Technische Universität München

Institut für Toxikologie
GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Neuherberg

Bioavailability of ethylene glycol and its metabolites glycolic acid and oxalic acid in volunteers following inhalation and dermal exposure to ethylene glycol

Swapna Upadhyay

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzender: **Univ.-Prof. Dr. rer. nat. Bertold Hock**

Prüfer der Dissertation:

1. **Univ.-Prof. Dr. rer. nat. Erich F. Elstner, em.**
2. **apl.Prof. Dr. rer. nat., Dr. med.habil. Johannes G. Filser**
3. **Univ.-Prof. Dr. rer. nat. Michael Schemann**

Die Dissertation wurde am 13.06.06 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 07.08.06 angenommen.

I dedicate this work to my parents

This work was carried out at the Institut für Toxikologie,
GSF-Forschungzentrum für Umwelt und Gesundheit GmbH, Neuherberg,
under the supervision of Prof. Dr. Johannes G. Filser.

I express my profound indebtedness and deepest gratitude to Prof. Dr. J. G. Filser for giving me the opportunity to carry out my doctoral dissertation under his valuable guidance and supervision, for his continuous interest and encouragement in my work, and for critically evaluating this manuscript.

I am grateful and like to express my most sincere thanks to Prof. Dr. E. F. Elstner for his continuous inspiration.

My special thanks to Prof. Dr. H. J. Leppelsack for his continuous encouragement.

I sincerely thank Prof. Dr. G. Csanády, Dr. C. Hutzler, and Mrs. E. Erbach for their helpful suggestion, as well as for the compilation and correction of the manuscript preparation.

I am grateful to Prof. Dr. S. Halbach and Dr. W. Kirchinger for their kind medical attendance.

I thank to Mrs. B. Semder and Mr. C. Pütz for their excellent technical support provided during my work.

I also take this opportunity to express my warmest thanks to all my colleagues for their constant cooperation, suggestion and help that has made this work easier.

I am grateful to the almighty for all the happenings and I sincerely thank my parents, my brothers, my husband, family members and all well-wishers who stood beside me, as always, during the course of this work.

Table of Contents

1. Introduction

1.1 Objective

Ethylene glycol (EG) is a high production volume chemical. The liquid having a low vapour pressure of 0.092 mm Hg at 25 °C is widely used for antifreeze formulations, de-icing of airplanes, hydraulic fluids, surface coatings, surfactants, and emulsifiers and is a chemical intermediate in the production of polyethylene terephthalate (PET) and polyester fibres and films. Occupational exposure of workers to EG mostly occurs through dermal uptake and inhalation of vapours and mists during handling of EG-containing solutions. The general population is also exposed to EG due to dermal contact with products such as antifreeze solutions. Additionally, food or beverages can be contaminated by trace amounts of EG migrating from the package material PET or regenerated cellulose film.

Toxicity of EG in humans as a result of oral intake of high doses either accidentally or intentionally is well known. Effects on the central nervous system, metabolic acidosis and renal toxicity are characteristic. Moreover, the substance is embryotoxic in mice and rats. Most of these effects result from the EG metabolites, glycolic acid (GA) and oxalic acid (OA).

EG, GA and OA are also produced naturally as endogenous metabolites in the intermediary pathway and are natural constituents of food. Consequently, there is some background burden in humans.

Based on only limited information, the Commission for the Investigation of Health and Hazards of Chemical Compounds in the Work Area established a maximum workplace concentration (MAK) for a daily eight-hour exposure (40 h per week) to EG of 26 mg/m^3. This “MAK-value” is equivalent to a concentration of 10 ppm of vaporous EG. Under workplace exposure conditions, there is also a chance for wetting of hands and arms by liquid EG. There are only very few data available on human body burden by inhaled EG and its toxic metabolites under exposure conditions as might occur at the workplace. No data are published on dermal uptake of humans exposed to liquid EG. Consequently, for validating the current “MAK-value”, it was the aim of the present work to quantify in volunteers the burdens by EG, GA and OA resulting from uptake of EG following inhalation and dermal

exposure to $^{13}C_2$-EG. In order to establish a risk evaluation, the body burdens were compared with those representing the unavoidable background. For differentiating background from exposure-derived substances, exogenous EG was administrated as the ^{13}C-labelled isotope. Unlabelled and labelled EG and GA were measured from plasma and urine of volunteers after inhalation of small amounts of vaporous $^{13}C_2$-EG and during and after application of liquid $^{13}C_2$-EG onto skin. In addition, unlabelled and labelled OA were determined in the urine of the exposed subjects. For quantitative analysis, mass selective and sensitive methods were used.

1.2 Properties and use of ethylene glycol

EG (IUPAC name: ethane-1,2-diol; Cas No.: 107-21-1) is a colourless, odourless, and slightly viscous hygroscopic liquid of sweet taste. The chemical is highly soluble in water, ethanol, acetone, acetic acid, glycerine, pyridine, and in aldehydes. It is hardly soluble in diethylether (1:200) and practically insoluble in benzene, chlorinated hydrocarbons, petrol ether or oils (IPCS, 2000).

Physicochemical property	Value
Molecular weight	62.07 g/mol
Density	1.11 g/ml
Boiling point	198 °C at 760 mm Hg
Melting point	-13 °C
Vapour pressure	0.092 mm Hg at 25 °C

Table 1: Physicochemical properties of ethylene glycol (NTP, 2004).

About 60 % of EG consumed are used as a chemical intermediate in the plastic industry for the manufacture of PET (particularly plastic bottles for soft drinks), polyester fibres and films, and unsaturated polyester resins. The remaining EG is predominately used as an industrial coolant, antifreeze in heating and cooling

systems, de-icing fluid in aircrafts, in surface coatings, hydraulic fluids, surfactants and emulsifiers, and in the formulation of colours and inks. A smaller percentage is used in the synthesis of safety explosives (e.g. dinitroethylene glycol) and of glyoxal (Budavari *et al*., 1996; NTP, 2004).

1.3 Metabolism of ethylene glycol

Intake of EG may occur through consumer products (including food) and from occupational exposure (NTP, 2004; see 1.6). EG is also formed in the intermediary metabolic pathway from ethylene oxide that derives from endogenous ethylene (Filser *et al*., 1994). EG is mainly metabolised in the liver. All enzyme systems required for the oxidation of EG are present in this organ (Richardson, 1973). The metabolic pathway of EG is illustrated in Figure 1 (summarised from Cavender and Sowinski, 2001; Jacobsen *et al*., 1988; McChesney *et al*., 1971, 1972; Carstens *et al*., 2003). EG metabolism is similar in humans and rodents (Carstens *et al*., 2003; Gessner *et al*., 1961; Frantz *et al*., 1996b; Hess *et al*., 2004). EG is first oxidised by the liver alcohol dehydrogenase (ADH; von Wartburg *et al*., 1964; Wagner *et al*., 1983). Chou and Richardson (1978) suggested ADH mediated metabolism of EG to glycolaldehyde to be one of the rate limiting enzymatic steps in the metabolism of EG. Studies on rat hepatic metabolism of EG by Rajagopal and Ramakrishnan (1994) revealed that the first step of EG catabolism was not only catalysed by cytosolic ADH but additionally (25 %) by the microsomal system leading to formaldehyde. The latter was demonstrated to be an NADPH dependent, carbon monoxide-inhibitable reaction, indicating a role of CYP450 in the EG oxidation (Kukielka and Cederbaum 1991, 1995; Clejan and Cederbaum, 1992).

Metabolism of glycolaldehyde to GA is catalysed by mitochondrial ADH and cytosolic aldehyde oxidase (Wiener and Richardson, 1988). Glycolaldehyde is also oxidised to glyoxal as has been demonstrated in rat liver microsomes by Loeppky and Goelzer (2002). A third pathway for glycolaldehyde might be the reaction with thiamine pyrophosphate resulting in “reactive glycolaldehyde” that could be used in the transketolase reaction for the synthesis of ketoses (Karlson, 1974).

GA, is not only the direct oxidation product of glycolaldehyde but is also formed very rapidly from glyoxal, a reaction that is catalysed by the cytosolic glutathione-dependent glyoxylase system (Thornalley, 1998).

Oxidation of GA to glyoxylic acid is catalysed by glycolic acid oxidase (GAO; Richardson and Tolbert, 1961) localised in peroxisomes (De Duve and Baudhuin, 1966; McGroarty and Tolbert, 1973; Jones *et al*., 2000). As reviewed in Yanagawa *et al*. (1990), GA and glyoxylic acid are readily interconvertible. The reduction of glyoxylic acid to GA is catalysed by lactate dehydrogenase (LDH; Sawaki *et al*., 1967; Duncan and Tipton, 1969; Romano and Cerra, 1969). When $NADH/NAD^+$ ratio is high, the LDH reaction favours the formation of GA from glyoxylic acid (Wiener and Richardson, 1988).

Glyoxylic acid is rapidly degraded to a variety of further products, especially to OA and glycine, a reversible reaction. Halabe *et al*. (2003) demonstrated that EG, glycine, and GA produce OA via intermediate formation of glyoxylic acid. According to Yanagawa *et al*. (1990), the oxidation to OA is catalysed by three enzymes, xanthine oxidase, LDH and GAO, as had been demonstrated *in-vitro*. Of these, GAO was the most relevant one in humans *in-vivo*. The conversion of glyoxylic acid to glycine is catalysed by four aminotransferases, which specifically use glyoxylic acid as the alpha-keto acid (reviewed in Wiener and Richardson, 1988). Metabolism to glycine is closely coupled with serine formation, a reversible reaction (Rowsell *et al*., 1982). Serine can be further biotransformed via ethanolamine, which is also produced from carbohydrates, to glycolaldehyde followed by GA and glyoxylic acid and OA formation (reviewed in McWhinney *et al*., 1987). Because glycine and serine are also formed in the catabolism of proteins, nutrition rich in animal protein and carbohydrates can increase the formation of GA and OA (Holmes *et al.,* 1993) and calcium oxalate stones (McWhinney *et al*., 1987). About 50 % of urinary background OA (either from L-ascorbate or from glyoxylate) are assumed to be produced through the intermediary metabolism (reviewed in Williams and Wandzilak, 1989).

Both GA and OA are also natural constituents of food. GA is found in fruits, vegetables, beverages, and in meat. Daily total GA intake via food has been estimated to be about 33 mg for an adult (Harris and Richardson, 1980). Furthermore, it is used in some skin care products (reviewed in NICNAS, 2000) and in a broad spectrum of other consumer and industrial applications (DuPont, 2005). High levels of OA are

found in some food e.g. in spinach, sorrel, rhubarb (Sanz and Reig, 1992), star fruit (Chen *et al*., 2003), chocolate, and tea (Ogawa *et al*., 1984).

Formic acid and CO_2 are further end products of the EG catabolism (Gessner *et al*., 1961; Frantz *et al*., 1996a; Marshall and Cheng, 1983; McChesney *et al*., 1972). Both metabolites are discussed to be directly formed from glyoxylic acid (Gessner *et al*., 1961). CO_2 can also be produced from the condensation of glyoxylic acid with the endogenous 2-ketoglutaric acid leading to 2-hydroxy-3-ketoadipic acid and its decomposition product 5-hydroxy-4-ketovaleric acid together with two molecules of CO_2 (both deriving from the glyoxylic acid; Schlossberg *et al*., 1968).

Additionally, intermediary products are possibly formed by aldol-addition of all of the aldehydes occurring in the EG catabolism with endogenous CH-acidic compounds. For instance, Gupta and Dekker (1984) described the reaction of glyoxylic acid with pyruvic acid leading to 2-keto-4-hydroxy-glutaric acid. The reaction of glyoxylic acid with 2-ketosuccinic acid resulting in 3-oxalomalic acid is described in several reviews, too (Cavender and Sowinski, 2001; DFG, 1991).

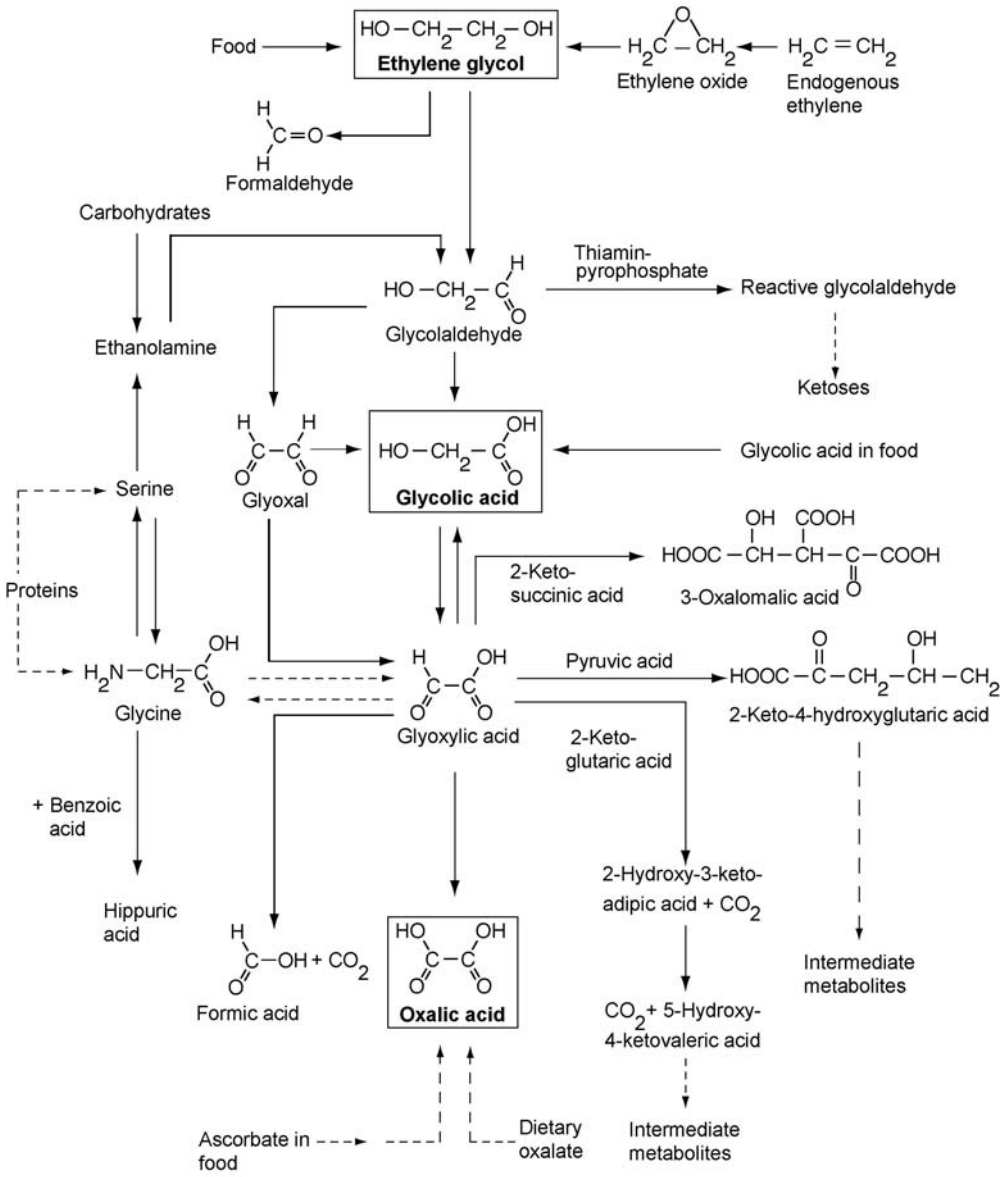

Figure 1: Scheme of ethylene glycol pathways. Substances marked by boxes are dealt with in the present work.

1.4 Toxicokinetics

Toxicokinetics of EG were investigated in animals and in some intoxicated humans in whom EG concentrations in blood were in the millimolar range. Concerning toxicokinetics of vaporous EG, there is only one inhalation study with two volunteers published. Additionally, there are some data on occupationally exposed persons (see 1.6).

1.4.1 Animals

Marshall and Cheng (1983) exposed Fischer 344 rats over 30 min to $^{14}C_2$-EG vapour (32 mg/m^3). The authors reported that the predominant share (63 %) of the dose taken up was exhaled as $^{14}CO_2$ during the first 4 days. In urine, 20 % of the dose was detected as unchanged $^{14}C_2$-EG. Species-specific toxicokinetics of $^{14}C_2$-EG were investigated in Sprague-Dawley rats and CD-1 mice following administration of doses in the range between 10 and 1000 mg/kg body weight (bw), via intravenous (i.v.), peroral (p.o.), and percutaneous routes (Frantz *et al.*, 1996a, 1996b, 1996c). Volumes of distribution of EG at steady-state, related to the EG concentration in plasma, were calculated to be between 0.3 and 0.6 l/kg bw in rats and 0.8 l/kg bw in mice. Almost the same terminal half-lives of EG were reported for rats (1.2-1.7 h) and mice (1.0-1.2 h), following i.v. administration of $^{14}C_2$-EG. In both species, orally administered EG was rapidly and almost completely absorbed (bioavailability 92-100 %). Following occlusive epicutaneous administration of $^{14}C_2$-EG over 6 h, about 80 % of the dose was biovailable in mice when measured over a time frame of 96 h. Less than 36 % was absorbed through rat skin within the same time period (see also 1.7). Exhaled $^{14}CO_2$ and urinary $^{14}C_2$-GA were major metabolites in rats and in mice following i.v. and p.o. administrations of EG. The metabolite pattern was dose-dependent in both species. At 1000 mg/kg bw p.o. dose, there was relatively less $^{14}CO_2$ exhalation and relatively more urinary ^{14}C excretion than at the 10 mg/kg bw p.o. dose. In urine of rats but not of mice, $^{14}C_2$-OA was detected after both the low (10 mg/kg bw) and the high (1000 mg/kg bw) dose levels by either the i.v. or p.o. routes. In another study, toxicokinetics of EG and its metabolites GA and OA were compared in pregnant and non-pregnant rats following p.o. administration of $^{13}C_2$-EG at gestation day (GD) 10 (Pottenger *et al.*, 2001). The authors demonstrated that toxicokinetic parameters of EG and its metabolites (GA and OA) were not affected by pregnancy. Blood levels of GA were roughly dose-proportional from 10 to 150 mg/kg bw of EG, but increased disproportionately from 500 to 1000 mg/kg bw of EG. EG and GA exhibited dose-dependent urinary elimination at EG doses of ≥500 mg/kg bw, probably due to saturation of metabolism of EG to GA and of GA to its metabolites. OA was only a minor metabolite in both blood and urine.

1.4.2 Humans

Carstens *et al*. (2003) performed an inhalation exposure study with vaporous EG in two male volunteers, A and B, who inhaled 1430 µmol and 1340 µmol of vaporous $^{13}C_2$-EG over 4 h, respectively. $^{13}C_2$-EG and its metabolites ($^{13}C_2$-GA and $^{13}C_2$-OA) together with the natural burden from background EG, GA and OA were determined using a gas chromatograph equipped with a mass selective detector. Background EG, GA and OA were detected in urine in which the corresponding ^{13}C-labelled compounds were also quantified. Within 28 h after starting the exposures, 6.4 % and 9.3 % of $^{13}C_2$-EG, 0.70 % and 0.92 % of $^{13}C_2$-GA, as well as 0.08 % and 0.28 % of $^{13}C_2$-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 % of background GA excreted over 24 h in urine (274 µmol and 88 µmol, respectively). The amounts of $^{13}C_2$-OA were 0.5 and 2.1 % of background urinary OA excreted over 24 h (215 µmol and 177 µmol, respectively). Maximum plasma concentrations of $^{13}C_2$-EG were 10.1 and 14.8 µmol/l and of $^{13}C_2$-GA were 0.9 and 1.8 µmol/l, respectively. Background GA concentrations were 25.8 and 28.3 µmol/l plasma. The distribution volume of EG, related to its concentration in plasma, was 0.78 and 0.91 l/kg bw. The plasma half-lives of $^{13}C_2$-EG were 2.1 and 2.6 h and of its metabolite $^{13}C_2$-GA were 2.9 and 2.6 h. Longer half-lives of between 3.0 and 8.6 h have been determined in EG derived from cases of EG intoxication where EG concentrations in blood were in the millimolar range (Eder *et al*., 1998; Jacobsen *et al*., 1988). These differences are likely to result from saturation of EG metabolism occurring at such high concentrations. The distribution volume of EG published by these authors ranging from 0.5 to 0.8 l/kg bw are similar to those determined by Carstens *et al*. (2003). The values are in agreement with the assumption that once absorbed, the highly water-soluble EG (The Merck Index, 1996) distributes predominantly into the aqueous phase of the body (Peterson *et al*., 1981; Jacobsen *et al*., 1982, 1984; Carney, 1994). Considering the toxicokinetic data gained by Carstens *et al*. (2003), Corley *et al*. (2005) and Corley and McMartin (2005) developed a physiologically based pharmacokinetic model for EG and its metabolite GA in humans.

1.5 Toxicity of ethylene glycol in animals and humans

1.5.1 Animals

Toxicity of EG has been examined in short- and long-term exposed experimental animals (summarised in e.g. ATSDR, 1997; NTP, 2004). Minimum acute lethal doses ranged between 1.0 g/kg (cats), 3.8 g/kg (rats), and 6.7 g/kg (dogs) as reported by LaKind *et al*. (1999).

Subchronic and chronic toxicity

A series of subchronic toxicity studies in different laboratory animal species demonstrated that kidney is the primary target organ for EG toxicity, male rats being rather sensitive as compared to female rats or mice (studies up to 1993; summarised in NTP, 1993). Cruzan *et al*. (2004) conducted a 16-weeks subchronic toxicity study to compare the toxicity of EG in male Fischer 344 and Wistar rats by administrating EG via diet at dosages of 0, 50, 150, 500, 1000 mg/kg bw/day. This study demonstrated in both species a No-Observed-Effect Level (NOEL) of 150 mg/kg/day concerning kidney toxicity.

