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TOXICOKINETICS AND METABOLISM

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Human inhalation exposure to ethylene glycol

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Abstract Two male volunteers (A and B) inhaled 1.43 and 1.34 mmol, respectively, of vaporous ^{13}C-labeled ethylene glycol ($^{13}C_2$-EG) over 4 h. In plasma, $^{13}C_2$-EG and its metabolite $^{13}C_2$-glycolic acid ($^{13}C_2$-GA) were determined together with the natural burden from background GA using a gas chromatograph equipped with a mass selective detector. Maximum plasma concentrations of $^{13}C_2$-EG were 11.0 and 15.8 μmol/l, and of $^{13}C_2$-GA were 0.9 and 1.8 μmol/l, for volunteers A and B, respectively. Corresponding plasma half-lives were 2.1 and 2.6 h for $^{13}C_2$-EG, and 2.9 and 2.6 h for $^{13}C_2$-GA. Background GA concentrations were 25.8 and 28.3 μmol/l plasma. Unlabeled background EG, GA and oxalic acid (OA) were detected in urine in which the corresponding ^{13}C-labeled compounds were also quantified. Within 28 h after the start of the exposures, 6.4% and 9.3% $^{13}C_2$-EG, 0.70% and 0.92% $^{13}C_2$-GA, as well as 0.08% and 0.28% $^{13}C_2$-OA of the inhaled amounts of $^{13}C_2$-EG, were excreted in urine by volunteers A and B, respectively. The amounts of $^{13}C_2$-GA represented 3.7% and 14.2% of background urinary GA excreted over 24 h (274 and 88 μmol). The amounts of $^{13}C_2$-OA were 0.5% and 2.1% of background urinary OA excreted over 24 h (215 and 177 μmol). From the findings obtained in plasma and urine and from a toxicokinetic analysis of these data, it is highly unlikely that workplace EG exposure according to the German exposure limit (MAK-value 10 ppm EG, 8 h) could lead to adverse effects from the metabolically formed GA and OA.

Keywords Ethylene glycol · Glycolic acid · Oxalic acid · Human · Risk · Metabolism · Toxicokinetics

This publication is dedicated to Prof. Dr. Dr. Hermann M. Bolt on the occasion of his 60th birthday.

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Introduction

Ethylene glycol (EG) is a widely used liquid with a low vapor pressure of 0.08 mbar at 20°C (DFG 1991). It is employed in antifreeze formulations, de-icing of airplanes, production of polyesters, and a series of other applications.

High doses of EG, a simplified metabolic scheme of which is given in Fig. 1, show various toxic effects. Symptoms related to the central nervous system, metabolic acidosis, nephrotoxic and embryotoxic properties are characteristic (Andrews and Snyder 1991). Nephrotoxicity is ascribed to acidic metabolites as glycolic acid (GA) and oxalic acid (OA) (reviewed, for example, in LaKind et al. 1999). Both acids also showed developmental toxicity in rat embryo cultures (Klug et al. 2001). In vivo, EG-exposed rats produced only minor amounts of OA in comparison with those of GA (Frantz et al. 1996). Embryotoxicity in EG- and GA-treated rats was related to GA (Carney et al. 1999). In rats, receiving EG in their food over 2 years, no-observed-effect-levels (NOELs) for EG-induced nephrotoxicity were 0.2% in food [about 80 mg/kg body weight (bw) per day for 2 years; Blood 1965] and 0.5% (about 200 mg/kg bw per day for 2 years; DePass et al. 1986). For B6C3F1 mice, the corresponding NOEL was 0.6% in food (about 1500 mg/kg bw per day for 2 years; National Toxicology Program 1993, cited in LaKind et al. 1999). NOELs for developmental toxicity have been reported to be 500 mg EG/kg bw per day in CD rats and 150 mg/kg bw per day in CD-1 mice (administration by gavage from gestation day 6 to gestation day 15; both species see Neeper-Bradley et al. 1995). For rats, the NOEL dose of 80 mg/kg bw can be calculated to lead to a maximum GA concentration in blood of 144 μmol/l (linear extrapolation from the maximum GA blood concentration of

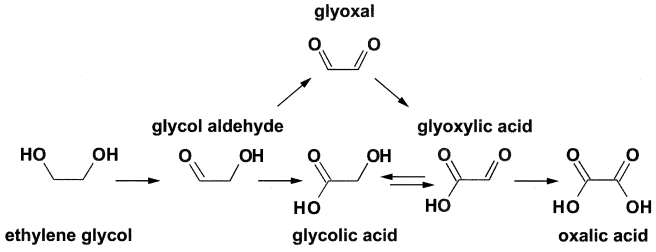

Fig. 1 Simplified scheme of ethylene glycol metabolism

271 μmol/l reached after a dose of 150 mg EG/kg bw; Pottenger et al. 2001). The dose of 500 mg EG/kg bw resulted in a maximum GA concentration of 1723 μmol/l blood (Pottenger et al. 2001).

