l^{-1})	C.V. (%)			Recovery (%)		
	EG	1,2-PG	DEG	EG	1,2-PG	DEG
Intra-assay (n=3)						
2.5	5.3	12.2	2.6	77	107	95
5.0	4.4	4.3	4.3	83	98	88
10.0	14.1	10.3	12.1	100	97	96

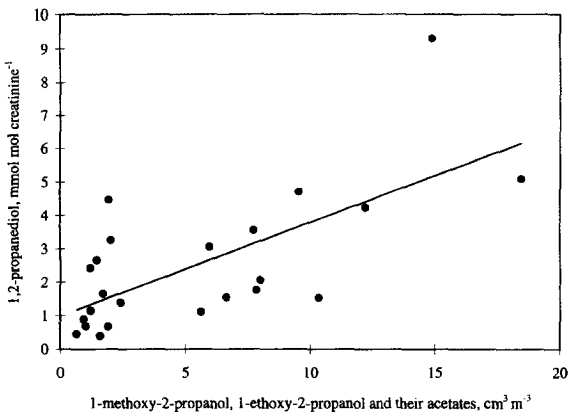

Fig. 2. Linear relationship of urinary 1,2-propanediol excretion (y, mmol mol^{-1} creatinine) with 8-h exposure to 1-methoxy-2-propanol and 1-ethoxy-2-propanol and their acetates (x, ppm): $y=0.99+0.28x$, $r=0.67$. There is a background excretion of 1,2-propanediol (1.2±0.9 mmol mol creatinine^{-1}) possibly because of the use of household chemicals and cosmetics.

The ethanediol excretion of the silkscreen printers was also compared with their 8-h exposure to ethylene glycol ethers. The silkscreen printers' 8-h exposure to ethylene glycol ethers was 3.28 (S.D. 3.02) cm^3 m^{-3} (median=2.26, $n=38$) and the average excretion of ethanediol was 0.93 (S.D. 0.80) mmol mol creatinine^{-1} (median=0.66, $n=38$). The correlation between 8-h exposure to ethylene glycol ethers and the excretion of ethanediol was 0.54. The excretion of ethanediol by the unexposed subjects was 0.67 (S.D. 0.72), mmol mol creatinine^{-1} (median=0.51, $n=41$). Alkoxyacetic acids are better known indicators of exposure to ethylene glycol ethers, but the ethanediol pathway is clinically interesting in nephrolithiasis [14]. On the other hand, urinalysis for ethanediol can be used successfully in evaluations of exposure to ethanediol in garages [13].

Our study confirms that 1-methoxy-2-propanol and 1-ethoxy-propanol are metabolized by humans to 1,2-propanediol. The initial methyl metabolite is probably formaldehyde, as is the case for the methyl tertiary butyl ether oxidation [15]. In the latter case, the ethereal bond is dissolved by a cytochrome P-450 dependent oxidation reaction, and it seems that CYP 2E1 and CYP 2B1 may both qualify [15].

Propanediol and 1-methoxy-2-propanol have a chiral carbon atom. The chiral compounds have two different configuration in space. Enantiomers are chemically identical in their reactions, but they differ in biochemical functions. Organic synthesis often produces a mixture of possible chiral forms at an equal rate, leading to an equimolar mixture [16]. Silkscreen printers used normally technical-grade 1-methoxy-2-propanol, which contained both enantiomers.

The living cells make chiral biomolecules in such a way that only one of the two enantiomers is formed. This occurs because the enzyme molecules themselves are chiral structures. The three-dimensional shape of biomolecule is extremely important in their biological function [16]. Are there two different enantiomers of 1-methoxy-2-propanol in urine? While specific data on the human metabolism of 1-methoxy-2-propanol stereospecificity are lacking, it is known that phenyl ethylene glycol produced as a metabolite in the biotransformation of styrene shows an approximate L/D enantiomer ratio of about 3 in occupationally exposed subject [17]. Biologically active amino acids are of L-configuration and it is possible that L forms are preferentially accepted as precursor in the keto- and amino acid metabolism.

The involvement of the mixed function oxidase in 1-methoxy-2-propanol metabolism may mean that even in prolonged exposure the solvent may not accumulate, as the system is very inducible by its substrates. Specific data on the capacity of 1-methoxy-2-propanol to induce its own oxidation are not available. Should this effect be great, there would theoretically be a more rapid formation of 1,2-propanediol from 1-methoxy-2-propanol in the workers with longer length of employment.

All of our subjects had already been employed in their current work for a long period and it is therefore likely that the quantitative model in our study would apply to experienced worker populations. According to our results the urinary BEI value for 1-methoxy-2-propanol would be below a 1,2-propanediol value of 10 mmol mol creatinine^{-1} if the 8-h exposure limit to 1-methoxy-2-propanol is lower than 20 cm^3 m^{-3}.

Another complicating factor is that all glycol ethers are easily absorbed by the skin. This phenomenon would explain the very high urinary 1,2-propanediol concentrations of the workers in charge of washing the silk screens.

In conclusion, biological monitoring of 1-alkoxypropanol exposure is possible by urinalysis for its metabolite 1,2-propanediol. At very low occupational exposure levels, the results may be confounded by background excretion of 1,2-propanediol originating from e.g., consumer cosmetics.

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