In an early 2-year study, decreased survival, kidney damage, and mild hepatocellular atrophy was found in male rats fed a diet containing 1 or 2 % EG. Female rats were less severely affected (Morris *et al.*, 1942; cited in NTP, 1993). Blood (1965) maintained Sprague-Dawley rats (males and females) for 2 years on a diet containing EG at concentrations of 0, 0.1, 0.2, 0.5, 1 and 4 %. The NOEL for the renal toxicity was determined at the EG concentration of 0.2 % in the diet of male and of 0.5 % in that of female animals. In another 2-year chronic toxicity/oncogenicity study, EG was provided by food to Fischer 344 rats and CD-1 mice with approximate EG dosages of 0, 40, 200, and 1000 mg/kg bw/day (DePass *et al*., 1986). In male and female rats, the NOEL for renal toxicity was about 200 mg EG/kg bw/day. At the high dose, kidney damage was less severe in female rats. No dose related clinical signs or any indications of toxicity were observed among mice at all dosages. NTP (1993) administered to male and female B6C3F1 mice over 13 weeks diets containing EG doses of 0, 3200, 6300, 12500, 25000, and 50000 mg/kg food. All mice survived. Chemical-related kidney and liver lesions consisting of nephropathy and

centrolobular hepatocellular hyaline degeneration were seen only in male mice receiving the diets containing 25000 and 50000 mg EG/kg food. In the following 2-year study, mice received diets containing 0, 6250, 12500, or 25000 mg EG/kg food (males) and 0, 12500, 25000, or 50000 mg EG/kg food (females). These concentrations corresponded to EG doses of 0, 1500, 3000, 6000 mg/kg bw/day (males) and 0, 3000, 6000 or 12000 mg/kg bw/day (females). NOELs for kidney and liver toxicity in male animals were 1500 mg/kg bw/day. For female mice no kidney effects were reported; NOEL for liver toxicity was 3000 mg/kg bw/day. In this gender, there were also hyperplastic changes in the lung at all doses. In agreement with negative mutagenicity data (summarised in NTP, 1993), the authors concluded from this long-term study that "there was no evidence of carcinogenic activity of ethylene glycol".

Generally, kidney damages are not attributed to the parent EG but to the action of metabolites particularly to GA. Male rats fed 1.0 % to 2.0 % GA in the diet for up to 35 weeks showed increseased mortality and renal lesions comparible with those observed from chronic EG feeding (Silbergeld and Carter, 1959). The role of the GA metabolite OA with respect to renal damages has been linked with the precipitation of calcium oxalate monohydrate in the tubular lumen (McMartin and Wallace, 2005). However, the causal relationship between calcium oxalate precipitation alone and renal damage is not without controversy (NTP, 1993). For instance, GA was assumed as the immediate cause of metabolic acidosis and kidney failure (NICNAS, 2000). In contrast, Green *et al*. (2005) concluded from a 4-weeks study with Sprague-Dawley rats consuming 0.75 % EG in their drinking water that "metabolic acidosis does not develop in conventional EG treatments but may ensue with renal insufficiency resulting from an oxalate load". Poldelski *et al*. (2001) investigated critical metabolites and EG pathways for kidney injury in isolated mouse proximal tubular segments. These authors discussed the relevance of the GA metabolite glyoxylic acid for kidney damage. Metabolism of EG was also assumed to be the cause for the hepatocellular hyaline degeneration detected in the long-term mouse study of NTP (1993). As leading cause for the apparent gender and species differences in EG toxicity, NTP (1993) discussed varying proportions of EG and metabolically formed acids.

Developmental toxicity

Neeper-Bradley *et al*. (1995) reported NOELs for developmental toxicity of EG at 500 mg/kg bw/day for rats and 150 mg/kg bw/day for mice, following p.o. administration of EG on GDs 6-15 at concentrations of 0, 150, 500, 1000 and 2500 mg/kg bw/day to CD rats and 0, 50, 150, 500, or 1500 mg/kg bw/day to CD-1 mice. Similar results were found in other studies on rats and mice (Price *et al.* 1985; Tyl *et al.*, 1995a, 1995b, 1995c; Yin *et al.*, 1986). Rabbits seem to be less sensitive than mice. In the developmental toxicity study by Tyl *et al*. (1993), EG was administrated to New Zealand white rabbits by gavage on GDs 6-19 at doses of 0, 100, 500, 1000, or 2000 mg/kg bw/day. Profound maternal toxicity of EG was observed at 2000 mg/kg bw/day (82 % kidney damage, 42 % mortality). However, there was no indication of developmental toxicity at any dosages used. Therefore, the NOEL for maternal toxicity was 1000 mg/kg bw/day and for developmental toxicity was at least 2000 mg/kg bw/day.

EG and GA were comparatively assayed for developmental toxicity in Sprague-Dawley rats. Deionised water (controls) or 40.3 mmol/kg (2500 mg/kg) of EG or 8.5 mmol/kg (650 mg/kg) of GA were given daily at GDs 6-15 via gavage (Carney *et al*., 1999). Foetuses were evaluated on GD 21. Both EG and GA caused developmental effects, including numerous axial skeletal malformation. According to the authors, the results indicate GA as the proximate developmental toxicant.

Klug *et al.* (2001) demonstrated also OA to be embryotoxic. The authors exposed rat whole embryo cultures for 48 h (GDs 9.5-11.5) to EG (50, 100 and 200 mmol/l), GA (3 mmol/l), OA (1 mmol/l and 2 mmol/l) and several further EG metabolites. EG showed no adverse effects at the lowest concentration. Both GA and OA were embryotoxic at the administered concentrations. The authors concluded that their findings did not militate against the hypothesis GA to be the most prominent factor for EG related developmental effects in rats. In agreement, toxicokinetic findings showed by far higher $^{14}C_2$-GA than $^{14}C_2$-OA in urine of rats treated i.v. and p.o. with $^{14}C_2$-EG (Frantz *et al*., 1996b). Furthermore, in urine of mice treated i.v. and p.o. with $^{14}C_2$-EG, only $^{14}C_2$-GA was detected (Frantz *et al*., 1996b).

1.5.2 Humans

Toxicity of EG has been examined following accidental or intentional ingestion (summarised in ATSDR, 1997; NTP, 2004).

The minimum lethal dose is reported to be 1.6 g/kg bw (reviewed in LaKind *et al*., 1999). According to NTP (2004) the relative high human sensitivity compared to rats (3.8 g/kg bw) and dogs (6.7 g/kg bw) is questionable because the dose in poisoned humans is often uncertain.

Acute EG poisoning proceeds usually in three clinical stages that may also overlap. Generally, an acute effect on the central nervous system (CNS) is followed by metabolic acidosis and cardiopulmonary effects, and finally by renal toxicity (summarised in DFG, 1991; Cavender and Sowinski, 1994; LaKind *et al*., 1999; IPCS, 2002; NTP, 2004; 75 cases of ethylene glycol poisonings are reported in Rzepecki *et al.*, 1998).

Central nervous system stage

The initial stage of EG poisoning is characterised by effects on the CNS. They usually occur within 30 min to about 12 h after ingestion. The primary symptoms are drunkenness, but without the characteristic alcoholic breath odour, nausea, and vomiting. At “larger” EG doses, ataxia, slurred speech, coma followed by convulsions, and even occasional deaths are reported. Ocular signs such as opthalmoplegia, papilledema, and optic atrophy have also been described. Most of these effects are attributed to the presence of unchanged EG and the formation of aldehydes that are normally found within 6 h to 12 h after ingestion.

Cardiopulmonary stage

The second stage is known as cardiopulmonary phase, which lasts from 12 h to 36 h post ingestion. This stage is characterised by severe metabolic acidosis. Symptoms include mild hypotension, tachycardia, tachypnea, and cyanosis. Haemolysis, pulmonary edema, disseminated intravascular coagulation syndrome, bronchopneumonia, cardiac enlargement and congestive failure are also reported in severe cases. Hypocalcaemia may occur due to the precipitation of the calcium salt of the metabolically formed oxalate.

Renal stage

The third stage, the 'renal failure' occurs usually 24 h to 72 h post ingestion. It is characterised by polyuria, followed by oligo-, and anuria that can be followed by terminal kidney failure. Urine analysis shows a low specific gravity with proteinuria, microscopic haematuria, pyuria and cylindruria. The stage is characterised by tubular epithelial degeneration, necrosis and crystal depositions in the kidney. Profound acidosis is primarily caused by the accumulation of the EG metabolites GA and glyoxylic acid which are finally metabolised to OA that forms calcium oxalate crystals.

Crystals of calcium oxalate precipitating in the proximal convoluted tubules and renal interstitial tissue were considered to be closely associated with nephropathy in EG poisoned patients (Jacobsen and McMartin, 1986; Wiley, 1999). The same conclusion was drawn from recent studies on human proximal tubular cells (Guo and McMartin, 2005). However, kidney damage is also discussed to result from acidosis caused from bicarbonate consumption by the acid EG metabolites (LaKind *et al*., 1999; see also 1.5.1).

Acute effects of EG on mucosa and skin

EG is irritating to the mucosa. In an experimental study (Wills *et al*., 1974), 19 volunteers were exposed for 20-22 h per day to aerosolised EG during 30 days to mean daily EG concentration between 0.8 and 67 mg/m^3 (weekly mean concentration: 17-49 mg/m^3). Blood and urine tests showed no significant differences into the 10 control subjects. There were no signs of toxic effects except complaints of irritation of the throat and fauces. Slight headache was also reported. Irritative effects were common at ambient EG concentrations of about 140 mg/m^3. Included in this study were additionally short-term exposure peaks of 188, 244 and 308 mg/m^3, which could be tolerated for 15 min, 1-2 min and 1-2 breaths, respectively.

An accidental EG splash into the eye of a gasoline station attendant damaged the conjunctiva, resulted in oedema, reduced light reflexes and strong keratitis. The symptoms had disappeared not until 4-weeks later (Sykowski, 1951; cited in DFG, 1991 and in Caravati *et al.*, 2005).

Two cases of skin sensitisation are reported: A 17-year-old man using a 25 % EG solution developed after 4 months an exanthema on hands and forearms

(Dawson, 1976; cited in DFG, 1991). A 31-year-old woman using occupationally a 25 % EG solution that resulted daily in extensive wetting of her clothes developed after 3 months dermatitis on arms, breast and abdomen. In both cases patch tests with EG were positive (Hindson and Ratcliffe, 1975; cited in DFG, 1991).

Chronic toxicity

In a case study, complaints were reported among 38 female workers in an electrolytic condenser factory who worked at open containers with a 105 °C hot mixture containing 40 % EG, 55 % boric acid, and 5 % ammonia (Troisi, 1950; according to the citations in DFG, 1991 and in Cavender and Sowinski, 2001). Nine of the women suffered from frequent attacks of loss of consciousness and nystagmus, five of them showed an absolute lymphocytosis. Further examination revealed 5 additional cases of nystagmus among the residual 29 workers. There was no information on air concentrations of EG. All but the two most severely affected women, who were removed to other work, recovered completely after enclosing the system in order to prevent exposure.

1.6 Exogenous sources of ethylene glycol in humans

There exists always some unavoidable background load by EG in the body. Besides endogenous and occupational exposure, there is also exposure via food and consumer products.

EG can be present in food due to its approved use as an indirect food additive. Food that have been disinfected and preserved with ethylene oxide may contain residual EG. EG is also found in drinks packaged in PET bottles (contain trace amounts of unreacted ethylene glycol) and in various cakes, and sweets (<10-34 ppm) wrapped in regenerated cellulose film, which may contain polyethylene glycol as a softening agent (NTP, 2004). Additionally, ubiquitous environmental ethylene as well as ethylene and ethylene oxide in cigarette smoke and at workplaces can be considered as indirect EG sources because ethylene is biotransformed to ethylene oxide, which is hydrolysed to EG by epoxide hydrolase (Törnqvist *et al.*, 1986; Filser *et al.*, 1992).

Because EG is widely used in paints, in antifreeze solutions for motor vehicles and for de-icing of aeroplanes, not only workers but also the general population are

exposed. For instance, Percy (1992) reported EG concentrations of 3.2 mg/m^3 and 4.1 mg/m^3 in the atmospheric air of an airport.

Concerning EG exposed workers, it has been estimated for the USA that 1.5 million workers had been possibly exposed to EG in the time frame between 1981 and 1983 (NIOSH, 1990). Unfortunately, there are not many data published on EG concentrations at workplaces. In one study, total airborne EG concentrations during bridge de-icing operations, were up to 10.57 mg/m^3 (Abdelghani *et al*., 1990; cited in IPCS, 2002). In another study, EG was measured over a winter period of 2 months in breathing zone air samples and in urine samples of 33 workers exposed at an airport to an EG-containing de-icing fluid. Vaporous EG did not exceed 22 mg/m^3. EG mist was quantified at higher levels (76-190 mg/m^3) in 3 air samples. In 16 of 117 urine samples, EG exceeded 5 mmol/mol creatinine amounting up to 129 mmol/mol creatinine. No EG related kidney damage was found. From the high urine concentrations in workers, some of whom had been accidentally sprayed with the de-icing fluid, the authors concluded "other routes of absorption than inhalation, such as the percutaneous route, may be important" (Gérin *et al*., 1997).

Together with some other urinary parameters, Laitinen *et al*. (1995) determined EG in inhaled air and in urine of 10 car mechanics who were frequently exposed to EG-containing cooling liquid. Ten office workers served as controls. Although no EG could be detected in the inhaled air (detection limit 1.9 ppm=4.9 mg/m^3), urinary excretion of EG by the mechanics (7.3±4.7 mmol/mol creatinine; mean±SD) was significantly ($p<0.05$) higher than by the controls (1.7±0.7 mmol/mol creatinine). The authors concluded an EG uptake by skin contact to be probable. Additionally, glycosaminoglycans in urine were significantly lower in the mechanics (3.1±1.0 g/mol creatinine) than in the office workers (4.7±1.9 g/mol creatinine). The excretion of OA by the mechanics (47±11 mmol/mol creatinine) differed slightly from that by the controls (36±14 mmol/mol creatinine). These findings were interpreted as hint for possible kidney problems in EG exposed workers.

1.7 Transdermal uptake of ethylene glycol in animals and humans

Potential dermal contact to liquid EG exists during many of its industrial and consumer uses that could result in transdermal uptake. Because of the low vapour

pressure of EG, this path might represent a higher health risk than the inhalation of vaporous EG. Several authors essayed to quantify the dermal penetration of liquid EG in mouse and human skin *in-vitro*. Also, transdermal EG uptake was studied in laboratory rodents.

Frantz *et al*. (1996a, 1996b, 1996c) comparing $^{14}C_2$-EG uptake and elimination in female mice and female and male rats treated i.v., p.o., and epicutaneously (6 h) with $^{14}C_2$-EG concluded that transdermal EG uptake was slow and rather poor in rats. Mice showed a significant higher EG uptake. In both species, radioactively labelled EG was administered occlusively onto 1 cm^2 surface of shaved skin. In mice, 77 % and 84 % of the administered dose was percutaneously absorbed following the application of 100 mg/kg bw and of 1000 mg/kg bw, respectively (Frantz *et al*., 1996b). In rats, 23-36 % of the radioactivity was absorbed following both the application of 10 mg/kg bw and 1000 mg/kg bw (independently of the dose). After applying 1000 mg/kg bw $^{14}C_2$-EG as 50 % aqueous solution to skin of both species, the authors detected 60 % (mice; Frantz *et al*., 1996b) and 26 % as well as 22 % (rats; Frantz *et al*., 1996b; 1996c) to be bioavailable.

After thawing stored frozen human skin, Lodén (1986) applied liquid ^{14}C-labelled EG onto it and determined after a lag-phase of about 0.5 h the steady-state transdermal flux in a flow-through diffusion cell to be 118 $\mu g/cm^2 \cdot h$ of EG at 30 °C.

Driver *et al*. (1993) applied ^{14}C-labelled EG in acetone as vehicle onto dermatomed, 500 µm thick thigh skin of 3 male human cadavers that had been stored up to one week in Eagle Minimum Essential Medium at 4 °C. While incubating the skin at 32 °C for 24 h in flow-through diffusion cells in contact with the receptor medium phosphate buffer (flow: 3 ml/h), radioactivity was determined in the receptor fluid. An average flux of approximately 0.09 $\mu g/cm^2 \cdot h$ was calculated for ^{14}C-labelled EG. Most of the applied EG evaporated under these conditions. Therefore, this study cannot be used for estimating quantitatively the transdermal uptake of liquid EG. In a third study, fresh, full-thickness skin from the dorsal trunks of female CD-1 mice and from the abdomen of 6 female human donors was used (Sun *et al*., 1995). After applying ^{14}C-labelled EG (97 % purity) undiluted or as a 50 % aqueous solution to the skin at target doses high enough to represent "infinite doses", ^{14}C penetration was measured for 6 h in the receptor medium of the flow-through diffusion cells. At

steady-state conditions, fluxes were 0.52 mg/cm^2·h (undiluted) and 0.22 mg/cm^2·h (50 % dilution) in mouse skin and were 0.013 mg/cm^2·h (undiluted) and 0.007 mg/cm^2·h (50 % dilution) in human skin. Because the fluxes were proportional to the concentrations, calculation of permeability constants (K_p values) was meaningful. The values were in mouse skin $47 \cdot 10^{-5}$ cm/h (undiluted) and $44 \cdot 10^{-5}$ cm/h (50 % dilution) and in human skin $1.2 \cdot 10^{-5}$ cm/h (undiluted) and $1.4 \cdot 10^{-5}$ cm/h (50 % dilution).

In summary, transdermal transport of liquid EG was demonstrated in animals *in-vivo* and *in-vitro* and in human skin *in-vitro*. Although this uptake route might reflect a health risk for people handling EG, *in-vivo* data in humans is missing.

1.8 Problems concerning a health based threshold value ("MAK-value") for ethylene glycol

The German Commission for the Investigation of Health and Hazards of Chemical Compounds in the Work Area (MAK Commission) established a "MAK-value" for a daily eight-hour exposure (40 h per week) to EG of 26 mg/m^3 (10 ppm of vaporous EG).

"The MAK-value is defined as the maximum concentration of a chemical substance (as gas, vapour or particulate matter) in the workplace air, which generally does not have known adverse effects on the health of the employee nor cause unreasonable annoyance (e.g. by a nauseous odour) even when the person is repeatedly exposed during long periods, usually for 8 hours daily but assuming on average a 40 hours working week. As a rule, the MAK-value is given as an average concentration for a period of up to one working day or shift" (DFG, 2001).

The "MAK-value" for EG is founded only on the irritating effects observed in a volunteers study with 19 male volunteers exposed continuously over 30 days to EG (Wills *et al*., 1974). It was assumed by the MAK Commission the systemic adverse effects observed in highly dosed animals to become relevant only at doses far above those leading to mucosa irritation in humans. However, no data on the systemic burdens by EG, GA, and OA in EG exposed humans were available to verify this

assumption. In 2003, the first study was published describing the blood burden by EG and GA and the urinary excretion of EG, GA, and OA in two male volunteers exposed by the inhalation route to vaporous EG (Carstens *et al*., 2003). Concerning the uptake of vaporous EG, results of the study supported the assumption of the MAK commission that the systemic burdens by EG, GA, and OA were low in humans compared to those in animals when EG was systemically toxic. However, the relevance of skin uptake of liquid EG remains to be investigated.

1.9 Aim of the work

No data are reported on skin uptake of humans exposed to liquid EG. Concerning exposure to vaporous EG, quantitative data in only two volunteers are published. Therefore, it was the aim of the present work to quantify in volunteers the burdens by EG, and its two metabolites GA and OA resulting from inhaled and from dermally applied EG. In order to establish a risk evaluation, these burdens were compared with those representing the unavoidable background. For differentiating background from exposure-derived substances, exogenous EG was given as the ^{13}C-labelled isotope. After inhalation of small amounts of vaporous $^{13}C_2$-EG by volunteers and after application of liquid $^{13}C_2$-EG onto forearms of volunteers, respectively, labelled EG, and unlabelled and labelled GA were measured from plasma and unlabelled and labelled EG, GA, and OA from urine. For the determination of the unlabelled and labelled compounds, mass selective and sensitive procedures were used (Carstens, 2003).