In the US National Occupational Exposure Survey, conducted during 1981–1983, it was estimated that about 1.5 million workers were potentially exposed to EG in the USA, each year (National Institute of Occupational Safety and Health 1990, cited in IPCS 2002). Exposure may lead to uptake of EG. Only limited experimental or occupational data are available on humans exposed to EG in spite of its occupational relevance. Urinary EG was found in volunteers exposed (20–22 h/day, 30 days) to weekly air concentrations of EG between 17 and 49 mg/m^3 (Wills et al. 1974), in workers using EG for de-icing of airplanes (Gérin et al. 1997) and in motor servicing workers (Laitinen et al. 1995). None of these studies established a reliable correlation between EG concentrations in urine and air. In order to obtain such a relationship and to enable a comparison between background and additional exposure-derived burdens, we determined EG, GA and OA levels and those of their ^{13}C-labeled analogues in plasma and urine of human volunteers inhaling small amounts of vaporous $^{13}C_2$-EG at exposure conditions below the German MAK-value (maximum workplace concentration of EG 10 ppm = 25.7 mg/m^3; DFG 1991).

Materials and methods

Chemicals

$^{13}C_2$-EG (99 atom% ^{13}C) was obtained from Aldrich (Steinheim, Germany). All other chemicals were of analytical grade.

Exposure experiments

The study was approved by the ethics committee of the Technical University, Munich.

Two healthy, non-smoking, male volunteers (volunteer A: 54 years old, 96 kg; B: 44 years old, 57 kg) avoided oxalate- and ascorbate-rich foods for 2 days before the individual experiments. On the exposure days, each volunteer inhaled vaporized $^{13}C_2$-EG from an all-glass vessel (250 ml, Fig. 2). Vapors of $^{13}C_2$-EG were generated by repeatedly (16 times at intervals of 15 min over 4 h) injecting small amounts of liquid $^{13}C_2$-EG (6.6 μl each) via a septum into the closed vessel using a calibrated 10 μl syringe (SGE, Darmstadt, Germany). The air in the vessel was warmed to about 140°C (boiling point of EG 198°C) to facilitate the evaporation. At the beginning of the time intervals, the volunteer inhaled the generated vapor via the mouth. To start the inhalation, the volunteer successively opened the outlet and then the inlet valve and took a deep breath. The inhaled air stream entered the vessel by the inlet valve and transported the $^{13}C_2$-EG vapor into the respiratory tract. Immediately thereafter, the valves were closed. This inhalation procedure was repeated three to four times. At selected time points exhaled air was collected in gas bags (polyethylene-coated aluminum bags; Linde, Unterschleißheim, Germany) in order to quantify the fraction of exhaled $^{13}C_2$-EG. The gas bags were sealed immediately after collection. At the end of the exposure period (after 4 h) residual $^{13}C_2$-EG was determined from the vessel. For this purpose, 10 ml deionized water were injected into the vessel via the septum. The vessel was thoroughly shaken in order to wet all glass surfaces. Correspondingly, to determine the amounts of exhaled $^{13}C_2$-EG, 5 ml deionized water were injected into the gas bags. The aqueous solutions where stored at −80°C until analyzed for $^{13}C_2$-EG.

Prior to the exposure, an intravenous indwelling cannula (B. Braun, Melsungen, Germany) was positioned in the cubital vein. Samples of about 5 ml venous blood were collected before the start of the exposure, during the exposure at each time interval of 15 min (about 7 min following each inhalation process), and up to 4 h post-exposure. Blood pH was determined in each sample. Plasma, obtained by centrifugation, was stored at −80°C until analyzed for labeled and unlabeled EG and GA. All urinary