2 Materials and Methods

2.1 Materials

2.1.1 Chemicals

Acetone, PESTANAL®, ≥99.8 %	Riedel-de Haën, Seelze, Germany
Acetonitrile, PESTANAL®, ≥99.8 %	Riedel-de Haën, Seelze, Germany
n-Butylboronic acid, 97 %	Aldrich, Steinheim, Germany
N-tert-Butyldimethylsilyl-*N*-methyltrifluoroacetamide, 97 % with 1 % *tert.*-butyl-dimethylchlorosilane	Aldrich, Steinheim, Germany
1,4-Diazabicyclo[2.2.2]octane, 98 %	Aldrich, Steinheim, Germany
N,N-Dimethylformamide, SUPRASOLV®	Merck, Darmstadt, Germany
Ethyl acetate, PESTANAL®, ≥99.8 %	Riedel-de Haën, Seelze, Germany
Ethylene glycol, puriss., ≥99.5 %	Fluka, Buchs, Switzerland
[$^{13}C_2$]Ethylene glycol, 99 atom percent ^{13}C	Aldrich, Steinheim, Germany
Glycolic acid, puriss., ~99 %	Fluka, Buchs, Switzerland
n-Hexane, p.a.	Riedel-de Haën, Seelze, Germany
Hydrochloric acid, p.a., 32 %	Merck, Darmstadt, Germany
(+/-) 2-Hydroxyisovaleric acid, puriss., ≥99 %	Fluka, Buchs, Switzerland
Liquemin® N 25000 (Heparin Sodium)	Hoffmann-La Roche,Grenzach-Wyhlen, Germany
Methanol, LiChrosolv®, ≥99.8 %	Merck, Darmstadt, Germany
Sodium chloride, p.a.	Merck, Darmstadt, Germany
Oxalic acid dihydrate, ≥99 %	Sigma, Steinheim, Germany
[$^{13}C_2$]Oxalic acid dihydrate, 99 atom percent ^{13}C	Aldrich, Steinheim, Germany
1,2-Propanediol, puriss., ACS reagent, ≥99.5 %	Fluka, Buchs, Switzerland

1,3-Propanediol, 98 %	Aldrich, Steinheim, Germany
Succinic acid, puriss., ≥99.5 %	Fluka, Buchs, Switzerland
[$^2H_6$]Succinic acid, 98 atom percent ^2H, ≥99.5 %	Aldrich, Steinheim, Germany
Toluene, for residue analysis, 99.8 %	Fluka, Buchs, Switzerland
Helium 5.0	Linde, Munich, Germany

2.1.2 Gas chromatography equipment

Gas chromatograph HP 5890 Series II	Agilent Technologies, Waldbronn, Germany
"Cool-on-column-injector" HP 5890	Agilent Technologies, Waldbronn, Germany
Autosampler HP 7673	Agilent Technologies, Waldbronn, Germany
Mass selective detector HP 5972	Agilent Technologies, Waldbronn, Germany
Precolumn, "fused silica", deactivated, 0.53 mm ID, 2.5 m length	Agilent Technologies, Waldbronn, Germany
Capillary column ZEBRON ZB-5, 0.5 mm ID, 30 m length, 1 µm film thickness	Phenomenex, Aschaffenburg, Germany
Column connector "press fit", glass	Agilent Technologies, Waldbronn, Germany
Autosampler syringe, 10 µl, needle gauge 23-26, part number: 002821	SGE, Darmstadt, Germany
Software: ChemStation G1701 BA Version B02.00	Agilent Technologies, Waldbronn, Germany
Mass spectral libraries: NIST98 and Wiley275	Agilent Technologies, Waldbronn, Germany
Thermostat DC1 equipped with cooler K20	Haake, Karlsruhe, Germany

2.1.3 Inhalation exposure systems

Flask (250 ml), round bottom, three necks with standard grounds, center joint 29, side joints 14	Schott, Mainz, Germany
Heating mantle for round bottom flask (0-120 W)	Neo Lab, Munich, Germany
Glass syringe, 10 µl, series 800 NE	Hamilton, Darmstadt, Germany

Silicon tube (diameter 10 mm)	K & K Laborbedarf, Munich, Germany
Pinch clamp	Neo Lab, Munich, Germany
Polyethylene-coated aluminium gas bag 2.5 l	Linde, Unterschleissheim, Germany
Aluminium foil	Igefa, Berlin, Germany

2.1.4 Dermal exposure systems

Sterilized gauze Amicus®, 6 cm·4 m	Michallik, Mühlacker, Germany
Silicon-free grease (Glisseal HV)	Borer Chemie, Zuchwil, Switzerland
Surgical tape Transpore™, 1.25 cm·9.1 m	3M Deutschland, Neuss, Germany
Bandage Leukosilk, 6 cm·4 m	BSN Medical, Hamburg, Germany
Polyethylene sheet, 30 cm·10 m	Melitta Haushaltsprodukte, Minden, Germany
Glass syringe, 1 ml, series 1000RN	Hamilton, Bonaduz, Switzerland

2.1.5 Laboratory ware for urine collection

Wide-mouth bottle, polyethylene, 1000 ml	Roth, Karlsruhe, Germany
Colour fixed indicator sticks, pH-Fix (pH 4.5-10.0)	Macherey-Nagel, Düren, Germany
Round bottom Falcon™ tube, polypropylene, 14 ml	BD Biosciences, Heidelberg, Germany
Conical bottom Falcon™ tube, polypropylene, 15 ml	BD Biosciences, Heidelberg, Germany

2.1.6 Medical ware for blood collection

Disposable hypodermic needles, Sterican® (0.55 mm·25 mm and 0.80 mm·40 mm)	B. Braun, Melsungen, Germany
Single-use syringes “Injekt” (10 and 20 ml)	B. Braun, Melsungen, Germany
Non ported i.v. cannula with fixation wings, Introcan®-W, (18G, 1.3 mm·32 mm)	B. Braun, Melsungen, Germany

Stylet for long-term closure of i.v. cannula, Introcan®-W, (18G, 1.3 mm·32 mm)	B. Braun, Melsungen, Germany
Alcohol pads for skin cleansing	B. Braun, Melsungen, Germany
Soft, hypoallergenic wound plaster, Hansamed® (6 cm·5 m)	BSN Medica, Hamburg, Germany

2.1.7 Personal computers

Hewlett Packard, Vectra 386	Agilent Technologies, Waldbronn, Germany
Apple Macintosh, iMac	Apple, Cupertino, USA

2.1.8 Printers

HP LaserJet 4L	Agilent Technologies, Waldbronn, Germany
Xerox Phaser 3450	Xerox, Neuss, Germany

2.1.9 Software

Adobe Illustrator® 10.0	Adobe Systems, San Jose, USA
CS ChemOffice 2001	CambridgeSoft®, Cambridge, USA
EndNote 6.0	Thomson ResearchSoft, Carlsbad, USA
Microsoft Office 2004	Microsoft, Redmond, USA
Sigma Plot 4.17	Jandel, Erkrath, Germany

2.1.10 Laboratory ware

Autosampler vial, 2 ml	Wicom, Heppenheim, Germany
Aluminium crimp cap with teflon/silicon septum for 11 mm autosampler vials	Wicom, Heppenheim, Germany
Blood gas analyser, Stat Profile® pHOx®	Nova Biomedical, Rödermark, Germany
Glass beaker of different volumes	Schott Duran, Mainz, Germany

Laboratory glass bottles with screw caps, Duran®, 100 ml and 500 ml	Schott Duran, Mainz, Germany
Eppendorf-cups, Safe-Lock, polypropylene, 1.5 and 2 ml	Eppendorf, Hamburg, Germany
Multi twist top vials, polypropylene, 2 ml	Sorenson™ Bioscience, Salt Lake City, USA
Volumetric flasks of different volumes with one ring mark and polyethylene stopper, DURAN®	Schott Duran, Mainz, Germany
Glass microinserts for autosampler vials (30.75 mm·6 mm·0.7 mm)	K&K Labordedarf, Munich, Germany
Pasteur pipettes, soda lime glass, disposable, with long and fine tip, 10 and 20 mm	Hirschmann, Eberstadt, Germany
Volumetric pipette with one mark of different volumes	Brand, Steinheim, Germany
Adjustable pipettes of different volumes, research series 2100	Eppendorf, Hamburg, Germany
Pipette "epTIPS", different volumes	Eppendorf, Hamburg, Germany
Ultrasonicator, Sonorex TK 30 (240 W)	Bandelin Electronics, Berlin, Germany
Analytical balance AB204-S (0-220 g)	Mettler-Toledo, Giessen, Germany

2.1.11 Centrifuges

Microcentrifuge, Sigma 1-15	Sigma, Osterode, Germany
Cooling centrifuge, Sigma 4K15C	Sigma, Osterode, Germany
Cooling centrifuge EBA 12R	Hettich, Tuttlingen, Germany
Vaccum concentrator centrifuge, Univapo 100H (390 W) with cold trap Unicryo MC2 L–60 °C	Uniequip, Martinsried, Germany

2.2 Volunteers

Four healthy, non-smoking adult male volunteers participated on the study. Two of them (A and B) had already taken an active part in the study of Carstens *et al.* (2003) by inhaling vaporous EG. These experiments were further extended in the present

study by additionally exposing volunteers C and D to EG by inhalation. All four subjects participated at the present dermal exposure study (Table 2). The Ethics Council of the Technical University of Munich, Germany, had approved the study. All volunteers avoided food, rich in oxalate and ascorbate, from 2 days before start of the experiments until 30 h thereafter.

Volunteer	Age [year]	Body weight [kg]
A	56	96
B	44	57
C	33	76
D	31	70
Mean±SD (n=4)	41±12	74.8±16

Table 2: Age and body weight of volunteers.

2.3 Analytical methods

2.3.1 Safety

General operating procedures of a chemical laboratory were followed. Special care was taken to comply with established safety standards (e.g. working under hood and using gloves).

2.3.2 Principle procedure

Volunteers were exposed by inhalation or via the dermal route to ^{13}C-labelled EG ($^{13}C_2$-EG) for up to 6 h. Plasma and urine samples were collected during and following the exposure experiments. In collected body fluids, labelled ($^{13}C_2$-EG) and unlabelled EG and its two main metabolites (GA and OA) were measured using mass selective and sensitive analytical methods (Carstens, 2003; Carstens *et al*., 2003).

2.3.3 Determination of ethylene glycol from plasma, urine and water

The vicinal diol EG was determined following derivatisation with n-butylboronic acid (Figure 2). The resulting cyclic ester was extracted by means of ethyl acetate and analysed using a gas chromatograph equipped with a mass selective detector (GC/MSD). Propylene glycol (PG) and 1,3-propandiol (1,3-PD) were used as internal standards.

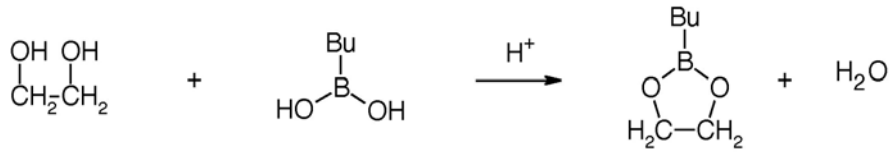

Figure 2: Reaction of ethylene glycol with n-butylboronic acid leading to a cyclic boronic ester.

2.3.3.1 Sample preparation

Plasma, urine and water samples were stored at –80 °C and were thawed at room temperature just prior to analysis. The internal standards were added, and the proteins were precipitated in the plasma samples. The protein-free samples were derivatised and the determination of EG by GC/MSD was carried out. Urine and water were analysed in triplicates, whereas determination in plasma samples were carried out once.

Plasma

To a plasma sample (100 µl) in a 1.5 ml Eppendorf-cup, 5 µl of an aqueous solution containing a mixture of PG and 1,3-PD (525 µmol/l each) was added. High molecular weight proteins were removed by addition of acetone (100 µl) followed by 20 sec vortexing and centrifugation (4 min, 14000 rpm, 17530·g; microcentrifuge Sigma 1-15) of the sample. The supernatant was then transferred to a 1.5 ml Eppendorf-cup and acidified with 10 µl of HCl (2 mol/l). To the acidified protein-free sample, 20 µl of acetonic n-butylboronic acid (400 mmol/l) was added. The sample was then

incubated at room temperature for 15 min under occasional shaking. To extract the boronic ester, 150 µl of ethyl acetate was added and the sample was then vortexed for 25 sec and centrifuged (2 min, 14000 rpm, 17530·g; microcentrifuge Sigma 1-15). The organic phase (upper layer) was transferred into an autosampler vial, which contained a micro insert. The vial was sealed with a crimp cap and placed on the cooled tray (15 °C) of the autosampler for analysis by GC/MSD.

Urine and water samples

Into an 1.5 ml Eppendorf-cup, 100 µl of water or an acidified urine sample (5 ml of concentrated HCl was added, see 2.4.3.2) and 5 µl of an aqueous solution of PG and 1,3-PD (1050 µmol/l each) were added. Water samples were additionally acidified with 10 µl of HCl (2 mol/l). Thereafter, 100 µl of acetonic n-butylboronic acid (80 mmol/l) was added, and the sample was incubated for 15 min at room temperature under occasional shaking. The extraction of the cyclic boronic ester was performed as described for the plasma samples.

2.3.3.2 Gas chromatographic analysis

EG, $^{13}C_2$-EG, PG and 1,3-PD were determined using a GC/MSD system. The samples were injected ino the "cool-on-column-injector" using the autosampler. The analytes were separated on a Zebron ZB-5 fused silica capillary column which was protected by a pre-column. The detailed configuration of the GC/MSD is given in Table 3. After elution from the column, the substances were analysed using the MSD in the electron impact ionisation (EI) mode. For the identification of the substances and to determine their retention times the scan-mode was used. To identify and quantify each compound, two ions (the molecular ion $M^{+\bullet}$ and a specific fragment; quantifier) were selected from the mass spectrum. Both ions were then determined in the experiments using the single ion monitoring (SIM) mode. The ions m/z=86 and m/z=128 ($M^{+\bullet}$) were selected for EG, and the ions m/z=88 and m/z=130 ($M^{+\bullet}$) for $^{13}C_2$-EG. For the internal standards, the ions m/z=113 (PG and 1,3-PD) and m/z=142 (PG and 1,3-PD; $M^{+\bullet}$) were used. The retention times were about 6.2 min (EG), 6.1 min ($^{13}C_2$-EG), 6.7 min (PG) and 8.9 min (1,3-PD). Figures 3a and 3b depict the

chromatograms of $^{13}C_2$-EG and the internal standards (PG and 1,3-PD) isolated from a plasma sample.

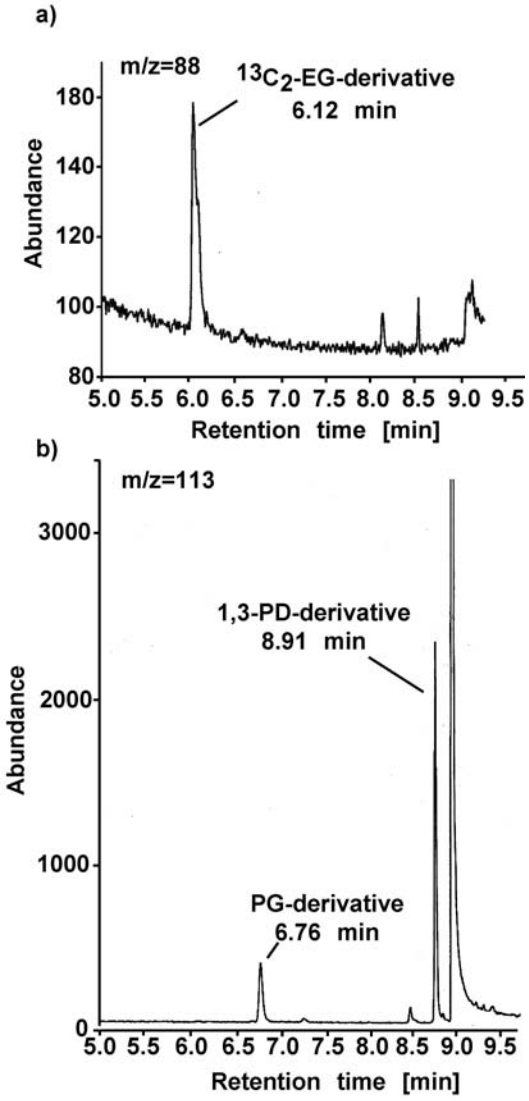

Figure 3: Single ion chromatograms for the determination of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) from a plasma sample (2 µmol/l) containing the internal standards (25 µmol/l) propylene glycol (PG) and 1,3-propandiol (1,3-PD) by monitoring the ion m/z=88 (a) and the ion m/z=113 (b).

The integration of the obtained signals was carried out using the manual integration-function of the ChemStation software. Prior to each measurement, the conditions and the parameters of the MSD were optimised with the calibration functions “autotune” and “usertune”.

Injector	"Cool-on-column-injector"
-Temperature	Track-on-oven-mode (i.e. the injector temperature is always 3 °C above the oven temperature)
-Injected volume	1 µl
-Washing solvent	Methanol
GC	HP 5890 Series II
-Precolumn	2.5 m·0.53 mm, fused silica, deactivated
-Capillary column	ZB-5, 30 m·0.5 mm, film thickness 1 µm
-Column temperature program	Starting temperature: 65 °C for 2 min Heating rate 1: 2.5 °C/min up to 80 °C Heating rate 2: 40 °C/min up to 250 °C Final temperature: 250 °C for 5 min Total run time: 17.25 min
-Carrier gas	Helium
-Pressure of carrier gas	0.8 bar at 65 °C, "constant-flow-mode" (flow calculated by software: 1.3 ml/min)
-Transfer line temperature	300 °C
MSD	HP 5972
-Ionisation	Electron impact, ionisation potential 70 eV
-Masses collected in SIM mode	EG-derivative: m/z=86 and m/z=128 $^{13}C_2$-EG-derivative: m/z=88 and m/z=130 PG- and 1,3-PD-derivative: m/z=113 and m/z=142
-Masses used for quantification	EG-derivative: m/z=86 $^{13}C_2$-EG-derivative: m/z=88 PG- and 1,3-PD-derivative: m/z=113
-Tuning of the MSD	"Usertune" (ions m/z=69, m/z=100, and m/z=131)

Table 3: Parameters used for the GC/MSD determination of unlabelled ethylene glycol (EG) and ^{13}C-labelled EG (^{13}C-EG), propylene glycol (PG), and 1,3-propandiol (1,3-PD).

Using the mass spectrum of perfluorotributylamine, the instrument varies different parameters in order to optimise the calibration of the mass axis (m/z), the signal width

and the sensitivity of the detector. The ions used for the "usertune" (m/z=69, m/z=100 and m/z=131) were selected from fragments of the calibration substance in order to cover the range of the masses of the analytes. After each sample run, methanol was injected into the GC for cleaning the system, especially from residues of n-butylboronic acid.

2.3.3.3 Mass spectra

The mass spectra of the cyclic boronic esters of EG, PG and 1,3-PD together with their fragmentation pattern have been already shown and discussed (Carstens, 2003).

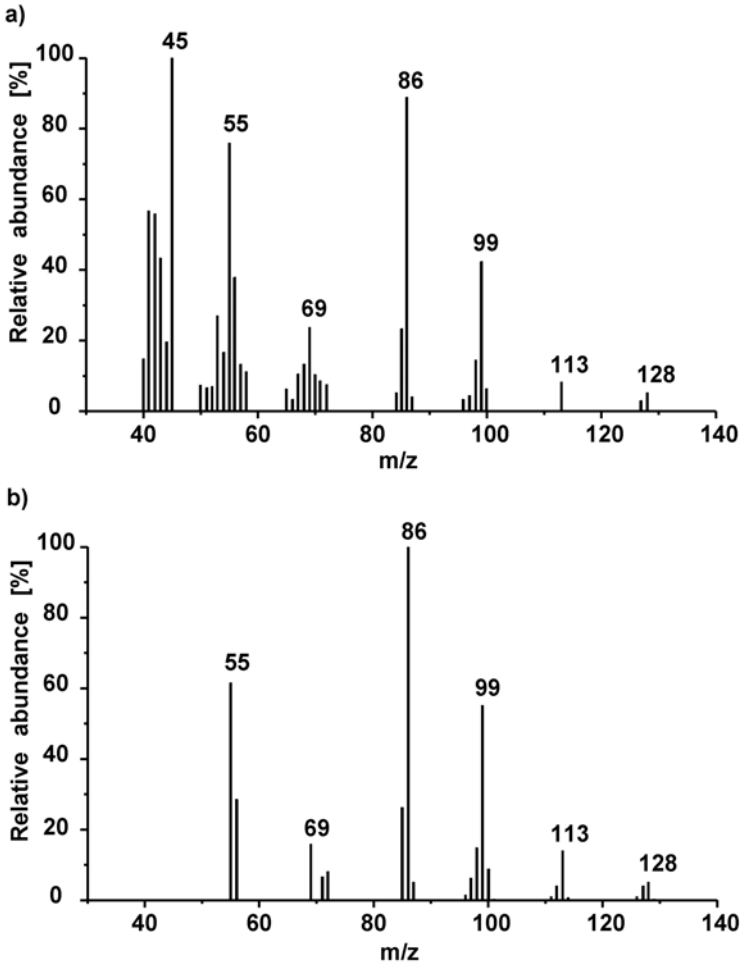

Figure 4: **a)** Mass spectrum of the n-butylboronic acid-ester of ethylene glycol.
b) Reference mass spectrum of the n-butylboronic acid-ester of ethylene glycol (Wiley275).

Here, only the mass spectrum of the boronic esters of EG is shortly presented. The largest m/z value observed in the mass spectrum (Figure 4a) corresponds to the molecular ion of the cyclic boronic ester ($M^{+\bullet}$, m/z=128, $C_6H_{13}BO_2^{+\bullet}$), from which further characteristic ions (Figure 5) are formed by the loss of $CH_3^{\bullet}$ (M-15), $C_2H_5^{\bullet}$ (M-29) and $C_3H_6^{\bullet}$ (M-42) leading to m/z=113 ($C_5H_{10}BO_2^{+}$), m/z=99 ($C_4H_8BO_2^{+}$) and m/z=86 ($C_3H_7BO_2^{+\bullet}$). The spectrum recorded in the scan-mode agrees fairly well with that found in the reference mass spectrum library (Figure 4b; Wiley275).

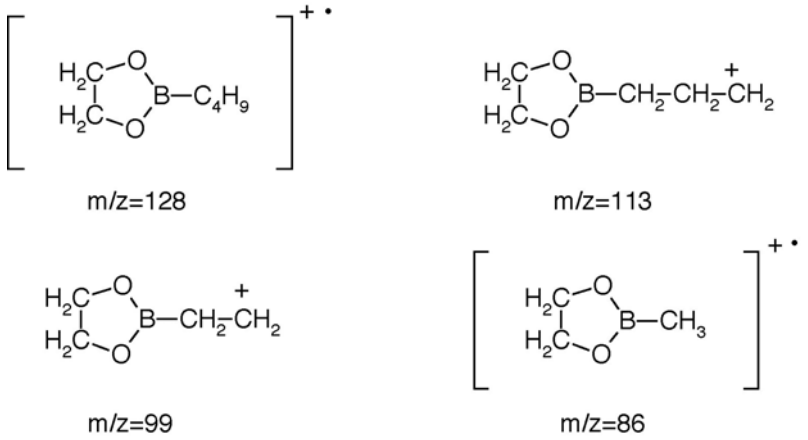

Figure 5: The molecular ion and characteristic ions of the n-butylboronic acid-ester of ethylene glycol.