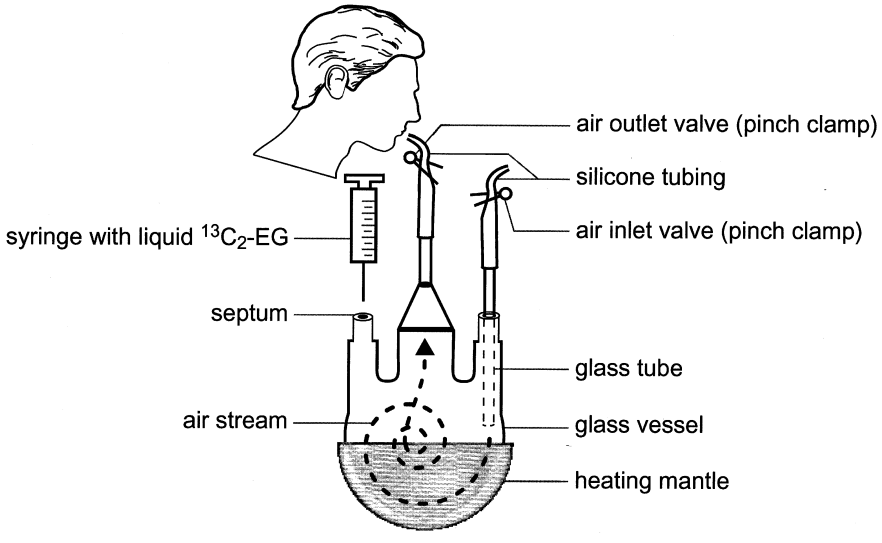

Fig. 2 Schematic set-up for inhalation exposure to ethylene glycol ($^{13}C_2$-EG) vapor

fractions were collected immediately prior to start of exposure and up to 30 h thereafter. Urinary pH was monitored immediately in each fraction, which was subsequently acidified. Then, fractions were stored at 4°C for up to 24 h. Within this period, specimens were taken for the determination of labeled and unlabeled OA. Immediately thereafter, fractions were stored at −80°C until analyzed for labeled and unlabeled EG and GA. A 5-week stability test demonstrated that the storage duration did not influence the extent of recovery of the analytes.

Analysis of ethylene glycol, glycolic acid and oxalic acid by gas chromatography

A detailed description of the analytical procedures is in preparation and will be submitted elsewhere. Therefore, only a brief description is given.

A gas chromatograph equipped with a mass selective detector (GC/MSD) was used for all analyses (GC: HP5890 Series II with cool-on-column inlet equipped with a HP-5MS column, 30 m length, 0.25 mm i.d., 0.25 μm film, carrier gas Helium; MSD: HP5972, electron impact ionization, 70 eV, selected ion monitoring). The complete system was obtained from Agilent Technologies, Waldbronn, Germany.

Sample preparation for ethylene glycol analysis

Both, propylene glycol and 1,3-propandiol served as internal standards in parallel. EG and the standards in the samples were derivatized with *n*-butylboronic acid, extracted with ethyl acetate and analyzed (limits of quantification in plasma: EG 7.6 μmol/l, $^{13}C_2$-EG 0.6 μmol/l; in urine: EG 1.1 μmol/l, $^{13}C_2$-EG 0.1 μmol/l; in water: $^{13}C_2$-EG 0.3 μmol/l).

Sample preparation for glycolic acid analysis

Deuterated succinic acid (D_6-SA) and 2-hydroxyisovaleric acid were added as internal standards to plasma and to urine, respectively. The corresponding samples were deproteinized with acetonitrile (only plasma) and dried using a vacuum concentrator. The residues were treated with *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA), and the derivatives were analyzed (limits of quantification in plasma: GA 1.2 μmol/l, $^{13}C_2$-GA 0.8 μmol/l; in urine: GA 32.6 μmol/l, $^{13}C_2$-GA 2.9 μmol/l).

Sample preparation for oxalic acid analysis

Succinic acid and D_6-SA served as internal standards for the determination of OA and of $^{13}C_2$-OA, respectively. After adding these standards, the samples were acidified and extracted with ethyl acetate. The residues obtained after drying with a vacuum concentrator were silylated with MTBSTFA and the derivatives were analyzed (limits of quantification in urine: OA 17.2 μmol/l, $^{13}C_2$-OA 0.2 μmol/l).

Results

The volunteers did not report any effects related to the exposure. The inhaled $^{13}C_2$-EG was completely taken up from the inhaled air as proven by its absence in the expired air. Therefore, the amount taken up during the entire exposure period equaled the difference between the sum of the administered doses and the residual $^{13}C_2$-EG, which was determined in the glass vessel at the end of exposure. The pH measurements in blood and urine showed normal physiological values being 7.45 ± 0.02 (volunteer A) and 7.39 ± 0.03 (B), and ranging from 6 to 7.5 (A) and from 6 to 8 (B). In plasma of the volunteers, $^{13}C_2$-EG, $^{13}C_2$-GA and GA could be determined. A background concentration of EG was not found at a limit of quantification of 7.6 μmol/l. This high value probably resulted from interference by other compounds in plasma. There was no interference for the determination of $^{13}C_2$-EG. In urine of both volunteers, labeled and unlabeled EG, GA and OA were quantifiable.