2.3.3.4 Calibration and quantification of ethylene glycol

For quantification of EG, calibration samples were prepared and included into each series of measurements. Calibration samples were prepared using freshly collected matrices (urine and blood from unexposed volunteers and water). They contained either $^{13}C_2$-EG or unlabelled EG in concentrations covering the ranges of the corresponding concentrations expected to result from exposures. These ranges were 1-20 µmol/l $^{13}C_2$-EG for plasma, 1-250 µmol/l $^{13}C_2$-EG and 1-50 µmol/l EG for urine, and 1-1000 µmol/l $^{13}C_2$-EG for water. Three aliquots of each concentration were prepared and analysed in the same way as described for the exposure sample (see 2.3.3.1 and 2.3.3.2). To obtain a calibration curve, the ratio between the peak

area of the analyte ($^{13}C_2$-EG or EG) to the peak area of the internal standard (PG) was calculated and plotted versus the analyte concentration (Figures 6-9).

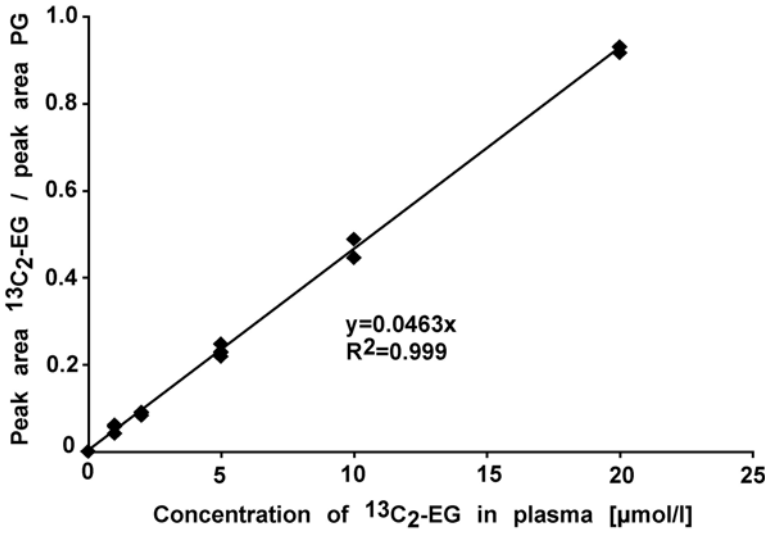

Figure 6: Calibration curve for the determination of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) from plasma; internal standard was propylene glycol (PG, 25 µmol/l).

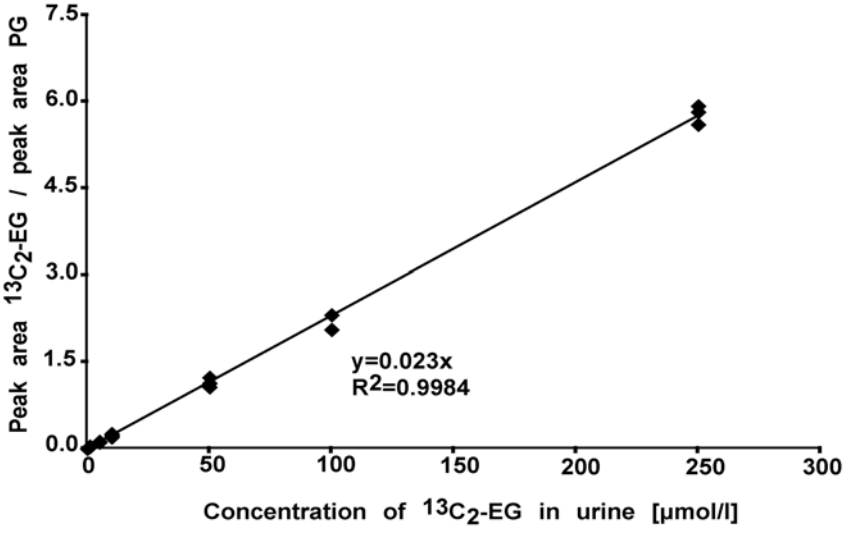

Figure 7: Calibration curve for the determination of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) from urine; internal standard was propylene glycol (PG, 50 µmol/l).

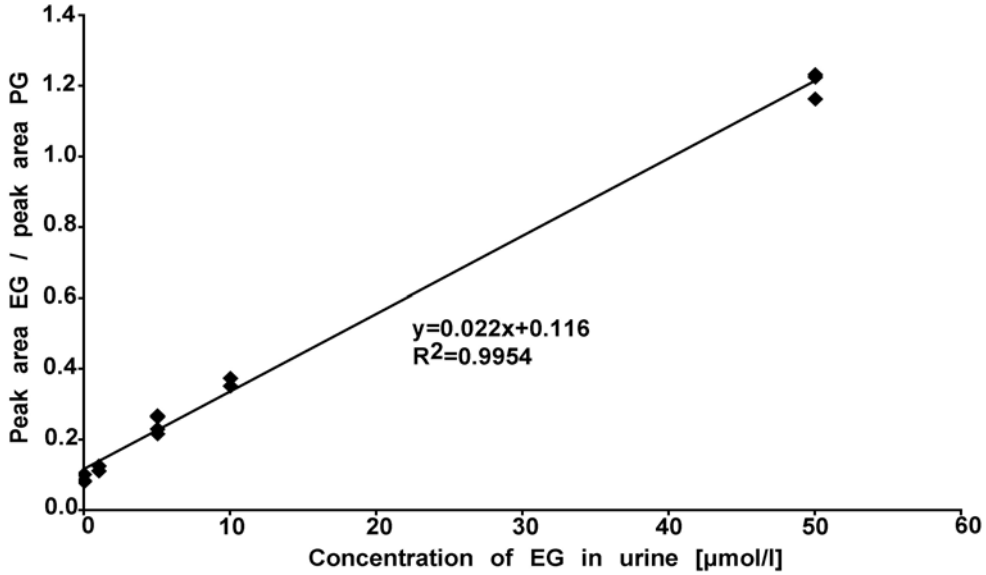

Figure 8: Calibration curve for the determination of ethylene glycol (EG) from urine; internal standard was propylene glycol (PG, 50 µmol/l).

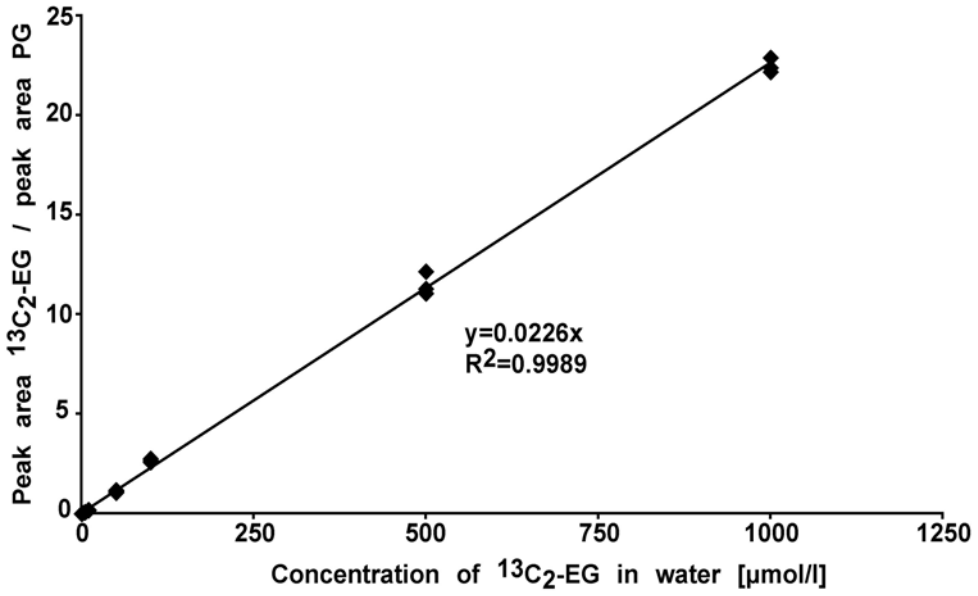

Figure 9: Calibration curve for the determination of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) from water; internal standard was propylene glycol (PG, 50 µmol/l).

The calibrations for EG and $^{13}C_2$-EG resulted in straight lines in all matrices over the investigated concentration range. Here, only the calibrations obtained for the internal standard PG are depicted. All samples analysed from one exposure experiment together with the calibration samples formed a series of measurements for GC/MSD analysis. First, a solvent blank was inserted into the autosampler followed by the calibration samples in duplicates where the concentrations were in an ascending order. Thereafter, a solvent blank preceded the exposure samples among which the residual calibration samples were distributed.

The slope of the calibration curve was calculated by linear regression analysis. If the matrix contained already a background of the analyte (e.g. EG in urine), the calibration curve did not pass through the origin. In order to determine the intercept accurately, triplicate blank samples were measured. The limits of quantification of EG were 7.6 and 1.1 µmol/l from plasma and urine, respectively. It was defined as the standard deviation of the background signal measured in samples to which no EG was added. The high quantification limit in plasma resulted from a highly variable background signal obtained in repeated measurements from the same sample. The determination of unlabelled EG from plasma was not improved further since it was not needed for the present study. For $^{13}C_2$-EG, no background signal was found. Therefore, the standard deviations of the lowest measured $^{13}C_2$-EG concentration in plasma, urine, and water, obtained in triplicates, were defined as the limits of quantification. They were 0.6 µmol/l in plasma, 0.1 µmol/l in urine and 0.3 µmol/l in water. Both peak areas and peak heights of EG and $^{13}C_2$-EG were quantified in each masurement. Generally, peak areas varied less than peak heights and therefore were chosen for the calculation of concentrations in both plasma and urine. However, following inhalation exposures of volunteers C and D to $^{13}C_2$-EG, peak heights varied less and were therefore used to calculate $^{13}C_2$-EG concentration in plasma.

2.3.3.5 Recovery, precision and stability

Recovery and precision of the method and the stability of the samples were determined previously by Carstens (2003). Recoveries of $^{13}C_2$-EG from plasma were 102±8 % and 94±8 % at 2 and 10 µmol/l, respectively. From urine, the recovery of $^{13}C_2$-EG was 94±14 % at 5 and 100 µmol/l. EG was assumed to have the same

recovery as $^{13}C_2$-EG. Precision of the method, defined by the coefficient of variation (CV), was on average 2.5 % for both plasma and urine samples. Analysis of $^{13}C_2$-EG in plasma and urine samples stored for 5-weeks at –80 °C revealed no statistical significant difference ($p \leq 5$ %) between the EG content of fresh and stored samples.

2.3.4 Determination of glycolic acid from plasma and urine

For the determination of labelled ($^{13}C_2$-GA) and unlabelled GA, a GC/MSD method was used. In order to carry out a gas chromatogarphic separation of the barley volatile GA, it was converted to a di-*tert*-butyldimethylsilyl (di-TBDMS)-derivative using a mixture called SILA, containing 99 % of *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) and 1 % of *tert*-butyldimethylchlorosilane (Figure 10). A tertiary amine, diazabicyclo[2.2.2]octane (DABCO) was used to catalyse this reaction. Since the derivatisation was carried out in acetonitrile (ACN), the water content of the plasma and urine samples was removed at first. Prior to the evaporation of water, GA was converted into its quinoline salt in order to prevent any loss. For quantification, [$^2H_6$]succinic acid ($^2H_6$-SA) and 2-hydroxyisovaleric acid (HIVA) were used as internal standards for plasma and urine, respectively.

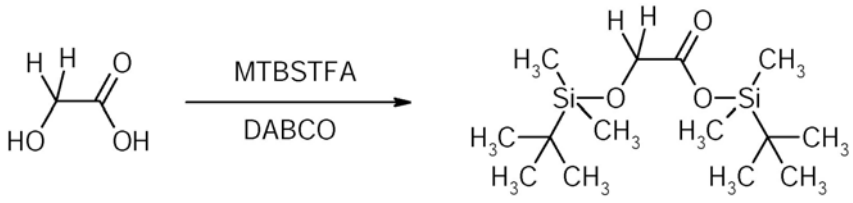

Figure 10: Reaction of glycolic acid with *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) catalysed by diazabicyclo[2.2.2]octane (DABCO), leading to its di-*tert*-butyldimethylsilyl-derivative.

2.3.4.1 Sample preparation

Plasma and urine samples were stored at –80 °C and were thawed at room temperature prior to sample analysis. The internal standards were added and the

proteins were precipitated in the plasma samples. The protein-free samples were derivatised and the determination of GA by GC/MSD was carried out. Urine was analysed in triplicates, whereas determination in plasma samples was carried out once.

Plasma

To a plasma sample (200 µl) in a 1.5 ml Eppendorf-cup, 5 µl of an aqueous solution of $^{2}H_{6}$-SA (206 µmol/l) was added. For precipitation of proteins, the sample was treated with ACN (500 µl), vortexed for 5 sec, ultrasonicated, vortexed again for 20 sec, and finally centrifuged (3 min, 14000 rpm, 17530·g; microcentrifuge Sigma 1-15). The supernatant was then transferred to a 1.5 ml Eppendorf-cup. The protein pellet was resuspended in 250 µl of ACN:water (4:1), ultrasonicated, vortexed for 20 sec, and again centrifuged (3 min, 14000 rpm, 17530·g; microcentrifuge Sigma 1-15). To the combained supernatants a solution of 40 µl of quinoline:ACN (1:9) was added. For solvent evaporation, the sample was placed in a vacuum concentrator centrifuge and the volume was reduced to about 5-10 µl. The residual water content in the sample was removed by adding 200 µl of toluene and followed by evaporation. This step was repeated two more times each with 100 µl of toluene. The solvent-free residue was then dissolved in an ACN solution containing 2.5 % of DABCO (50 µl). Then, 75 µl of MTBSTFA was added for derivatisation. The sample-containing Eppendorf-cup was closed, ultrasonicated for 15 sec, and placed over-night in a drying chamber at 40 °C for at least 12 h. After the derivatisation was completed, the sample was ultrasonicated again for 15 sec and the di-TBDMS-derivative was extracted by adding 25 µl of ACN and 85 µl of n-hexane, followed by 15 sec vortexing. Thereafter, the sample was centrifuged (30 sec, 14000 rpm, 17530·g; microcentrifuge Sigma 1-15) and then placed on ice for 10 min. The supernatant n-hexane phase was transferred into an autosampler vial, which contained a micro insert. The vial was sealed with a crimp cap and placed on the cooled tray (15 °C) of the autosampler for analysis by GC/MSD.

Urine

In an 1.5 ml Eppendorf-cup, 100 µl urine sample and 5 µl of an aqueous solution of

HIVA (10500 µmol/l) were mixed. To form the quinoline-salt, 50 µl of quinoline:ACN (1:9) was added. The further sample preparation steps are the same as described for the plasma samples with the exception of one point: After derivatisation, colourless crystal needles were found in the n-hexane phase of the cooled sample. Therefore, for separation of colourless crystal needles from the analyte, an additional centrifugation was necessary at 0 °C (3 min, 14000 rpm, 17530·g; centrifuge EBA 12R).

2.3.4.2 Gas chromatographic analysis

GA, $^{13}C_2$-GA, HIVA and $^{2}H_6$-SA were analysed by GC/MSD (for configuration, see Table 4). The substances eluted from the column were analysed using the MSD in the EI mode. For identification of the substances and for determining their retention times, the scan-mode was used. To identify and quantify a selected compound, two ions were selected from its mass spectrum and determined in experiments using the SIM mode. In contrast to the method developed for EG, the molecular ion could not be used due to its low abundance. To select the specific fragments, their specificity, abundance, and an eventual disturbing signal in the chromatogram were considered. The ions m/z=247 and m/z=289 were selected for GA, and m/z=249, and m/z=291 for $^{13}C_2$-GA. For HIVA as internal standard, the ions m/z=289 and m/z=331, and for $^{2}H_6$-SA the ions m/z=293 and m/z=335 were determined. The retention times were 12.9 min (GA-derivative), 14.6 min (HIVA-derivative), and 16.4 min ($^{2}H_6$-SA-derivative). Figure 11 shows the separation of di-TBDMS-derivative of $^{13}C_2$-GA and of the$^{2}H_6$-SA isolated from plasma.

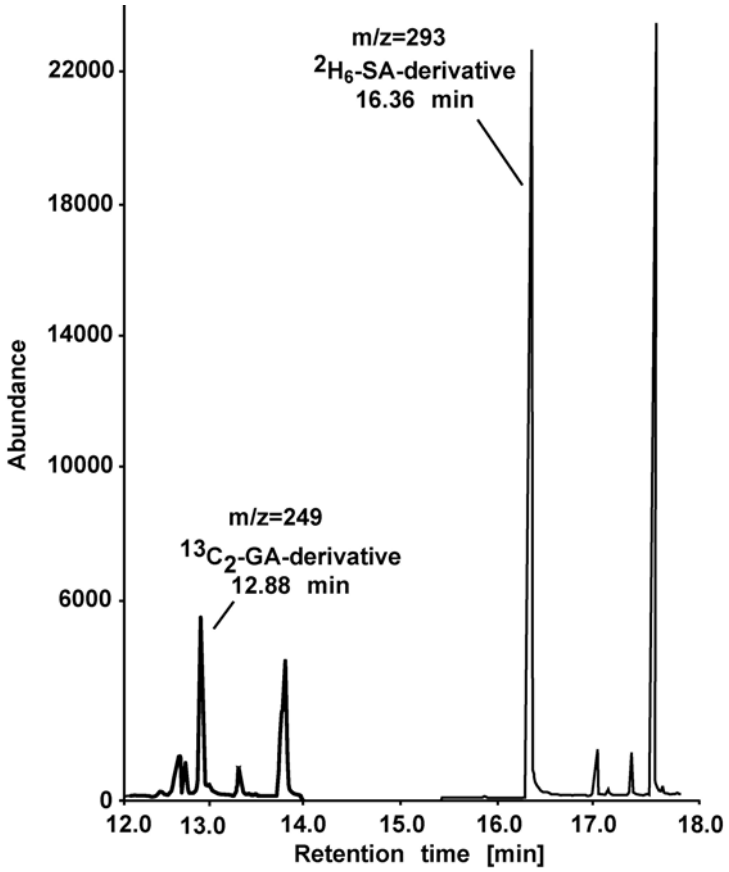

Figure 11: Single ion chromatogram for the determination of ^{13}C-labelled glycolic acid ($^{13}C_2$-GA) from plasma (2 µmol/l) containing the internal standard [$^{2}H_6$]succinic acid ($^{2}H_6$-SA, 5 µmol/l) by monitoring the ion m/z=249 and m/z=293.

The integration of the signals was carried out using the manual integration-function of the ChemStation software. The parameters of the method used for sample analysis are given in Table 4. Prior to each measurement, the conditions and the parameters of the MSD were optimised with the calibration functions “autotune” and “usertune”. The ions used for “usertune” (m/z=131, m/z=219 and m/z=414) were selected from the available fragments of the calibration substance perfluorotributylamine to cover the range of the masses used for quantification of the analytes.

After each sample run, ethyl acetate was injected into the GC in order to clean the column.

Injector	"Cool-on-column-injector"
-Temperature	Track-on-oven-mode (i.e. the injector temperature is always 3 °C above the oven temperature)
-Injected volume	1 µl
-Washing solvent	Ethyl acetate
GC	HP 5890 Series II
-Precolumn	2.5 m·0.53 mm, fused silica, deactivated
-Capillary column	ZB-5, 30 m·0.5 mm, film thickness 1 µm
-Column temperature program	Starting temperature: 75 °C for 1.5 min Heating rate 1: 7 °C/min up to 170 °C Heating rate 2: 30 °C/min up to 270 °C Final temperature: 270 °C/min for 2 min Total run time: 21.40 min
-Carrier gas	Helium
-Pressure of carrier gas	1.41 bar at 75 °C, "constant-flow-mode" (flow calculated by software: 2.17 ml/min)
-Transfer line temperature	300 °C
MSD	HP 5972
-Ionisation	Electron impact, ionisation potential 70 eV
-Masses collected in SIM mode	GA-derivative: m/z=247 and m/z=289 $^{13}C_2$-GA-derivative: m/z=249 and m/z=291 HIVA-derivative: m/z=289 and m/z=331 $^{2}H_6$-SA-derivative: m/z=293 and m/z=335
-Masses used for quantification	GA-derivative: m/z=247 $^{13}C_2$-GA-derivative: m/z=249 HIVA-derivative: m/z=289 $^{2}H_6$-SA-derivative: m/z=293
-Tuning of the MSD	"Usertune" (ions m/z=131, m/z=219, and m/z=414)

Table 4: Parameters used for the GC/MSD determination of unlabelled glycolic acid (GA) and ^{13}C-labelled GA ($^{13}C_2$-GA), 2-hydroxyisovaleric acid (HIVA), and [$^{2}H_6$]succinic acid ($^{2}H_6$-SA).

2.3.4.3 Mass spectra

The mass spectra of the di-TBDMS-derivatives of GA, HIVA, and $^{2}H_{6}$-SA together with their fragmentation patterns were already given by Carstens (2003).

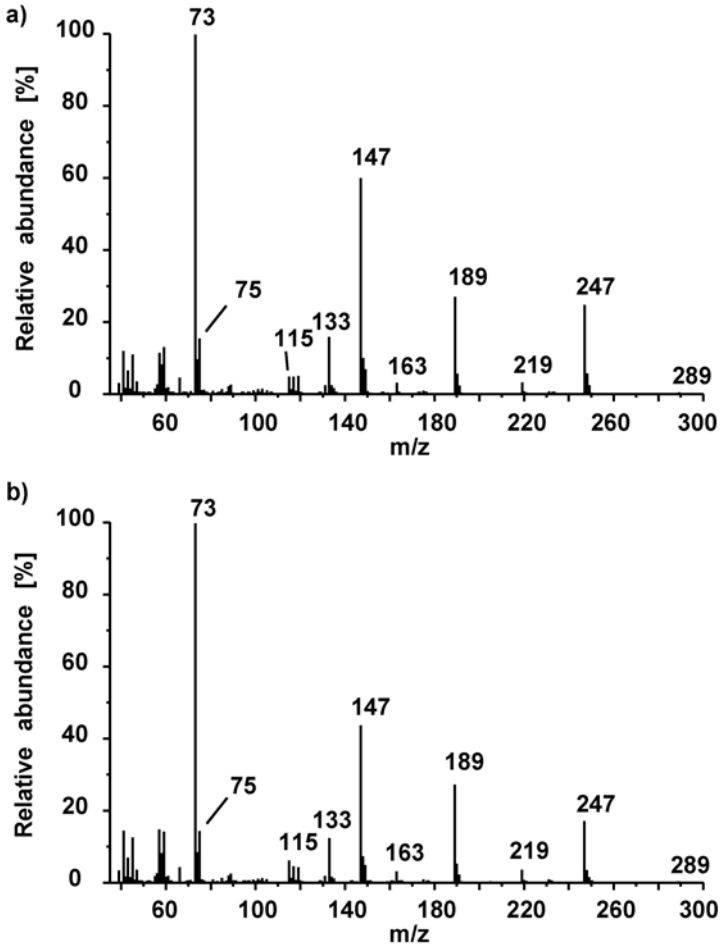

Figure 12: **a)** Mass spectrum of the di-*tert*-butyldimethylsilyl-derivative of glycolic acid.

b) Reference mass spectrum of the di-*tert*-butyldimethylsilyl-derivative of glycolic acid (Wiley275).

Here, only the mass spectrum (Figure 12a) of the di-TBDMS-derivative of GA is presented. Due to the excessive fragmentation, the molecular ion ($M^{+\bullet}$, m/z=304, $C_{14}H_{32}O_3Si_2^{+\bullet}$) is not present in the mass spectrum. Most probably, α-cleavage of a $CH_3^{\bullet}$ and a $C_4H_9^{\bullet}$ group led to the characteristic ions m/z=289 for $C_{13}H_{29}O_3Si_2^{+}$ and

m/z=247 for $C_{10}H_{23}O_3Si_2^+$, respectively (Figure 13). The ions m/z=189, m/z=147, m/z=115, m/z=75 and m/z=73 (Figures 12a and b) are formed upon rearrangements from the TBDMS-group and are not specific for GA (Little, 1999). The spectrum of di-TBDMS-derivative of GA recorded in the scan-mode is consistent with that in the reference mass spectrum library (Figure 12b; NIST98).

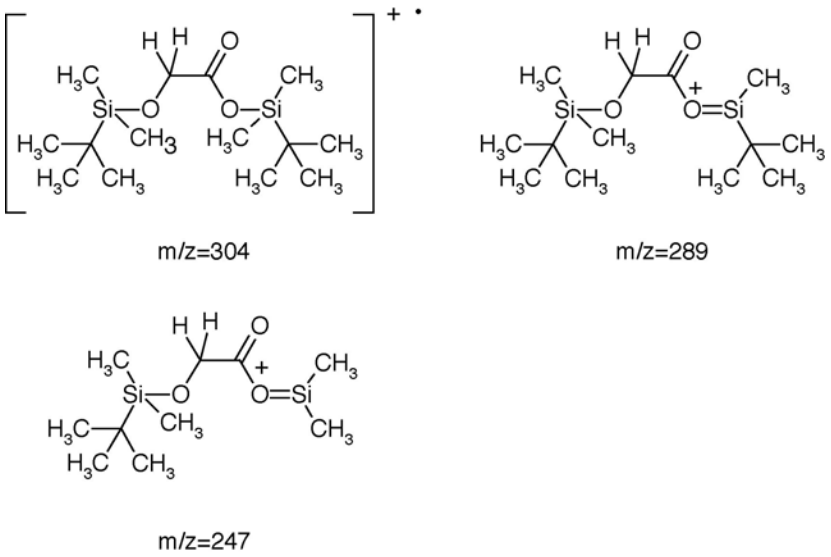

Figure 13: The molecular ion and characteristic ions of the di-*tert*-butyldimethylsilyl-derivative of glycolic acid.

2.3.4.4 Calibration and quantification of glycolic acid

For quantification of GA, calibration samples were included into each series of measurements. Calibration samples were prepared using freshly collected matrices (urine and blood from unexposed volunteers). The calibration curve of unlabelled GA was used for the quantification of both GA and $^{13}C_2$-GA, since no $^{13}C_2$-GA was available commercially. In the calibration samples, the concentrations of GA were 0.5-20 µmol/l and 10-500 µmol/l in plasma and urine, respectively. Three aliquots of each concentration were prepared and analysed in the same way as described for the exposure sample (see 2.3.4.1 and 2.3.4.2). All samples from one exposure experiment together with the calibration samples represented one series of measurements for GC/MSD analysis. The sequence was assorted as described for EG (see 2.3.3.4). For

the quantification of $^{13}C_2$-GA, it was considered that the specific ion m/z=249 was also present in the spectrum of the di-TBDMS-derivative of unlabelled GA as a result of the natural isotope distributions, especially of ^{13}C (1.1 %), ^{29}Si (4.7 %) and ^{30}Si (3.1 %). The intensity of this ion (m/z=249) is about 9.4 % of the intensity of the ion m/z=247 originating from the di-TBDMS-derivative of unlabelled GA (Software ISOFORM, part of the ChemStation package; see also Figure 12). Before performing a $^{13}C_2$-GA quantification, the intensity of the GA derived ion m/z=249 had to be determined. For this purpose the signal ratio ($A_{control}$) of m/z=249 to m/z=247 was determined (equation 1) in control samples (plasma and urine) free of $^{13}C_2$-GA, containing only endogenous (unlabelled) GA:

equation 1: $$A_{control} = \frac{\text{peak area}_{m/z=249}}{\text{peak area}_{m/z=247}}$$

This ratio was considered constant as far as the sample did not contain $^{13}C_2$-GA. Higher values than this ratio indicated the presence of $^{13}C_2$-GA in the sample. A statistical criterion based on the standard deviation of $A_{control}$ was used to judge whether $^{13}C_2$-GA was present in the exposure sample. To calculate the exposure related peak area of the signal m/z=249, the actual $\text{peak area}_{m/z=249}$ measured in the exposure sample was corrected for the portion of the $^{12}C_2$-GA using the $\text{peak area}_{m/z=247}$ (equation 2) measured in the exposure sample and the value of $A_{control}$:

equation 2: $$\text{peak area}_{^{13}C_2\text{–GA}} = \text{peak area}_{m/z=249} - (A_{control} \cdot \text{peak area}_{m/z=247})$$

In order to obtain the concentration of $^{13}C_2$-GA in the sample, the peak area of $^{13}C_2$-GA obtained by means of equation 2 was divided by the peak area of the corresponding internal standard. The ratio was then divided by the slope of the corresponding calibration cuvre of GA, assuming the slope of a theoretical calibration curve for $^{13}C_2$-GA to be identical with that of GA. As $^{13}C_2$-GA was not commercially available, no calibration curve could be constructed with this compound. To obtain a calibration curve for GA, the ratio between the peak area of the analyte (GA) to the peak area of the internal standard ($^2H_6$-SA or HIVA) was calculated and plotted versus the analyte concentration (Figures 14 and 15). In both plasma and urine, the

calibrations for GA resulted in straight lines over the investigated concentration ranges.

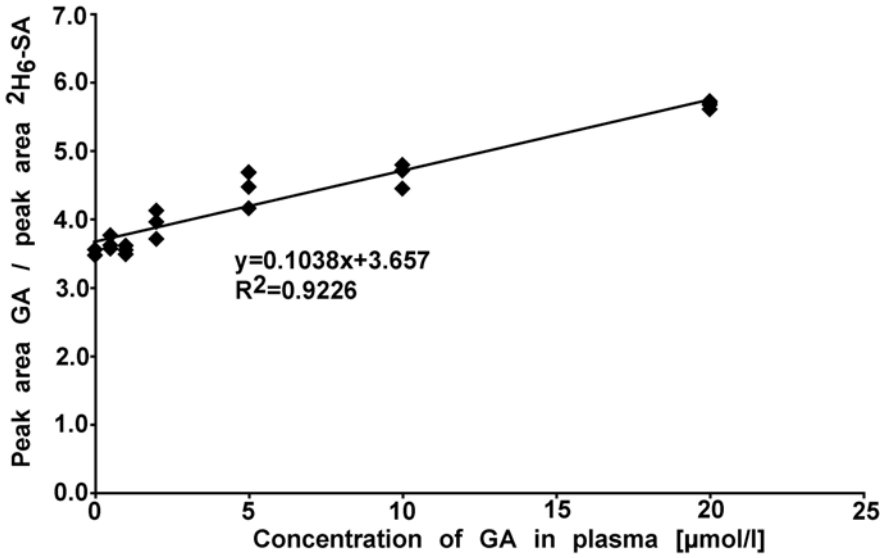

Figure 14: Calibration curve for the determination of glycolic acid (GA) from plasma; internal standard was [$^2H_6$] succinic acid ($^2H_6$-SA, 5 µmol/l).

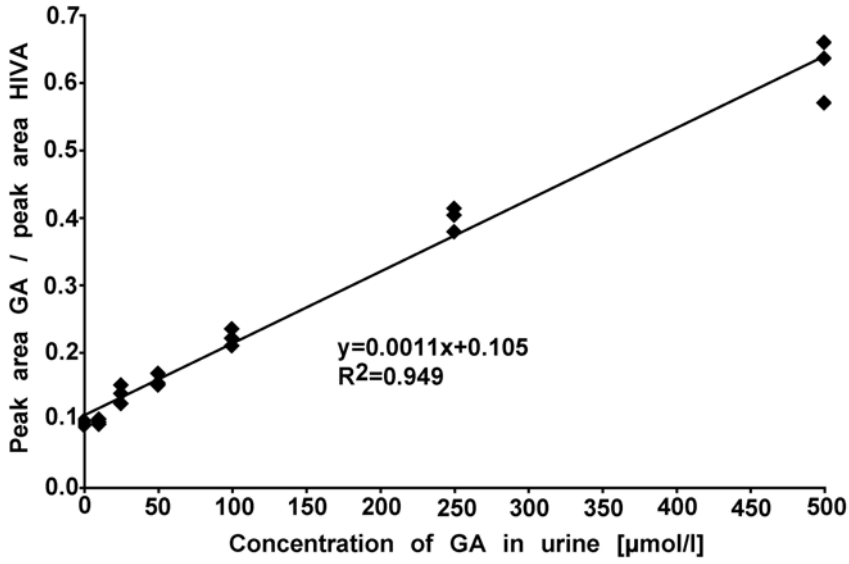

Figure 15: Calibration curve for the determination of glycolic acid (GA) from urine; internal standard was 2-hydroxyisovaleric acid (HIVA, 500 µmol/l).

The slopes were calculated by linear regression analysis. The calibration curves in Figures 14 and 15 did not pass through the origins, because the matrices contained backgrounds of the analyte (GA is taken up with food and is also formed in the intermediary metabolism). In order to determine the intercept accurately, blank samples were measured as triplicates. The contribution of the reagents to the background value (intercept) was negligible as was determined separately by the measurement of a matrix-free sample.

The limits of quantification of GA were 1.2 µmol/l from plasma and 33 µmol/l from urine. For $^{13}C_2$-GA, the values were 0.8 µmol/l and 2.9 µmol/l from plasma and urine, respectively. To calculate the limits of quantification, the standard deviations of the background signals, measured in triplicates, were divided by the slopes of the corresponding calibration curves.

2.3.4.5 Recovery, precision and stability

Recovery and precision of the method and the stability of the samples were already determined by Carstens (2003). The recovery of GA from plasma was between 83-120 % at 25 µmol/l. In urine, at a concentration of 250 µmol/l GA, the recovery was 51±4 %. Precision of the method, as defined by the CV, was on average 4 % for both plasma and urine samples. Analysis of GA in plasma and urine samples stored for 5-weeks at –80 °C revealed no statistical significant difference ($p \leq 5$ %) between the GA content of fresh and stored samples.

2.3.5 Determination of oxalic acid from urine

In plasma, OA could not be quantified because its recovery was not reproducible. Carstens *et al*. (2003) had made this observation, too. In urine, OA was determined by GC/MSD. For this purpose, the non-volatile OA was converted to its volatile di-TBDMS-ester (Figure 16). For quantification, succinic acid (SA) and $^2H_6$-SA were used as internal standards for unlabelled OA and labelled ($^{13}C_2$-OA), respectively.

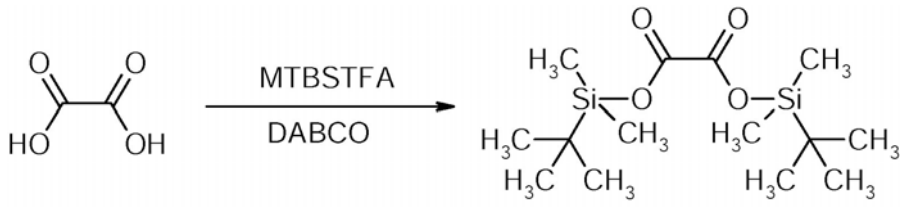

Figure 16: Reaction of oxalic acid in the organic phase with *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) catalysed by diazabi-cyclo[2.2.2]octane (DABCO), producing di-*tert*-butyldimethylsilyl-derivative of oxalic acid.

2.3.5.1 Sample preparation

Prior to the analyses, the urine samples stored at –80 °C were thawed at room temperature, and the internal standards were added. Analyses were performed in triplicates.

In a 14 ml Falcon™ tube, urine sample (300 µl) was mixed with 3 µl of an aqueous solution containing both internal standards SA (50000 µmol/l) and $^{2}H_{6}$-SA (500 µmol/l). After acidifying the sample with 6 drops of concentrated HCl (32 %), 300 µl of HCl (2 mol/l) and approximately 200 mg of NaCl were added. Thereafter, the sample was cooled on ice and the following extraction was carried out in a cold room (at 4 °C): The cooled sample was treated with ice-cold 1.5 ml of ethyl acetate, vigorously shaken by hand for 1 min, vortexed 20 sec, again vigorously shaken by hand for 1 min, and centrifuged at 0 °C (2 min, 2400 rpm, 1000·g; cooling centrifuge, Sigma 4K15C). The supernatant (organic phase) was transferred in a 7 ml glass tube. The extraction step was repeated twice, and the organic phases were combined. After adding 70 µl of quinoline:ACN (1:9) and 250 µl of toluene to the combined organic extract, the volume was reduced to about 300 µl in the vacuum concentrator centrifuge and then transferred into an 1.5 ml Eppendorf-cup. The glass tube was rinsed with 500 µl of ACN, which was also added into the Eppendorf-cup. The volume of the sample was again reduced to 5-10 µl using the vacuum concentrator centrifuge. Thereafter, 100 µl of toluene was added. In order to remove any residual

water and the solvent, the sample was then evaporated in the vacuum concentrator centrifuge. To the solvent-free residue, 50 µl of DABCO solution (2.5 % in ACN) and 75 µl of MTBSTFA were added. The mixture was then ultrasonicated for 15 sec and placed over-night for at least 12 h in a drying chamber at 40 °C. The mixture was then again ultrasonicated for 15 sec, and the formed di-TBDMS-derivative was extracted by adding 25 µl of ACN and 85 µl of n-hexane, followed by vortexing for 15 sec. To obtain two liquid phases, the sample was centrifuged (30 sec, 14000 rpm, 17530·g; microcentrifuge Sigma 1-15) and placed on ice for 10 min. The supernatant (hexane phase) was transferred into an autosampler vial that contained a micro insert. The vial was sealed with a crimp cap and placed on the cooled tray (15 °C) of the autosampler for the analysis by GC/MSD.

2.3.5.2 Gas chromatographic analysis

OA, $^{13}C_2$-OA, SA, and $^{2}H_6$-SA were analysed by GC/MSD. The GC/MSD-parameters are given in Table 5.

The substances eluted from the column were analysed using the MSD in the EI mode. For the identification of the substances and to determine their retention times, the scan-mode was used. To identify and quantify a selected compound, two ions were selected from its mass spectrum and determined in the experiments using the SIM mode. The molecular ion of the di-TBDMS-derivative could not be used due to its low abundance. To select the specific fragments, their specificity, abundance and eventual disturbing signal in the chromatogram were considered. The ions m/z=261 and m/z=303 were selected for OA, and m/z=263 and m/z=305 for $^{13}C_2$-OA. For SA, the ions m/z=289 and m/z=331, and for $^{2}H_6$-SA the ions m/z=293 and m/z=335 were used. The retention times were about 13.6 min (OA-derivative), 16.4 min (SA-derivative) and 16.4 min ($^{2}H_6$-SA-derivative). Figure 17 shows the separation of the di-TBDMS-derivative of $^{13}C_2$-OA and of the $^{2}H_6$-SA isolated from plasma.

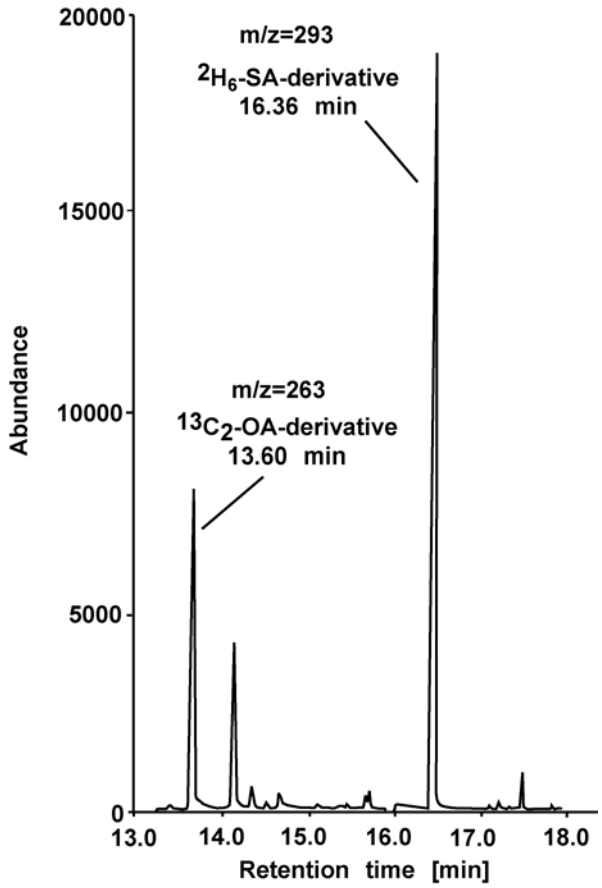

Figure 17: Single ion chromatogram for the determination of ^{13}C-labelled oxalic acid ($^{13}C_2$-OA) from urine (5 µmol/l) containing the internal standard [$^{2}H_6$]succinic acid ($^{2}H_6$-SA, 5 µmol/l) by monitoring the ions m/z=263 and m/z=293.

The integration of the signals was carried out using the manual integration-function of the ChemStation software. Prior to each measurement, the conditions and the parameters of the MSD were optimised using the calibration functions “autotune” and “usertune”. The ions used for the “usertune” (m/z=131, m/z=219 and m/z=414) were selected from the available fragments of the calibration substance perfluorotributylamine to cover the range of the masses used for quantification of the analytes.

After each sample, ethyl acetate was injected into the GC in order to clean the column.

Injector	"Cool-on-column-injector"
-Temperature	Track-on-oven-mode (i.e. the injector temperature is always 3 °C above the oven temperature)
-Injected volume	1 µl
-Washing solvent	Ethyl acetate
GC	HP 5890 Series II
-Precolumn	2.5 m·0.53 mm, fused silica, deactivated
-Capillary column	ZB-5, 30 m·0.5 mm, film thickness 1 µm
-Column temperature program	Starting temperature: 75 °C for 1.5 min Heating rate 1: 7 °C/min up to 170 °C Heating rate 2: 30 °C/min up to 270 °C Final temperature: 270 °C/min for 2 min Total run time: 21.40 min
-Carrier gas	Helium
-Pressure of carrier gas	1.41 bar at 75 °C, "constant-flow-mode" (flow calculated by software: 2.17 ml/min)
-Transfer line temperature	300 °C
MSD	HP 5972
-Ionisation	Electron impact, ionisation potential 70 eV
-Masses collected in SIM mode	OA-derivative: m/z=261 and m/z=303 $^{13}C_2$-GA-derivative: m/z=263 and m/z=305 HIVA-derivative: m/z=289 and m/z=331 $^{2}H_6$-SA-derivative: m/z=293 and m/z=335
-Masses used for quantification	OA-derivative: m/z=261 $^{13}C_2$-OA-derivative: m/z=263 SA-derivative: m/z=289 $^{2}H_6$-SA-derivative: m/z=293
-Tuning of the MSD	"Usertune" (ions m/z=131, m/z=219, and m/z=414)

Table 5: Parameters used for the GC/MSD determination of unlabelled oxalic acid (OA) and ^{13}C-labelled OA ($^{13}C_2$-OA), and of succinic acid (SA) and $^{2}H_6$-SA.

2.3.5.3 Mass spectra

The mass spectra of the di-TBDMS-derivative of OA, SA, and $^{2}H_6$-SA together with their fragmentation pattern have been already shown in detail by Carstens (2003).