Table 1 Doses of $^{13}C_2$-ethylene glycol inhaled by the two volunteers

Volunteer	Total dose (mmol)	Normalized dose (mg/kg body weight)
A	1.43	0.96
B	1.34	1.51

Table 1 shows the inhaled doses of $^{13}C_2$-EG, both total (in millimoles) and normalized for body weight of each volunteer (as milligrams per kilogram body weight).

Figure 3 depicts the concentration-time courses of $^{13}C_2$-EG in plasma during and after exposure. The ratio of the maximum $^{13}C_2$-EG concentration found in volunteer A to that in B reflects that of the doses, as given in Table 1. The half-lives of $^{13}C_2$-EG, derived from the elimination phase of Fig. 3, are 2.1 h (A) and 2.6 h (B). Twentyfour hours after start of the exposure $^{13}C_2$-EG was no longer detectable. With $^{13}C_2$-EG half-lives of ≥2 h and dosing intervals of 15 min, the exposure can be considered as quasi-continuous.

Figure 4 displays the concentration-time courses of $^{13}C_2$-GA in plasma during and after exposure. The maximum $^{13}C_2$-GA concentrations were reached shortly after the end of exposure (calculated for volunteer A about 1 h; for B 0.5 h). These short time-spans are in agreement with the observation that the $^{13}C_2$-GA half-lives of 2.9 h (A) and 2.6 h (B) were similar (A) or identical (B) with those of $^{13}C_2$-EG. The maximum $^{13}C_2$-GA concentration was lower in volunteer A than in B, as was also observed for the maximum $^{13}C_2$-EG concentration (see above). The background plasma concentrations of GA were 25.8 ± 3.7 and 28.3 ± 2.8 μmol/l for volunteer A and B, respectively.

Figures 5, 6 and 7 depict the cumulative amounts of unlabeled (background) and labeled EG, GA and OA excreted in the urine that was collected during and after exposure.

Table 2 shows the amounts of $^{13}C_2$-EG, $^{13}C_2$-GA and $^{13}C_2$-OA in urine, expressed as a percentage of the inhaled dose of $^{13}C_2$-EG (see Table 1). Obviously, EG is predominantly biotransformed to other metabolites presumably via intermediary metabolism. This conclusion is supported by data from rat studies. Following a 30-min inhalation exposure of Fischer-344 rats to gaseous ^{14}C-EG at a mean air concentration of 12.5 ppm, Marshall and Cheng (1983) determined the amounts of exhaled $^{14}CO_2$. From the initial ^{14}C-burden, about 30% were exhaled as $^{14}CO_2$ within the first 12 h. This metabolite amounted to 60% after 4 days.

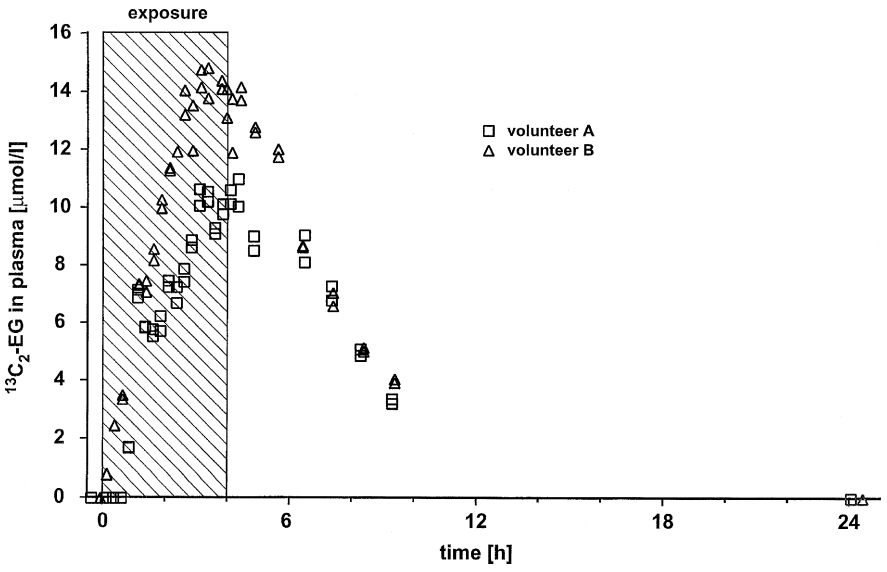

Fig. 3 Plasma concentrations of $^{13}C_2$-ethylene glycol ($^{13}C_2$-EG) during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor

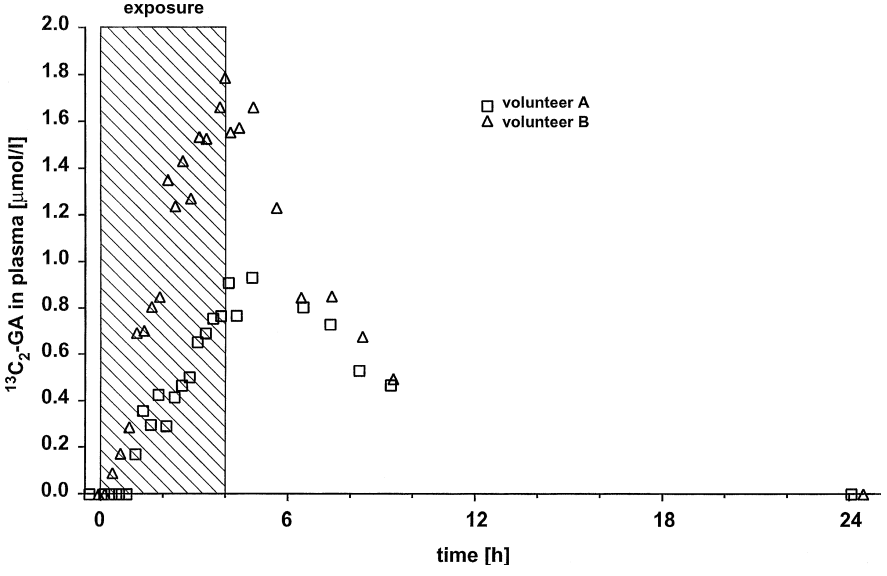

Fig. 4 Plasma concentrations of $^{13}C_2$-glycolic acid ($^{13}C_2$-GA) in plasma during and after a 4-h inhalation exposure to $^{13}C_2$-ethylene glycol vapor

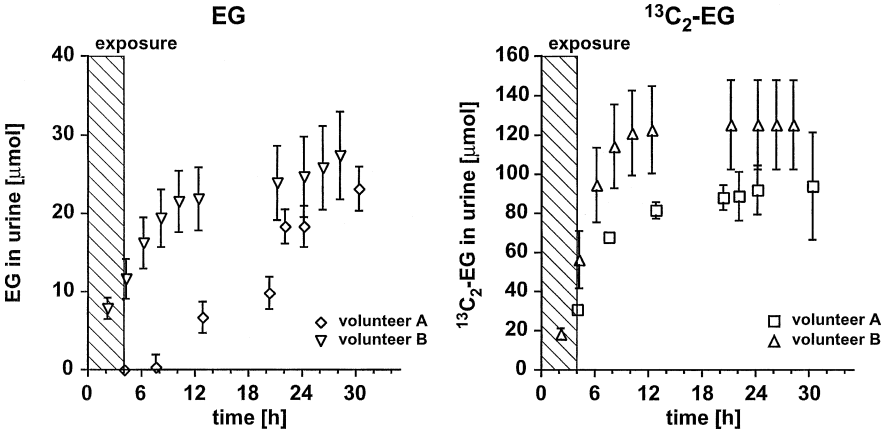

Fig. 5 Background ethylene glycol (EG) and $^{13}C_2$-EG in urine during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor, expressed as cumulative excretion (means ± SD, $n = 3$)

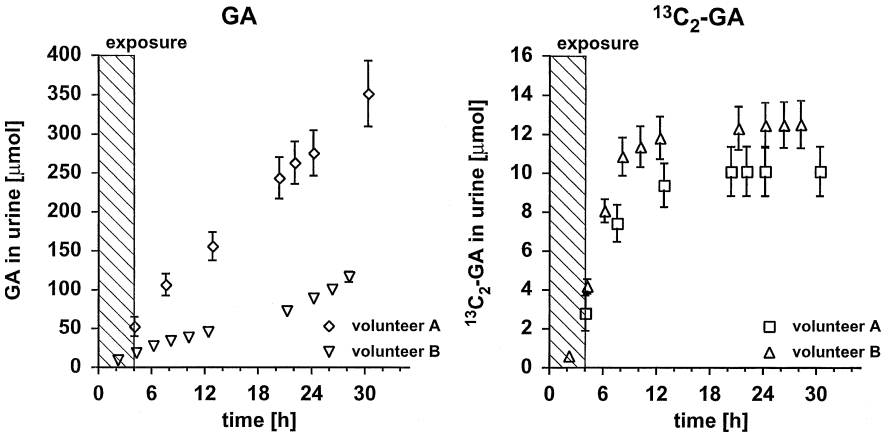

Fig. 6 Background glycolic acid (GA) and $^{13}C_2$-GA in urine during and after a 4-h inhalation exposure to $^{13}C_2$-ethylene glycol vapor, expressed as cumulative excretion (means ± SD, $n = 3$)