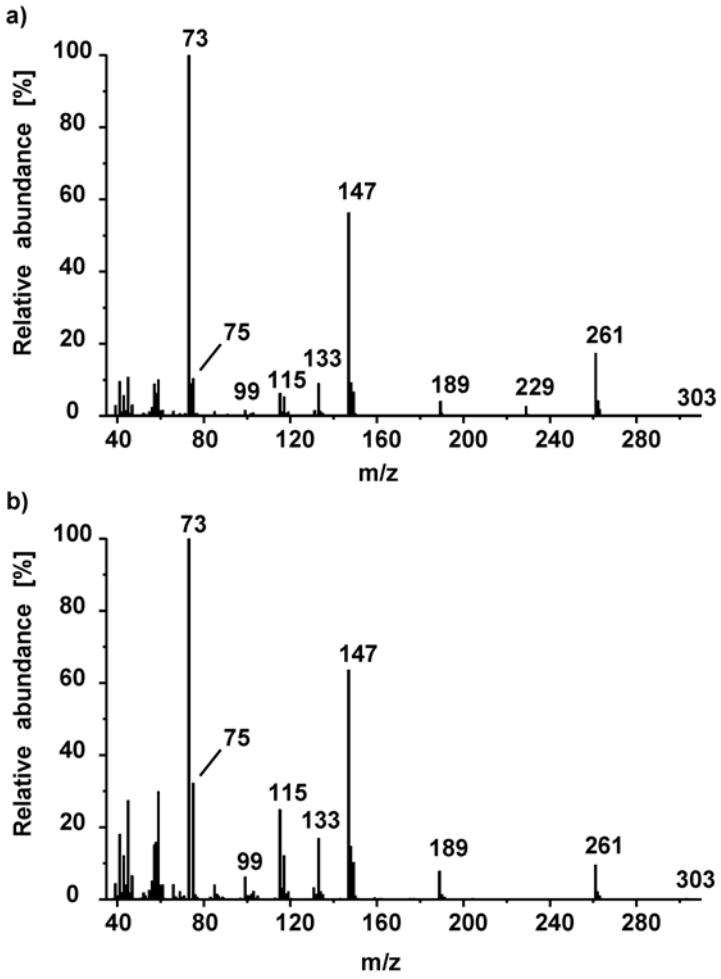

Figure 18: **a)** Mass spectrum of the di-*tert*-butyldimethylsilyl-derivative of oxalic acid.

b) Reference mass spectrum of the di-*tert*-butyldimethylsilyl-derivative of oxalic acid (NIST98).

Here, only the mass spectrum of the di-TBDMS-derivative of OA is presented (Figure 18a). Due to the excessive fragmentation, the molecular ion ($M^{+\bullet}$, m/z=318; $[C_{14}H_{30}O_4Si_2]^{+\bullet}$) is not present in the mass spectrum. The observed characteristic ions (Figure 19), m/z=303 for $C_{13}H_{27}O_4Si_2^{+}$ and m/z=261 for $C_{10}H_{21}O_4Si_2^{+}$, could result

from losses of $CH_3^{\bullet}$ (M-15) and $C_4H_9^{\bullet}$ (M-57) in a TBDMS-group by α-cleavage. The ions m/z=189, m/z=147, m/z=115, m/z=75, and m/z=73 (Figures 18a and 18b) could be formed upon rearrangements from the TBDMS-group and are not specific for OA (Little, 1999). The spectrum of the di-TBDMS-derivative of OA recorded in the scan-mode is consistent with that found in the reference mass spectrum library (Figure 18b; NIST98).

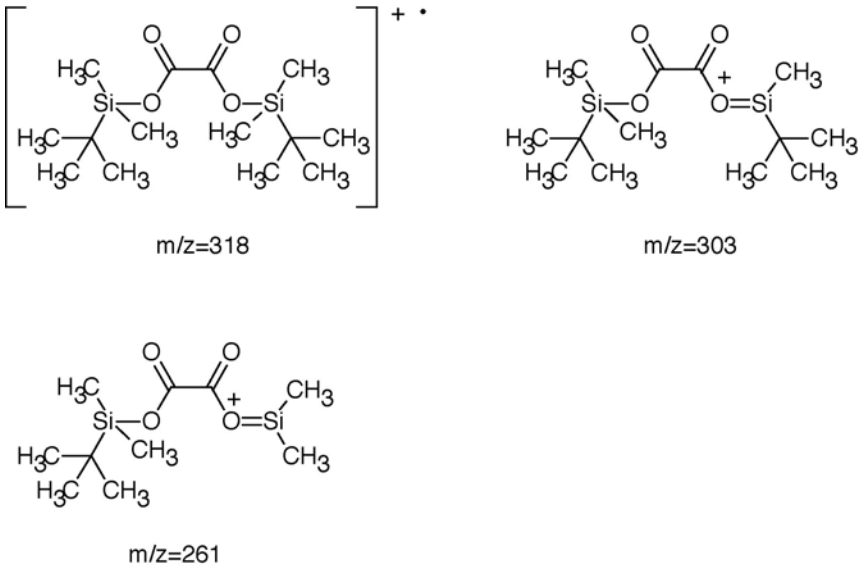

Figure 19: The molecular ion and characteristic ions of the di-*tert*-butyldimethylsilyl-derivative of oxalic acid.

2.3.5.4 Calibration and quantification of oxalic acid

For quantification of OA, calibration samples were included in each sequence. Calibration samples were prepared using freshly collected urine. In the calibration samples, the concentration of OA and $^{13}C_2$-OA were 10-300 and 1-10 µmol/l, respectively. Three aliquots for each concentration were prepared and analysed in the same way as described for the exposure sample (see 2.3.5.1 and 2.3.5.2). All samples from one exposure experiment together with the calibration samples represented one series of measurements for GC/MSD analysis. The sequence was assorted as described for EG (see 2.3.3.4). When quantifying $^{13}C_2$-OA, it was taken into account that the specific ion m/z=263 was also present in the spectrum of the di-TBDMS-

derivative of unlabelled OA (Figure 18a), resulting from the natural distribution of isotopes (see 2.3.4.4). Before performing $^{13}C_2$-OA quantification, the intensity of the OA derived ion m/z=263 was determined. For this purpose the ratio ($A_{control}$) of the $\text{peak area}_{m/z\,263}$ to the $\text{peak area}_{m/z\,261}$ (equation 1, see 2.3.4.4) was determined in control samples (urine) free of $^{13}C_2$-OA, containing only endogenous (unlabelled) OA. The exposure related peak area of the $^{13}C_2$-OA derived signal m/z=263 was obtained as follows: The $\text{peak area}_{m/z\,263}$ measured in the exposure sample was corrected according to the equation 2 (see 2.3.4.4) for the portion that resulted from the unlabelled OA using the $\text{peak area}_{m/z\,261}$ measured in the exposure sample and the value of $A_{control}$. The obtained exposure related peak area of $^{13}C_2$-OA was then divided by the peak area of the corresponding internal standard, which was used to calculate the concentration of $^{13}C_2$-OA from the slope of the respective calibration curve. To obtain a calibration curve, the ratio between the peak area of the analyte ($^{13}C_2$-OA or OA) to the peak area of the internal standard ($^2H_6$-SA or SA) was calculated and plotted versus the analyte concentration. The calibration curves resulted in straight lines in all matrices over the investigated concentration range (Figures 20 and 21).

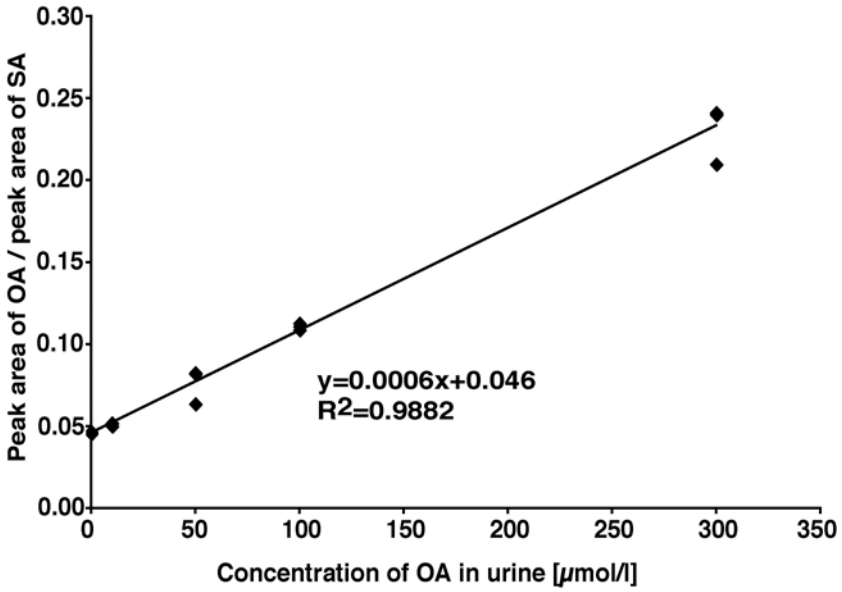

Figure 20: Calibration curve for the determination of oxalic acid (OA) from urine; internal standard was succinic acid (SA, 500 µmol/l).

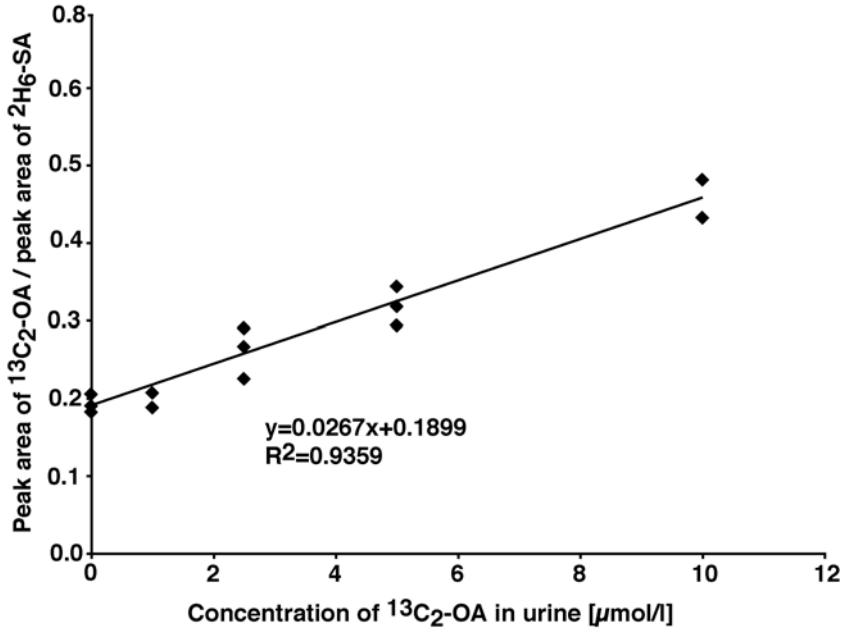

Figure 21: Calibration curve for the determination of ^{13}C-labelled oxalic acid ($^{13}C_2$-OA) from urine; internal standard was [$^2H_6$]succinic acid ($^2H_6$-SA, 5 µmol/l).

The slopes were calculated by linear regression analysis. The calibration curves in Figures 20 and 21 did not pass through the origins. This was because the matrices contained unavoidable backgrounds of the OA that is taken up with food and also formed in the intermediary metabolism. In order to determine the intercept accurately, blank samples were measured in triplicates. The limits of quantification from urine were 17.2 and 0.2 µmol/l for OA and $^{13}C_2$-OA, respectively. To calculate the limits of quantification, the standard deviations of the background signals, measured in triplicates, were divided by the slopes of the corresponding calibration curves.

2.3.5.5 Recovery, precision and stability

Recovery and precision of the method was determined by Carstens (2003). The recovery of OA from urine was in the range of 25±3 % at 250 µmol/l. The precision of the method, defined by the CV was on average 6 %. The stability of OA in urine samples was investigated by Duez *et al.* (1996) and von Unruh *et al*. (1998). These

investigators showed that the urine samples can be stored for at least three months at –20 °C without loss of OA.

2.4 Inhalation and dermal exposures to ^{13}C-labelled ethylene glycol

Volunteers were exposed either to vapours of $^{13}C_2$-EG via inhalation, exactly as described earlier (Carstens, 2003; Carstens *et al.*, 2003), or to liquid $^{13}C_2$-EG via skin using a new method developed in the present work for occlusive dermal exposure to liquids.

2.4.1 Inhalation experiment

For inhalation exposure, a closed system was used into which small amounts of liquid $^{13}C_2$-EG were administered at selected time points. Liquid $^{13}C_2$-EG was warmed up, evaporated quickly, and was inhaled by the volunteer. The whole $^{13}C_2$-EG amount inhaled by a volunteer during an exposure experiment was about 2 mmol, about half of the amount that is inhaled during an 8 h work shift under MAK-conditions (10 ppm EG; alveolar ventilation 1.25 m^3/h). The volunteer inhaled the vapours of $^{13}C_2$-EG through a mouthpiece from the system that consisted of a modified all-glass three neck round bottom flask and a heating mantle and an inside thermometer (Figure 22). The air temperature in the system was maintained at ≤140 °C (boiling point of EG is 198 °C). One of the vertical side necks was closed by a septum. The main neck was equipped with a tubular glass adapter. The mouthpiece was connected to it by means of silicon tube that was closed by a pinch clamp. To the other side neck, a similar glass adapter was mounted containing a longer glass tube that extended into the air space of the round bottom flask. On the outside of the flask, that glass tube was equipped with a silicon tube, closed by a second pinch clamp.

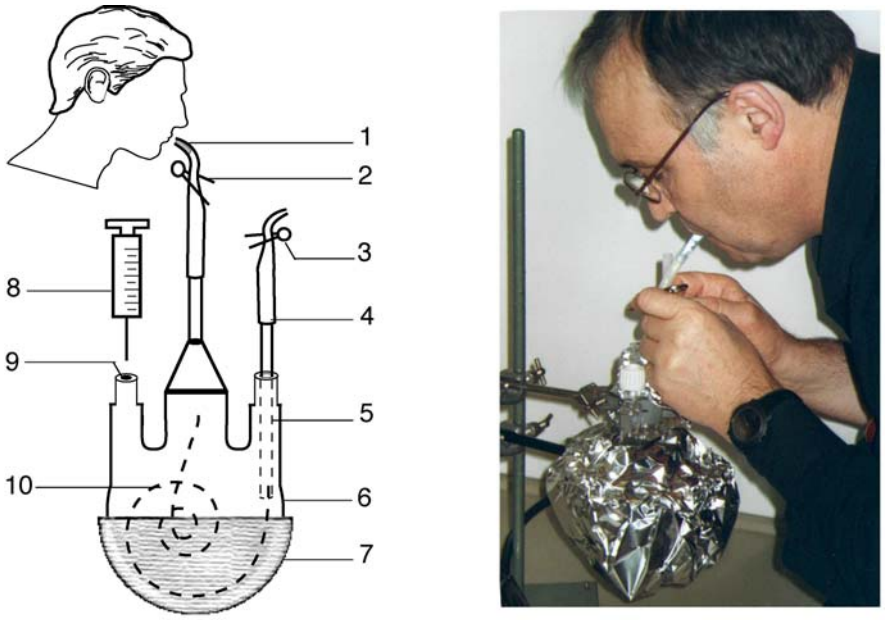

Figure 22: Exposure system for the inhalation of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG): 1. mouth piece, 2. air outlet valve (pinch clamp), 3. air inlet valve (pinch clamp), 4. silicon tubing, 5. glass tube, 6. glass vessel, 7. heating mantel, 8. syringe with liquid $^{13}C_2$-EG, 9. septum, 10. air stream.

One hour before start of the exposure, the heating mantle was switched on in order to warm up the exposure system with the pinch clamps opened. These clamps were closed immediately before starting the experiment. To generate $^{13}C_2$-EG vapour, 6.6 µl of liquid $^{13}C_2$-EG was injected repeatedly (16 times over 4 h) at intervals of 15 min via the septum into the round bottom flask using a calibrated 10 µl Hamilton syringe. Approximately one minute after the injection, the volunteer took the mouthpiece in his mouth, opened the outlet valve, and took a deep breath through the mouthpiece simultaneously opening the inlet valve. Hereby, the $^{13}C_2$-EG vapour from the flask was transported by the inhaled stream of room air into his respiratory tract. Immediately thereafter, the valves were closed, and the volunteer hold the inhaled breath for 1 min before the next inhalation step. By this procedure, the escape of $^{13}C_2$-EG vapour from the flask into the room air was prevented. Inhalation steps were repeated 3-4 times. In order to measure for each volunteer the fraction of exhaled $^{13}C_2$-EG during an inhalation step, exhaled air was collected in a polyethylene coated 2.5 l aluminium bag immediately after the 1 h time point. The same procedure was

repeated following the inhalation step at the 4 h time point. Each gas bag was quickly sealed and 5 ml of deionised water was injected through a septum of the bag. After thoroughly rinsing the inner side of the bag, defined amounts of the water were collected separately in two 14 ml Falcon™ tubes and stored at –80 °C until analysis. For quantifying the residual EG after the last inhalation step in the system, the round bottom flask was cooled to room temperature, and 10 ml of deionised water was injected via the septum. The flask was thoroughly shaken in order to moist the entire surfaces, and a defined amount of the water was collected in a 14 ml Falcon™ tube and then stored at –80 °C until analysis.

2.4.2 Dermal application experiment

The seated volunteer positioned his inner forearm upwards onto a table (Figure 23). A previously washed and dried cellulose gauze of 66 cm^2 was placed on the inner forearm and wetted evenly with 0.8 ml of liquid $^{13}C_2$-EG by using a calibrated 1 ml Hamilton syringe. Thereafter, the edges of the gauze were framed with silicon-free laboratory grease and occluded with a polyethylene sheet. This sheet was fixed to the skin by a surgical tape. During the entire exposure period of 4 to 6 h, the volunteer kept his forearm rested on a desk with the patch upwards. At the end of exposure, the cover and the gauze were removed and placed on a glass plate. In order to measure the non-absorbed EG, the exposed skin area together with the cellulose gauze and polyethylene sheet was washed thoroughly with deionised water. All aqueous solutions were combined and stored at –80 °C until analysis.

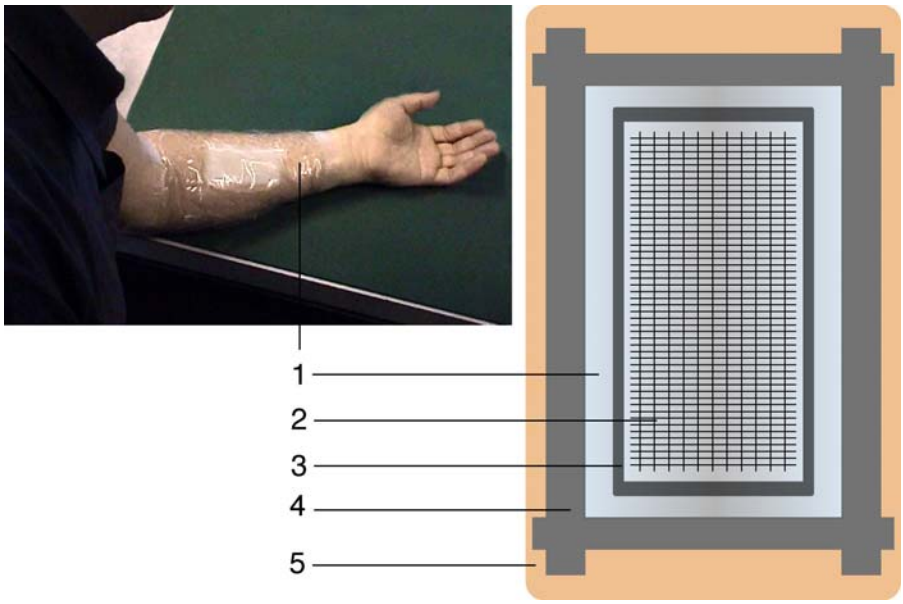

Figure 23: Dermal exposure system for liquid ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG):
1. polyethylene sheet, 2. cellulose gauze with $^{13}C_2$-EG, 3. silicon-free laboratory grease, 4. surgical tape, 5. skin.

2.4.3 Collection of biological material

In both the inhalation and the dermal uptake experiments, venous blood from one forearm and urine samples were collected before, during and after the end of the exposures.

2.4.3.1 Blood collection and plasma separation

Prior to the exposure, an indwelling cannula (Introcan®-W) was fixed to the cubital vein at the forearm of the volunteer (the non-exposed forearm, when epidermally $^{13}C_2$-EG exposed) and remained there for up to 13.3 h. Clogging was prevented by an inserted stylet. For blood collection, the stylet was removed, and a blood sample (8 ml) was taken using a heparinised disposable 10 ml syringe. The cannula was immediately closed by a new stylet. In inhalation experiments, blood samples were collected approximately 20 min before starting the exposure and 5 min after each

inhalation cycle (in 15 min periods). After the end of the 4 h inhalation exposures, blood samples were taken at the approximate time points of 4.3, 4.5, 5.0, 5.8, 6.5, 7.5, 8.5, 9.5, and 10.3 h. In the transdermal uptake experiments, blood samples were collected approximately 20 min before and in 15 min intervals after start of the exposure. After the end of the two 4 h exposures the same time points as given for the inhalation experiment were used for blood collection. After the end of the two 6 h exposures, blood samples were taken at the approximate time points of 6.3, 6.5, 7.0, 8.0, 9, 10, 11, 12, and 13 h. About 24 h after the start of each exposure, an additional venous blood sample (8 ml) was taken with a heparinised disposable syringe. Each blood sample was divided in two parts. About 7.5 ml was immediately transferred into a 15 ml Falcon™ tube. Plasma was obtained by centrifugation at 0 °C (4 min, 3760 rpm, 2500·g; cooling centrifuge, Sigma 4K15C) and then distributed in three multi twist top 2 ml vials and stored at –80 °C. The second blood sample (about 0.5 ml) was used to measure pH and blood gases (pCO_2, and pO_2,) by means of a blood gas analyser.