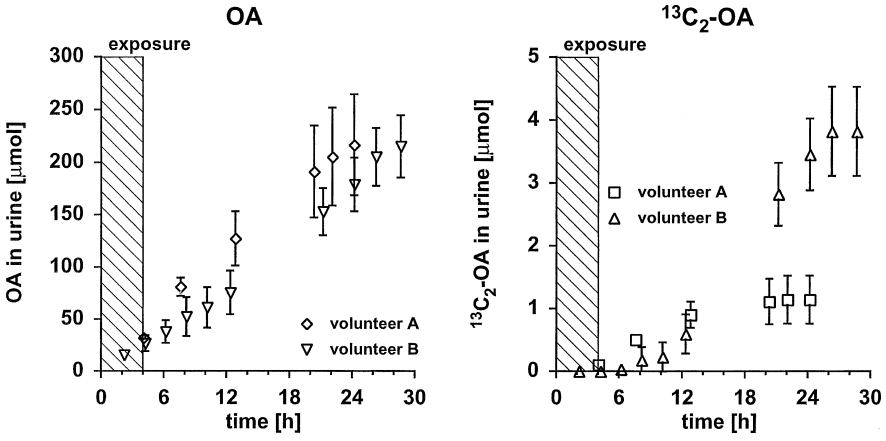

Fig. 7 Background oxalic acid (OA) and $^{13}C_2$-OA in urine during and after a 4-h inhalation exposure to $^{13}C_2$-ethylene glycol vapor, expressed as cumulative excretion (means ± SD, $n = 3$)

Table 2 ^{13}C-Labeled urinary ethylene glycol (EG), glycolic acid (GA) and oxalic acid (OA) expressed as a percentage of inhaled dose $^{13}C_2$-EG

Volunteer	Amount in urine (percentage of the inhaled dose of $^{13}C_2$-EG)		
	$^{13}C_2$-EG	$^{13}C_2$-GA	$^{13}C_2$-OA
A	6.4%	0.70%	0.08%
B	9.3%	0.92%	0.28%

Table 3 Unlabeled glycolic acid (GA) and oxalic acid (OA) excreted in urine over 24 h, together with urinary excretion of ^{13}C-labeled GA and OA after inhalation of about 1.4 mmol $^{13}C_2$-ethylene glycol, expressed as a percentage of the corresponding unlabeled acids

Volunteer	Background excretion (µmol/24 h) in urine		^{13}C-Labeled acid (percentage of the background)	
	GA	OA	$^{13}C_2$-GA	$^{13}C_2$-OA
A	274	215	3.7%	0.5%
B	88	177	14.2%	2.1%

Table 3 gives the amounts of background GA and OA excreted over 24 h. It shows also the relative increases in these acids as labeled metabolites formed from inhaled $^{13}C_2$-EG, the dose of which was 1.43 mmol (A) and 1.34 mmol (B).

Discussion

Background values

Ethylene glycol

Background EG was quantifiable in urine (volunteer A: 18.2 µmol/24 h; B: 24.5 µmol/24 h). These urinary EG excretions can be compared with ratios of urinary EG to urinary creatinine reported by other groups for non-exposed persons (Laitinen et al. 1995, 1997; Gérin et al. 1997; Letzel et al. 2000). Using these ratios and considering a normal creatinine excretion of 1.8 g/24 h in healthy men (Geigy Documenta 1975), EG excretions can be calculated to be between 0 and 77 µmol/24 h. These amounts match with those measured in volunteers A and B. In the study of Wills et al. (1974) EG background concentrations have been reported for both serum (1500–3400 µmol/l) and urine (260–1200 µmol/l). The values seem to be very high for unexposed controls, in the light of the other published data on urinary EG.

The difference might result from the rather unspecific methodology used by Wills et al. (1974), which was based on the periodate oxidation of EG to formaldehyde used by Russell et al. (1969). Since treatment with this reagent transforms a number of substances present in biological material into formaldehyde (Russell et al. 1969) an overestimation of EG probably occurred. Background EG could result from several sources. Endogenous and environmental ethylene and ethylene oxide could contribute via metabolism to background EG (reviewed in IARC 1994; Filser et al. 1994). A relevant EG source could be ethylene and ethylene oxide uptake via smoking (e.g. Törnqvist et al. 1986; Filser et al. 1992). A series of further possible sources for background EG, including food and consumer products, are discussed in IPCS (2002).

Glycolic acid

The GA background concentrations in plasma determined in the present study are in agreement with those (6.6–32.9 μmol/l) published by Chalmers et al. (1984). Other authors have reported lower GA background concentrations (between 4.4 and 12.2 μmol/l) in plasma of fasted subjects (Maeda-Nakai and Ichiyama 2000), and in plasma-ultrafiltrate of fasted (Hagen et al. 1993) and non-fasted individuals (Petrarulo et al. 1991). Urinary background amounts of GA, excreted over 24 h (Table 3) are consistent with values from 75 to 1220 μmol/24 h for healthy humans, published by a series of other groups (review by Petrarulo et al. 1998; Niederwieser et al. 1978; Marangella et al. 1992; Holmes et al. 1993; Maeda-Nakai and Ichiyama 2000). Only one publication reported a wider range from 0 to 1400 μmol/24 h (Hagen et al. 1993). Background GA can originate from several sources. GA is formed in the catabolism of proteins (Holmes et al. 1993) and carbohydrates (McWinney et al. 1987). It can also be taken up directly via food since it has been detected as a natural constituent of vegetables, fruits and meat (Harris and Richardson 1980). These authors estimated a daily total GA intake via food of 33 mg for an adult. Furthermore, GA is an ingredient of certain cosmetic products (reviewed in NICNAS 2000).

Oxalic acid

Unfortunately, we were not able to quantify OA in plasma because the recovery in plasma was not reproducible. The reason for this behavior is unclear. Recently, several methods for the determination of the OA background concentrations in plasma have been summarized (reviewed in Petrarulo et al. 1998; further citations in Hönow et al. 2002). The reported OA plasma concentrations in healthy subjects range from 0.4 to 6.0 μmol/l (Petrarulo et al. 1998) and from <0.68 to 15.9 μmol/l (Hönow et al. 2002). Petrarulo et al. (1998) recommend either enzymatic determination or HPLC separation followed by conductivity determination. Hönow et al. (2002) used HPLC separation coupled with an enzyme reactor. None of these methods allows discrimination between isotopically labeled and unlabeled analytes. Therefore, they were not applicable for the present study. The urinary background OA excretion reported here (Table 3) corresponds with the range of 86 to 622 μmol/24 h published by a series of other groups (Wandzilak et al. 1991; Marangella et al. 1992; Holmes et al. 1993; von Unruh et al. 1998; Maeda-Nakai and Ichiyama 2000; Kessler et al. 2002; Siener and Hesse 2002). Possible sources for background OA include GA and endogenous glyoxylic acid (Poore et al. 1997), catabolism of ascorbic acid (Levine et al. 1996) and uptake via food (Hönow and Hesse 2002). The contribution of intermediary metabolism to background urinary OA has been estimated to be about 50% (Williams and Wandzilak 1989).

Kinetics

Derivation of kinetic parameters

The concentration-time courses of $^{13}C_2$-EG and $^{13}C_2$-GA in plasma of volunteers A and B can be used to predict some important kinetic parameters, using a one-compartment model (for details see for example Filser 1996). For a constant uptake rate, half the plateau concentration is reached after the first half-life (for A 2.1 h; B 2.6 h). Consequently, from Fig. 3 it becomes evident that the $^{13}C_2$-EG plateau concentrations in blood amount to 14.4 μmol/l (A) and 24.0 μmol/l (B) when considering the respective "constant" inhalation rates of 358 μmol/h (A) and 335 μmol/h (B). The distribution volume (V_d)—related to the concentration in plasma—can be obtained using the plateau concentration (PC) and the half-life ($T_{1/2}$) since it is given by the expression V_d = inhalation rate$\times T_{1/2}/(\ln 2 \times PC)$. The distribution volumes are calculated to be 75 l (A) and 52 l (B), or 0.78 l/kg bw (A) and 0.91 l/kg bw (B). These values match with those of 0.5–0.8 l/kg bw (reviewed in Eder et al. 1998), and are in agreement with the assumption that the highly water soluble EG (Merck Index 1996) distributes predominantly into the aqueous phase of the body. Longer half-lives of between 3.0 and 8.6 h have been derived from cases of EG intoxication where EG concentrations in blood were in the millimolar range (reviewed in Eder et al. 1998). These differences are likely to result from saturation of EG metabolism occurring at such high concentrations.