2.4.3.2 Urine collection

In the inhalation and in the transdermal experiments, urine was collected in fractions. Fractions were gained subsequently from shortly before exposure until about 35 h thereafter. Urinary fractions were collected in 1 l wide-mouth-polyethylene bottles containing 5 ml of concentrated HCl (32 %) in order to prevent precipitation of calcium-oxalate and bacterial growth. During urine collection into the bottles, pH was measured using indicator sticks (pH-Fix). The volume of each urinary fraction was calculated from its mass considering a urine density of 1 g/ml. By means of the obtained value, it was possible to calculate for each fraction the actual amount of investigated compound from its concentration in a corresponding urine sample. All urinary fractions were stored at 4 °C for a maximum of 12 h. Within this time frame, they were analysed for EG and $^{13}C_2$-EG. Then, each fraction was subdivided into two 15 ml Falcon™ tubes and stored at –80 °C until analysis of the other compounds.

2.5 Statistics

The following equations (Sachs, 2002) were used to calculate the arithmetic mean ($\bar{x}$; equation 3), the standard deviation (SD; equation 4), and the CV (equation 5).

equation 3: $$\bar{x} = \frac{\sum_{i=1}^{i=n} x_i}{n}$$

$\bar{x}$ arithmetic mean

x_i value of the i^{th} sample

n number of measurements

equation 4: $$SD = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})^2}{n-1}}$$

SD standard deviation

equation 5: $$CV = \frac{SD}{\bar{x}} \cdot 100$$

CV coefficient of variation (%)

2.6 Toxicokinetics

For the toxicokinetic analysis of the concentration-time courses of $^{13}C_2$-EG and $^{13}C_2$-GA measured in plasma, a one-compartment model was used (Filser, 1996).

For $^{13}C_2$-EG, the volume of distribution (V_d) related to the matrix plasma, the plasma concentration at steady-state (C_{ss}), the elimination rate constant (k), the area under the curve (AUC), and the half-life ($t_{1/2}$) were calculated. For $^{13}C_2$-GA, area under the curve (AUC) and half-life ($t_{1/2}$) were determined. At the end of exposures the concentration of $^{13}C_2$-EG and $^{13}C_2$-GA in plasma declined according to first-order kinetics:

equation 6: $c(t) = C_0 \cdot e^{-k \cdot t}$

$c(t)$ concentration of the analyte at time point t after exposure (µmol/l)

C_o concentration of the analyte at the end of exposure (t=0, µmol/l)

k elimination rate constant (h^{-1})

t time (h)

The value of k was obtained by fitting equation 6 to the elimination phase of the concentration-time course measured in plasma using the program Sigma Plot 4.17.

The $t_{1/2}$ was calculated from the rate constant k:

equation 7: $t_{1/2} = \frac{\ln 2}{k}$

$t_{1/2}$ half-life (h)

The C_{ss} of $^{13}C_2$-EG in plasma following exposure to vaporous EG was calculated by equation 8:

equation 8: $C_{ss} = 2 \cdot c_{t_{1/2}}$

C_{ss} plasma concentration at steady-state (µmol/l)

$c_{t_{1/2}}$ plasma concentration at time point $t = t_{1/2}$ (µmol/l)

The C_{ss} is reached during a prolonged exposure when the rate of uptake (v) equals the elimination rate. This uptake rate was calculated by dividing the total amount of $^{13}C_2$-EG inhaled by the exposure time (4 h). The values of v, C_{ss} and k were used to calculate the V_d:

equation 9: $V_d = \frac{v}{C_{ss} \cdot k}$

V_d volume of distribution (l)

v inhalation rate (µmol/h)

The area (AUC_0^∞) under the concentration-time curve over the time interval (0, ∞) is considered as a measure of the body burden. It was calculated as the area under the measured concentration-time course (AUC_0^n) covering the time interval (0, t_n) plus the approximated area (AUC_n^∞) over the time interval (t_n, ∞):

equation 10: $$AUC_0^\infty = AUC_0^n + AUC_n^\infty$$

The AUC over the time interval (0, t_n) was calculated numerically by the trapeze integration method (Bronstein and Semendjajew, 1976):

equation 11: $$AUC_0^n = \sum_{i=0}^{n} \frac{t_{i+1} - t_i}{2} \cdot \left(c_i + c_{i+1}\right)$$

AUC_0^n area under curve between the time points t_0 and t_n (µmol/l·h)

t_i time point i (h)

c_i concentration of the analyte in the plasma sample i (µmol/l)

The approximated AUC over the time interval (t_n, ∞) was calculated by assuming a first order elimination:

equation 12: $$AUC_n^\infty = \int_{t_n}^{\infty} c(t_n) \cdot e^{-k \cdot t} dt = \frac{c(t_n)}{k}, \; t > t_n$$

AUC_n^∞ area under the curve between the time points t_n to t_∞ (µmol/l·h)

$c(t_n)$ plasma concentration of the analyte at the time point t_n (µmol/l)

The AUC_0^∞ of $^{13}C_2$-EG in plasma in inhalation experiments can also be estimated by using a one-compartment model. For this purpose the AUC_0^∞ is calculated as the area under the modelled concentration-time curve ($AUC_0^{t_{exp}}$) covering the exposure period (0, t_{exp}) plus the area ($AUC_{t_{exp}}^\infty$) over the time interval thereafter (t_{exp}, ∞):

equation 13: $AUC_0^{\infty} = AUC_0^{t_{exp}} + AUC_{t_{exp}}^{\infty}$

The first term $AUC_0^{t_{exp}}$ corresponds to the integral of the concentration-time curve representing the $^{13}C_2$-EG concentrations during the exposure period:

equation 14: $$AUC_0^{t_{exp}} = \int_0^{t_{exp}} \frac{v}{V_d \cdot k} \cdot (1 - e^{-k \cdot t})\, dt = \frac{v \cdot t_{exp}}{V_d \cdot k} \cdot (1 - e^{-k \cdot t_{exp}})$$

t_{exp} duration of exposure (h)

$AUC_0^{t_{exp}}$ corresponding to the exposure period (0, t_{exp}) in units (µmol/l·h)

The second term $AUC_{t_{exp}}^{\infty}$ corresponds to the integral of the concentration-time curve representing the compartmental concentration of $^{13}C_2$-EG after the end of exposure:

equation 15: $$AUC_{t_{exp}}^{\infty} = \int_{t_{exp}}^{\infty} \frac{v}{V_d \cdot k} \cdot (1 - e^{-k \cdot t_{exp}}) \cdot e^{-k \cdot (t - t_{exp})}\, dt = \frac{v}{V_d \cdot k^2} \cdot (1 - e^{-k \cdot t_{exp}})$$

By adding, the two terms results in:

equation 16: $$AUC_0^{\infty} = \frac{v \cdot (1 + k \cdot t_{exp})}{V_d \cdot k^2} \cdot (1 - e^{-k \cdot t_{exp}})$$

Substituting the inhalation rate in equation 16 by the product of the alveolar ventilation (Q_{alv}) with the constant atmospheric exposure concentration of $^{13}C_2$-EG (C_{exp}) and solving equation 16 for C_{exp}, yields equation 17:

equation 17: $$C_{exp} = \frac{AUC_0^{\infty} \cdot V_d \cdot k^2}{Q_{alv} \cdot (1 + k \cdot t_{exp}) \cdot (1 - e^{-k \cdot t_{exp}})}$$

Q_{alv} alveolar ventilation (l/h)

C_{exp} constant atmospheric exposure concentration (µmol/l)

For toxicokinetic analysis of the dermal exposure experiments, the dose taken up (D_{dermal}) had to be calculated at first. The elimination clearance ($V_d \cdot k$) of $^{13}C_2$-EG was assumed to be independent of the route of administration:

equation 18: $$V_d \cdot k = \frac{D_{inhalation}}{AUC_{inhalation}} = \frac{D_{dermal}}{AUC_{dermal}}$$

AUC_{dermal} area under the curve of $^{13}C_2$-EG following dermal exposure (µmol/l·h)

$AUC_{inhalation}$ area under the curve of $^{13}C_2$-EG following inhalation (µmol/l·h)

$D_{inhalation}$ amount of $^{13}C_2$-EG inhaled (µmol)

D_{dermal} amount of $^{13}C_2$-EG taken up via skin (µmol)

Reformulation of equation 18 yields D_{dermal}:

equation 19: $$D_{dermal} = \frac{AUC_{dermal}}{AUC_{inhalation}} \cdot D_{inhalation}$$

Thereafter, D_{dermal} was used to calculate the flux (J) of $^{13}C_2$-EG through the exposed skin area (A):

equation 20: $$J = \frac{D_{dermal}}{A \cdot t_{exp}}$$

J flux of $^{13}C_2$-EG through the skin (µmol/cm^2·h)

A exposed skin area (cm^2)

Finally, the permeability constant of $^{13}C_2$-EG (K_p) was calculated. Considering the transdermal uptake at steady-state, diffusion of liquid $^{13}C_2$-EG across skin was formulated using K_p according to Fick's first law:

equation 21: $$K_p = \frac{J}{\Delta c} \approx \frac{J}{c}$$

K_p permeability constant (cm/h)

J	flux of liquid $^{13}C_2$-EG through the skin (µmol/cm^2·h)
Δc	concentration difference of $^{13}C_2$-EG on both sides of the skin (µmol/l)
c	concentration of liquid $^{13}C_2$-EG on the skin surface (µmol/ml)

The C_{ss} of $^{13}C_2$-EG in plasma, which can be reached during continuous exposure to liquid $^{13}C_2$-EG, was calculated by the following equation:

equation 22: $$C_{ss} = \frac{J \cdot A}{V_d \cdot k}$$

3 Results

3.1 Inhalation exposure to vaporous ^{13}C-labelled ethylene glycol

Two healthy, non-smoking male volunteers C and D were exposed via inhalation to $^{13}C_2$-EG vapour as described in section 2.4.1. For a comparison, previous results obtained with volunteers A and B (Carstens *et al*., 2003) are also presented. None of the volunteers did report any health effects related to the exposure. The average pH values in blood and urine collected during and after the exposures (Table 6) were in the range of the corresponding reference ranges [blood: 7.37-7.43 (Thews *et al*., 1991); urine: 4.8-7.5 (Documenta Geigy, 1975)].

Volunteer	pH value (mean±SD)	
	Blood (n=25)	Urine (n=10)
A	7.45±0.02	6.75±0.59
B	7.39±0.03	6.59±0.66
C	7.42±0.02	6.92±0.53
D	7.43±0.02	6.62±0.51

Table 6: pH values in blood and urine of volunteers collected during and after inhalation exposure to ^{13}C-labelled ethylene glycol. Data for volunteers A and B are from Carstens *et al.* (2003).

3.1.1 Dose taken up

In order to determine the dose taken up, the amount of $^{13}C_2$-EG remaining in the exposure system at the end of exposure was subtracted from the administered amount. The administered amount was between 1890 and 2000 µmol (Table 7). In the exhaled air, $^{13}C_2$-EG could not be detected (limit of detection of $^{13}C_2$-EG given as a concentration in the exhaled air was 0.015 ppm). Consequently, inhaled $^{13}C_2$-EG was completely taken up from the inhaled air. The amount remaining in the system at the

end of the exposure was between 280 and 660 µmol, indicating that about 15-33 % of the administered dose did remain in the system (Table 7). Therefore, about 67-85 % of the dose administered was taken up in the inhalation experiments corresponding to a normalised dose of about 1.0-1.5 mg/kg bw.

The amounts taken up in the inhalation experiments can be compared with an 8 h exposure under workplace conditions. For this purpose, the amount of $^{13}C_2$-EG taken up was divided by the air volume of 9600 l that is inhaled within 8 h by a reference worker of 70 kg at a workload of 50 W (Åstrand, 1983). The obtained concentration (µmol/l) was converted to ppm by multiplying with 25.13 l/mol. According to this calculation, an 8 h exposure of a 70 kg reference worker to about 3.8 ppm EG (range: 3.5-4.2 ppm; Table 7) results in the same uptake of EG as was the case with $^{13}C_2$-EG in the experiments carried out here.

Volunteer	**$^{13}C_2$-EG administered into the exposure system [µmol]**	**Residual $^{13}C_2$-EG in the exposure system [µmol]**	**$^{13}C_2$-EG inhaled [µmol]**	**Equivalent EG concentration* [ppm]**
A	1890	460	1430	3.7
B	2000	660	1340	3.5
C	1890	280	1610	4.2
D	1890	400	1490	3.9

Table 7: Amount of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) administered into the exposure system, residual amount of $^{13}C_2$-EG in the system at the end of exposure, and amount of $^{13}C_2$-EG inhaled as calculated for the experimental exposure conditions. Data for volunteers A and B are from Carstens *et al.* (2003).

***** Equivalent EG vapour concentration was calculated considering the uptake of the same amount of EG by a 70 kg reference worker exposed at workplace conditions (8 h, 50 W).

3.1.2 Ethylene glycol in plasma and urine

The concentration-time courses of $^{13}C_2$-EG in plasma of volunteers A, B, and C showed two phases (Figure 24). During exposures, $^{13}C_2$-EG increased to maximum values that are reached at the end of exposures (absorption phase). Thereafter, $^{13}C_2$-EG declined rapidly (elimination phase) and was no more detectable after 24 h following start of the exposures. For volunteer D, only the elimination phase of the curve could be evaluated.

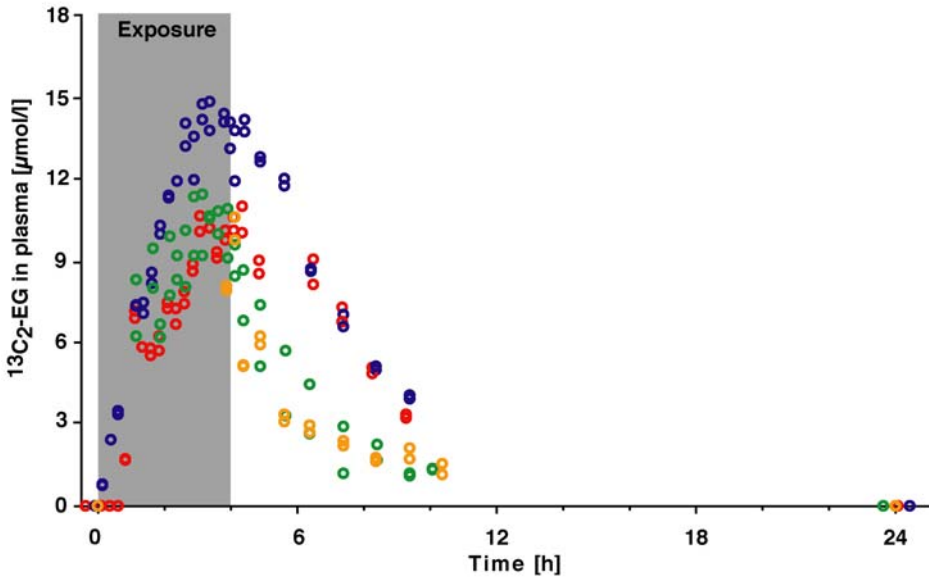

Figure 24: Concentration-time courses of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) in plasma during and after 4 h inhalation exposures to $^{13}C_2$-EG vapour (red: volunteer A; blue: volunteer B; green: volunteer C; orange: volunteer D). Symbols represent individual data points. For volunteer D, only the elimination phase of the curve could be evaluated. Data for volunteers A and B are from Carstens *et al.* (2003).

The $t_{1/2}$ of $^{13}C_2$-EG, calculated from the data representing the elimination phase, was in the range between 1.6 and 2.6 h (Table 8). The area under curve (AUC_0^∞; Table 8) was calculated from the concentration-time course and $t_{1/2}$, using equations 7 and 11-13 (see 2.6). The AUC_0^∞ values varied about a 1.6fold among the volunteers. The

same variation was observed in the maximum concentrations (10.1-14.8 µmol/l) measured at the end of exposure. Table 8 also displays the constant inhalation rates and the expected steady-state concentrations of $^{13}C_2$-EG in plasma considering a continuous exposure. These steady-state concentrations (calculated using equation 8; see 2.6 were between 14.4 and 24.0 µmol/l and varied about 1.7fold amoung the volunteers. After an 8 h exposure, 88-97 % of the steady-state plasma concentrations was calculated to be reached. The volume of distribution (calculated with equation 9; see 2.6 was on average 87 % of the body weight. Not all of these values were available for volunteer D, since only the elimination part of the concentration-time course could be reasonably evaluated.

Volunteer	**$t_{1/2}$ [h]**	**AUC_0^∞ [µmol/l·h]**	**C_{ss} [µmol/l]**	**v [µmol/h]**	**V_d [l]**
A	2.1	76	14.4	358	75
B	2.6	100	24.0	335	52
C	1.6	61	17.0	403	69
D	2.2	n.a.	n.a.	373	n.a.

Table 8: Toxicokinetic parameters of ^{13}C-labelled ethylene glycol obtained in volunteers following 4 h inhalation exposures; half-life ($t_{1/2}$), area under curve over the time interval 0 to ∞ (AUC_0^∞) in plasma, concentration at steady-state (C_{ss}) in plasma, inhalation rate (v), and volume of distribution (V_d). Data for volunteers A and B are from Carstens *et al.* (2003).
n.a.: not available.

The cumulative amounts of $^{13}C_2$-EG and unlabelled EG excreted in urine are shown in Figure 25.

The excretion of unlabelled EG results from endogenous and dietary sources. It increased almost linearly over time if shorter excretion intervals are considered

(Figure 25, part I). The amounts excreted over 24 h were between 16.5 and 38.7 µmol and showed a 2.3fold variation among the volunteers (Table 11).

The excretion of $^{13}C_2$-EG increased over time and reached a plateau after about 12 h (Figure 25, part II). The excreted amounts of $^{13}C_2$-EG were between 44.2 and 125 µmol showing a 2.8fold variation among the volunteers (Table 10). These amounts represented a small portion (3.0-9.3 %) of the inhaled $^{13}C_2$-EG dose (Table 10).

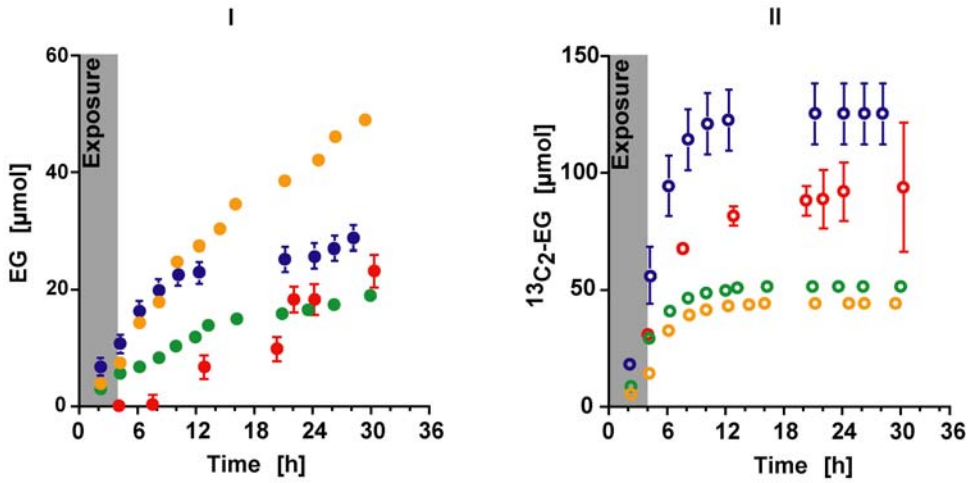

Figure 25: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) ethylene glycol (EG) during and after 4 h exposures to $^{13}C_2$-EG vapour (red: volunteer A; blue: volunteer B; green: volunteer C; orange: volunteer D). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size. Data for volunteers A and B are from Carstens *et al.* (2003).

3.1.3 Glycolic acid in plasma and urine

The concentration-time courses of $^{13}C_2$-GA in plasma of the four $^{13}C_2$-EG exposed volunteers show two phases similar to that of EG (Figure 26). During the exposures, $^{13}C_2$-GA increased to maximum values that were reached at about the end of the exposure periods (absorption phase). Thereafter, $^{13}C_2$-GA declined rapidly; 24 h after the start of the exposures it was no more detectable (elimination phase). The elimination half-lives varied between 1.8 and 2.9 h (Table 9) and were similar to

those of the metabolic precursor $^{13}C_2$-EG (Table 8). Hereof, one can conclude that the elimination rate of $^{13}C_2$-GA is limited by its production from $^{13}C_2$-EG. The maximum $^{13}C_2$-GA concentrations were between 0.9 and 2.6 µmol/l (Figure 26) showing a 2.9fold variation among the volunteers.

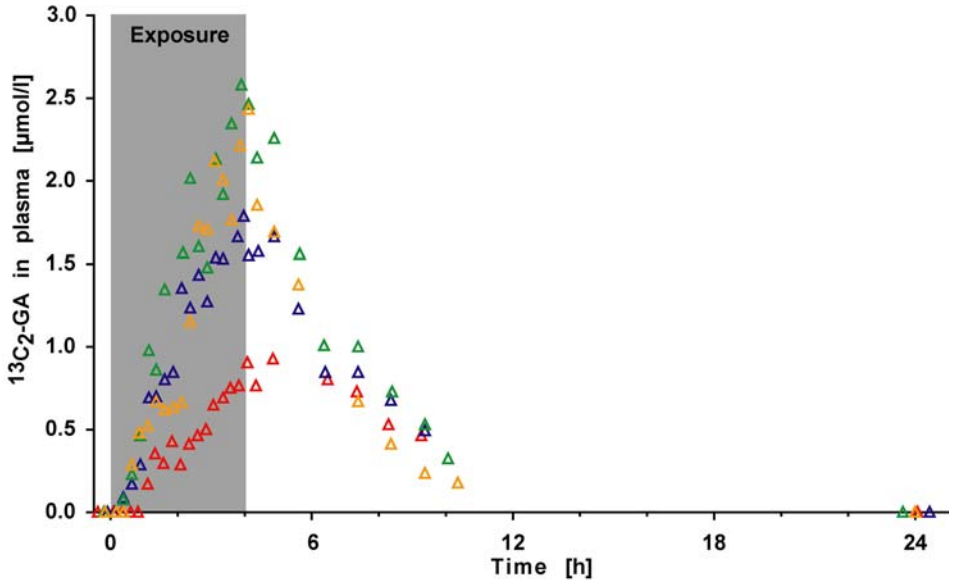

Figure 26: Concentration-time courses of ^{13}C-labelled glycolic acid ($^{13}C_2$-GA) in plasma during and after 4 h exposures to ^{13}C-labelled ethylene glycol vapour (red: volunteer A; blue: volunteer B; green: volunteer C; orange: volunteer D). Symbols represent individual data points. Data for volunteers A and B are from Carstens *et al.* (2003).