Concerning $^{13}C_2$-GA, such kinetic calculations cannot be made, since the production rate and the distribution volume of this second $^{13}C_2$-EG metabolite are not available from the measured data. However, a worst-case estimate for the maximum $^{13}C_2$-GA concentration achievable as a consequence of an 8-h exposure to $^{13}C_2$-EG can be made from the linearly increasing parts of the

measured $^{13}C_2$-GA concentration and by assuming a further continuous linear increase of the $^{13}C_2$-GA concentration with the $^{13}C_2$-EG-exposure time. In the present experiments, the 4-h $^{13}C_2$-EG exposures of volunteers A and B led to maximum $^{13}C_2$-GA plasma concentrations of 0.9 and 1.8 μmol/l, respectively (Fig. 4). For 8-h $^{13}C_2$-EG exposures, and considering the $^{13}C_2$-EG inhalation rates given above, a linear increase of the maximum $^{13}C_2$-GA concentrations would lead to 1.8 (A) and 3.6 (B) μmol/l plasma. It has to be stressed that these values are overestimates since the increase of the unknown $^{13}C_2$-GA production rate is not constant but becomes smaller with the flattening slope of the rising $^{13}C_2$-EG concentration. From the observation that in both volunteers the half-lives of $^{13}C_2$-GA were very similar (A) or identical (B) to those of the metabolic precursor $^{13}C_2$-EG, one can conclude that the elimination rate of $^{13}C_2$-GA is determined by its production from $^{13}C_2$-EG. Consequently, an even shorter half-life of GA should be expected if GA was to be administered as such.

Kinetic extrapolations

Based on the above data, an extrapolation to workplace conditions according to the MAK-value (10 ppm, 8 h, alveolar ventilation 20 l/min per 70 kg bw; Åstrand 1983) can be carried out. Allometrically, the alveolar ventilation is calculated for volunteer A to be 24.7 l/min [20×(bw of A/70)$^{2/3}$], and for B to be 17.4 l/min [20×(bw of B/70)$^{2/3}$]. Using these values together with the individual volumes of distribution and the obtained half-lives, an exposure to 10 ppm EG (25.7 mg/m^3 = 0.414 μmol/l) should result in maximum EG plasma concentrations of 23.0 μmol/l (A) and 27.5 μmol/l (B) reached on 8 h of exposure. Taking into account that the EG inhalation rate of volunteer A (24.7×60×0.414 = 614 μmol/h) would be 1.72 times that of the present 4-h exposure (358 μmol/h), the resulting maximum (worst-case) GA plasma concentration would amount to 3.1 μmol/l (1.72×1.8), which has to be added to the GA background concentration. Corresponding calculations for volunteer B yield a maximum increase over the background GA plasma concentration of 4.6 μmol/l. These values represent 12.0% (A) and 16.3% (B) of the corresponding background GA plasma concentrations.

Based on the relative urinary excretion data of $^{13}C_2$-GA and $^{13}C_2$-OA given in Table 3, corresponding increases over the urinary backgrounds resulting from EG uptake of 4.91 mmol (A) and 3.46 mmol (B)—equivalent to the 8-h exposure to 10 ppm—are predicted to be for GA and OA 12.7% and 1.7% (A), and 36.7% and 5.4% (B), respectively.

Conclusion

Workplace exposure to EG vapors under MAK conditions (10 ppm, 8 h/day, 50 W) leads to a daily EG uptake of 247 mg in a reference human of 70 kg bw. The resulting additional maximum GA and OA burdens are in the range of, or below, the unavoidable background levels. Furthermore, maximum plasma concentrations of EG and GA are rapidly decreasing after end of exposure, which becomes obvious from their short half-lives. Hereof, it has to be deduced also that both compounds cannot accumulate in daily-exposed humans (under MAK conditions). According to our data, an 8-h exposure to 10 ppm EG could lead to a maximum increase of the GA plasma concentration of less than 5 μmol/l. With the highest background GA concentration of 32.9 μmol/l, reported by Chalmers et al. (1984), this would summate to about 38 μmol/l. Considering that in rats the lowest NOEL for EG-induced nephrotoxicity was 80 mg/kg bw per day (Blood 1965), which leads to maximum GA concentrations of 144 μmol/l blood, and that the NOEL for developmental toxicity was 500 mg/kg bw resulting in a maximum GA concentration in blood of 1723 μmol/l, we conclude that GA- and OA-induced nephrotoxic or developmental toxic effects resulting from human exposure to 10 ppm EG are highly unlikely.

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