From comparing the AUC_0^∞ values of $^{13}C_2$-GA with those of $^{13}C_2$-EG it becomes obvious that only 9-24 % of $^{13}C_2$-EG appeared as $^{13}C_2$-GA in plasma. In contrast to $^{13}C_2$-EG, the expected steady-state concentrations of $^{13}C_2$-GA in plasma could not be calculated based on the data collected. However, assuming a linear increase of $^{13}C_2$-GA concentration over time and constant $^{13}C_2$-EG inhalation rates, $^{13}C_2$-GA concentrations could reach between 1.8 and 5.2 µmol/l (volunteer A: 1.8 µmol/l; volunteer B: 3.6 µmol/l; volunteer C: 5.2 µmol/l; volunteer D: 4.8 µmol/l) at the end of a theoretical 8 h exposure experiment. These values represent "worst case" estimates for two reasons: First, 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 and, second, the $^{13}C_2$-GA elimination rate increases with the $^{13}C_2$-GA concentration. The concentration of unlabelled GA was also determined in plasma. Its concentration is related to its endogenous origin and to dietary uptake. The average plasma concentrations were between 25.8 and 30.3 µmol/l (Table 9). They were several times higher than the maximum $^{13}C_2$-GA concentrations reached at the end of the $^{13}C_2$-EG exposures ($^{13}C_2$-GA concentrations in plasma: volunteer A: 0.9 µmol/l; volunteer B: 1.8 µmol/l; volunteer C: 2.6 µmol/l; volunteer D: 2.4 µmol/l).

Volunteer	**$t_{1/2}$ [h]**	**AUC_0^∞ [µmol/l·h]**	**Unlabelled GA [µmol/l] (mean±SD, n=25)**
A	2.9	6.7	25.8±3.7
B	2.6	11.1	28.3±2.8
C	2.0	13.3	29.9±4.7
D	1.8	9.4	30.3±7.4
Mean±SD	2.3±0.51 (n=4)	10.1±2.8 (n=4)	28.8±5.20 (n=100)

Table 9: Calculated toxicokinetic parameters of ^{13}C-labelled glycolic acid (GA) obtained in volunteers following 4 h exposures to ^{13}C-labelled ethylene glycol via inhalation; half-life ($t_{1/2}$) and area under curve over the time interval 0 to ∞ (AUC_0^∞) in plasma. Additionally, average concentrations of unlabelled GA determined in plasma of the volunteers are presented. Data for volunteers A and B are from Carstens *et al.* (2003).

The cumulative amounts of $^{13}C_2$-GA and unlabelled GA excreted in urine during and after exposure are given in Figure 27.

The excretion of unlabelled GA results from its endogenous formation in the intermediary metabolic pathway and from its intake via food. The excretion increased almost linearly over time if shorter excretion intervals are considered (Figure 27,

part I). The amounts excreted over 24 h were between 88.0 and 550 µmol and showed a considerable variation (6.2fold) among the volunteers (Table 11).

The excretion of $^{13}C_2$-GA increased over the time and reached a plateau after about 12 h (Figure 27, part II). The excreted amounts of $^{13}C_2$-GA were between 9.50 and 12.5 µmol showing only a 1.3fold variation among the volunteers (Table 10). These amounts represented a very small portion (0.59-0.92 %; Table 10) of the inhaled $^{13}C_2$-EG dose.

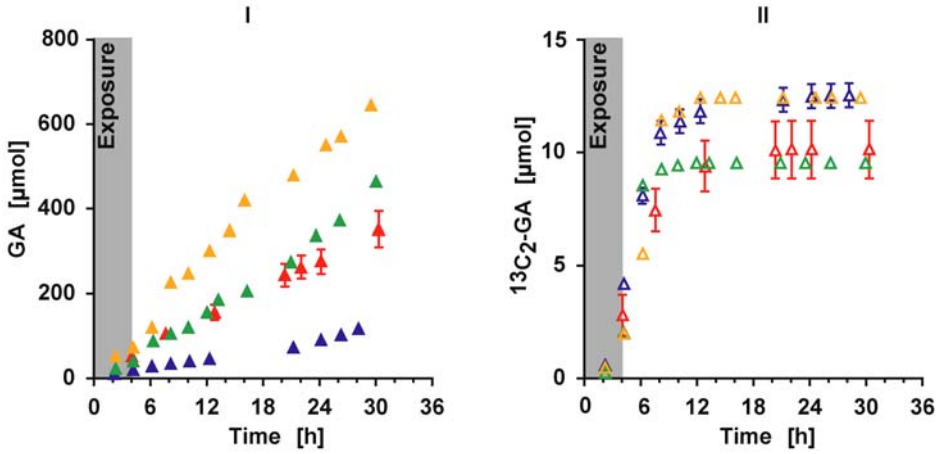

Figure 27: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) glycolic acid (GA) during and after 4 h exposures to ^{13}C-labelled ethylene glycol vapour (red: volunteer A; blue: volunteer B; green: volunteer C; orange: volunteer D). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size. Data for volunteers A and B are from Carstens *et al.* (2003).

3.1.4 Oxalic acid in urine

The cumulative amounts of $^{13}C_2$-OA and unlabelled OA excreted in urine during and after exposure are given in Figure 28.

The excretion of unlabelled OA results from its endogenous formation in the intermediary metabolism and from its intake via food. The excretion increased almost linearly over time (Figure 28, part I). The amounts excreted over 24 h were between

121 and 232 µmol and showed an about twofold variation among the volunteers (Table 11).

The excretion of $^{13}C_2$-OA increased over time and reached a plateau after about 12 h in two volunteers (volunteers C and D; Figure 28, part II). In the other two (volunteers A and B), the plateau was reached considerably later. The excreted amounts of $^{13}C_2$-OA were between 0.25 and 3.70 µmol showing a considerable, 14fold variation (Table 10). These amounts represented only 0.02-0.28 % of the inhaled $^{13}C_2$-EG dose (Table 10).

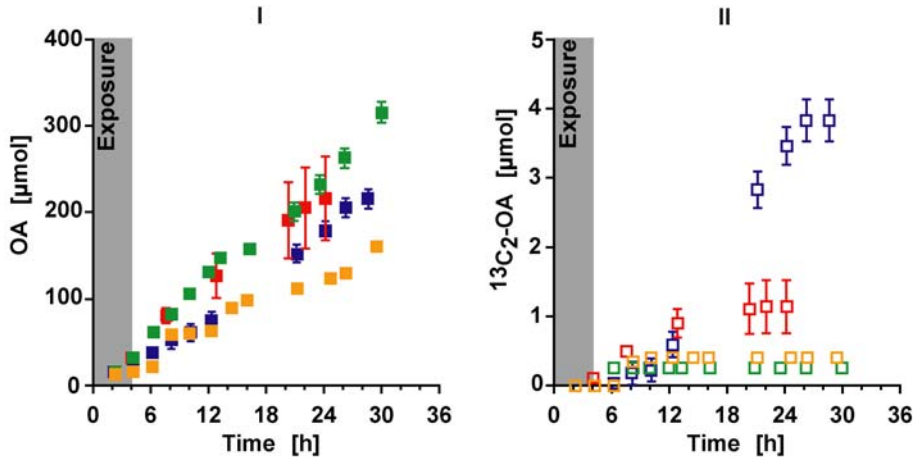

Figure 28: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) oxalic acid (OA) during and after 4 h of exposures to ^{13}C-labelled ethylene glycol vapour (red: volunteer A; blue: volunteer B; green: volunteer C; orange: volunteer D). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size. Data for volunteers A and B are from Carstens *et al.* (2003).

3.1.5 Comparative excretion of ethylene glycol, glycolic acid, and oxalic acid in urine

The total amounts of $^{13}C_2$-EG and its metabolites excreted in urine are shown in Table 10. On average 78, 11, and 1.3 µmol were excreted as $^{13}C_2$-EG, $^{13}C_2$-GA, and $^{13}C_2$-OA, respectively. The Table 10 shows also the percentages that these amounts

represent of the inhaled doses of $^{13}C_2$-EG. On average, 5.5, 0.76, and 0.1 % of appeared in urine as $^{13}C_2$-EG, $^{13}C_2$-GA, and $^{13}C_2$-OA, respectively. This indicates that on average only 6.4 % of the inhaled dose was excreted in urine. Consequently, inhaled EG is extensively biotransformed to other metabolites via the intermediary metabolism. The total amounts of endogenous, unlabelled EG, GA and OA excreted in urine within 24 h are shown in Table 11. On average, 24.5, 312, and 186 µmol were excreted in urine as EG, GA, and OA, respectively. The excreted ^{13}C-labelled compounds normalised for the excreted unlabelled ones are also presented in Table 11.

Volunteer	**^{13}C-labelled compound in urine [µmol]**			**^{13}C-labelled compound [% of inhaled $^{13}C_2$-EG]**		
	$^{13}C_2$-EG	**$^{13}C_2$-GA**	**$^{13}C_2$-OA**	**$^{13}C_2$-EG**	**$^{13}C_2$-GA**	**$^{13}C_2$-OA**
A	91.3	10.1	1.13	6.4	0.70	0.08
B	125	12.5	3.70	9.3	0.92	0.28
C	51.7	9.50	0.25	3.2	0.59	0.02
D	44.2	12.4	0.40	3.0	0.82	0.03

Table 10: ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG), ^{13}C-labelled glycolic acid ($^{13}C_2$-GA), and ^{13}C-labelled oxalic acid ($^{13}C_2$-OA) excreted in urine after 4 h exposures to vaporous $^{13}C_2$-EG given absolutely and as percentages of the inhaled doses of $^{13}C_2$-EG. Data for volunteers A and B are from Carstens *et al.* (2003).

It is interesting to calculate, how the amounts of ^{13}C-labelled EG, GA and OA excreted in urine after 4 h exposures to $^{13}C_2$-EG vapours compare to the corresponding background levels (Table 11). Following inhalation of $^{13}C_2$-EG, on average 359 % of EG, 5.7 % of GA and 0.75 % of OA appeared in urine as labelled compound if compared to the corresponding endogenous amounts excreted within 24 h. These numbers indicate that the additional burden by the metabolites GA and

OA resulting from an uptake of about 1500 µmol EG is very low if compared with the unavoidable background.

Volunteer	Unlabelled compound in urine [µmol]			^{13}C-labelled compound [% of unlabelled compound]		
	EG	GA	OA	$^{13}C_2$-EG	$^{13}C_2$-GA	$^{13}C_2$-OA
A	18.2	274	215	502	3.7	0.5
B	24.5	88.0	177	508	14	2.1
C	16.5	337	232	313	2.8	0.1
D	38.7	550	121	114	2.3	0.3
Mean±SD (n=4)	24.5±10.1	312±191	186±49.2	359±187	5.7±5.6	0.75±0.91

Table 11: Amounts of endogenous, unlabelled ethylene glycol (EG), glycolic acid (GA) and oxalic acid (OA) excreted in urine within 24 h. Comparatively, total amounts of urinary ^{13}C-labelled EG, ^{13}C-labelled GA, and $^{13}C_2$-labelled OA, excreted after 4 h exposures to $^{13}C_2$-EG vapours, are presented as percentages of the corresponding endogenous compounds. Data for volunteers A and B are from Carstens *et al.* (2003).

3.1.6 Extrapolation to workplace conditions

Based on the presented data, an extrapolation of blood concentrations and urinary excretions can be done for workplace conditions as described by Carstens *et al*. (2003) considering the MAK value of 10 ppm, an 8 h exposure and the alveolar ventilation (Q_{alv}) of 20 l/min which is determined for a reference man of 70 kg bw with a physical activity of 50 W (Åstrand, 1983). At workplace, it is assumed that Q_{alv} is considerably higher than at rest. The allometrically calculated Q_{alv} for the volunteers [$20 \cdot (\text{individual body weight}/70)^{2/3}$] ranged from 17.4 to 24.7 l/min (volunteer A: 24.7 l/min; volunteer B: 17.4 l/min; volunteer C: 21.1 l/min; volunteer D: 20 l/min). The plasma concentration of EG at the end of an 8 h exposure (C_8) to

10 ppm EG (corresponding to 25.7 mg/m^3 or 0.414 µmol/l) are expected to be [$C_8 = \frac{Q_{alv} \cdot 60 \cdot 0.414}{V_d \cdot k} \cdot (1 - e^{-k \cdot 8})$], thus between 17.0 and 27.5 µmol/l (volunteer A: 23.0 µmol/l; volunteer B: 27.5 µmol/l; volunteer C: 17.0 µmol/l; volunteer D: no data) using the individual calculated values of Q_{alv} and the measured values of V_d and $t_{1/2}$ of EG. The inhalation rates of EG (individual $Q_{alv} \cdot 60 \cdot 0.414$) in the four volunteers under workplace conditions are predicted to be between 432 and 614 µmol/h (volunteer A: 614 µmol/h; volunteer B: 432 µmol/h; volunteer C: 524 µmol/h; volunteer D: 497 µmol/h; mean±SD: 517±75 µmol/h), which is 1.3 to 1.7 times higher than those obtained in the experiments with the resting volunteers (between 335 and 403 µmol/h; Table 8). The corresponding maximum GA concentrations in plasma are also expected to be 1.3 to 1.7 times higher than the above estimated "worst case" concentrations of GA that are reached after an assumed 8 h exposure experiment at rest. Under workplace conditions of EG exposure (10 ppm, 8 h, 50 W), GA concentrations in plasma should amount to values between 3.1 and 6.8 µmol/l (volunteer A: 3.1 µmol/l; volunteer B: 4.7 µmol/l; volunteer C: 6.8 µmol/l; volunteer D: 6.4 µmol/l; mean±SD: 5.25±1.70 µmol/l). These maximum GA concentrations represent 12-23 % of the background GA concentrations in plasma. The dose of EG taken up from each volunteers at workplace ($Q_{alv} \cdot 60 \cdot 0.414 \cdot 8$) is expected to be between 3500 and 4910 µmol (volunteer A: 4910 µmol; volunteer B: 3500 µmol; volunteer C: 4160 µmol; volunteer D: 3980 µmol; mean±SD: 4138±586 µmol), which is 2.6 to 3.4 times higher than in the experiments lasting only 4 h. Consequently, under conditions of workplace exposure to 10 ppm EG, the AUC_0^∞ of EG in plasma and the amounts of EG, GA, and OA excreted in urine is predicted to be 3.40 (volunteer A), 2.60 (volunteers B and C), and 2.66 (volunteer D) times the corresponding values of the ^{13}C-labelled compounds shown in Tables 8 and 10 (AUC_0^∞: volunteer A: 258 µmol·h/l; volunteer B: 260 µmol·h/l; volunteer C: 159 µmol·h/l; volunteer D: not available; mean±SD: 226±58 µmol·h/l. EG in urine: volunteer A: 310 µmol; volunteer B: 325 µmol; volunteer C: 134 µmol; volunteer D: 118 µmol; mean±SD: 223±112 µmol. GA in urine: volunteer A: 34.3 µmol; volunteer B: 32.5 µmol; volunteer C: 24.7 µmol; volunteer D: 33.0 µmol; mean±SD: 31.1±4.35 µmol. OA in urine: volunteer A: 3.8 µmol; volunteer B: 9.6 µmol; volunteer C: 0.65

µmol; volunteer D: 1.1 µmol; mean±SD: 3.79±4.12 µmol). The same increases are expected in the relative urinary excretion given in Table 11. Compared to the corresponding background excretion within 24 h, the EG metabolites excreted from an 8 h workplace exposure are predicted to range from 6.0 to 36.9 % of the corresponding background for GA and from 0.28 to 5.4 % for OA. For EG, a 3.0 to 17.2fold increase over the urinary background is calculated.

3.2 Dermal exposure to liquid ^{13}C-labelled ethylene glycol

In separate experiments, the four volunteers were exposed over 4 h (volunteer A and D) and 6 h (volunteer B and C) to liquid $^{13}C_2$-EG, which was applied to cellulose gauze placed on the inner forearm (see 2.4.2). During and after the experiments, the volunteers did not report any adverse effect related to the exposure. The pH measurements in blood and urine (Table 12) showed normal physiological values [blood: 7.37-7.43 (Thews *et al*., 1991) and urine: 4.8-7.5 (Documenta Geigy, 1975)].

Volunteer	pH value (mean±SD)	
	Blood (n=20)	Urine (n=10)
A	7.41±0.02	6.75±0.59
B	7.42±0.02	6.20±0.21
C	7.43±0.02	6.92±0.53
D	7.40±0.02	6.38±0.48

Table 12: pH values in blood and urine of volunteers collected during and after dermal exposures to $^{13}C_2$-ethylene glycol.

3.2.1 Dose of ethylene glycol taken up and further uptake parameters

In order to determine the dose taken up, the amounts of $^{13}C_2$-EG, remaining on the skin and in the cellulose gauze and polyethylene sheet, were measured at the end of the exposures and subtracted from the administered amounts of $^{13}C_2$-EG (Table 13). Unfortunately, this simple method did not lead to reliable results since the recovery of

EG was 94±14 %. The error (14 %) in the determination of the dose corresponds to the amount of 2000 µmol being about 10 times larger than the actual amount taken up. The amount was calculated on the basis of the AUC_0^∞ of $^{13}C_2$-EG in plasma. Considering that the AUC represents the internal $^{13}C_2$-EG burden, the $^{13}C_2$-EG dose (D_{dermal}) taken up following epidermal $^{13}C_2$-EG administration, was calculated using equations 18 and 19 (see 2.6 by comparing the resulting AUC_{dermal} of $^{13}C_2$-EG with the $AUC_{inhalation}$ of $^{13}C_2$-EG after inhalation of a defined $^{13}C_2$-EG dose. The amount of D_{dermal} taken up transdermally was rather small (150-189 µmol; Table 13). Only a very small percentage (1.0-1.4 %) of the dermally administered dose was taken up during the occlusive exposure. D_{dermal} obtained for volunteer B was about 1.3 times higher than that for volunteer A corresponding to the longer exposure time. However, such an effect was not observed for volunteer C. The excreted amounts of $^{13}C_2$-EG and $^{13}C_2$-GA could also be used for calculating D_{dermal} by assuming that the excreted amounts and D_{dermal} parallel in the inhalation and dermal experiments. However, from the dose of $^{13}C_2$-EG inhaled, on average only 5.5 % and 0.76 % appeared in urine as $^{13}C_2$-EG and $^{13}C_2$-GA, respectively (Table 10). These values are comparable with the precision of the corresponding analytical method defined by its CV (2.5 and 4 % for $^{13}C_2$-EG and $^{13}C_2$-GA, respectively). Consequently, a precise dose estimation could not be done on the basis of the urinary excretion data and was therefore omitted. Using D_{dermal}, further uptake parameters of dermal applied EG were accessible (Table 13). The flux (J) of EG was calculated between 0.39 and 0.57 µmol/cm^2·h (Table 13) using equation 20 (see 2.6 by considering D_{dermal}, the exposed skin area (66 cm^2), and the duration of exposure (see 2.4.2). The flux represents an average value over the whole exposure time. The permeability constant of EG (K_p) was obtained by dividing the flux with the concentration of the pure $^{13}C_2$-EG (17950 µmol/ml) on the skin surface (equation 21; see 2.6). Both J and K_p show about a 1.5fold variation.

Volunteer	Applied amount [µmol]	Residual amount [µmol] (mean±SD, n=3)	D_{dermal} [µmol]	J [µmol/cm²·h]	K_p [cm/h·10⁻⁵]
A	14000	10795±181	150	0.57	3.2
B	13454	10838±271	189	0.48	2.7
C	14000	9783±193	156	0.39	2.2
D	14000	11420±238	n.a.	n.a.	n.a.

Table 13: Amount of epidermally applied liquid ^{13}C-labelled ethylene glycol (EG), residual amount of ^{13}C-labelled EG at the end of exposure, and amount of ^{13}C-labelled EG taken up (D_{dermal}), as calculated for the individual dermal exposure experiments. The flux (J), and the permeability constant (K_p) of EG were calculated using equations 20 and 21.
n.a.: not available.

3.2.2 Ethylene glycol in plasma and urine

The concentration-time courses of $^{13}C_2$-EG in plasma are shown in Figure 29 for volunteers A and D, who had been epidermally exposed over 4 h and, in Figure 30, for volunteers B and C, who had been exposed over 6 h. $^{13}C_2$-EG could not be quantified in plasma before 2.5 h (volunteer A and D) and 4 h (volunteer C) after starting the exposure. $^{13}C_2$-EG increased continuously with the exposure time to maximum values reached at about the end of the exposures (absorption phase). Maximum concentrations in plasma of volunteers A, B, and C were between 1.1 and 2.0 µmol/l. Though $^{13}C_2$-EG could be detected in plasma of volunteer D (Figure 29), its concentrations were at each time point below the limit of quantification (0.6 µmol/l). After the end of the exposures, $^{13}C_2$-EG concentrations declined rapidly (elimination phase). 24 h after start of the exposures, $^{13}C_2$-EG was no longer detectable. The elimination phase was used to calculate $t_{1/2}$ of $^{13}C_2$-EG. It was between 2.8 and 4.0 h (Table 14). The values are higher than those obtained after

inhalation exposure, indicating that epidermally applied EG was delivered from skin into blood even after the end of exposure, when the skin had been washed with water.

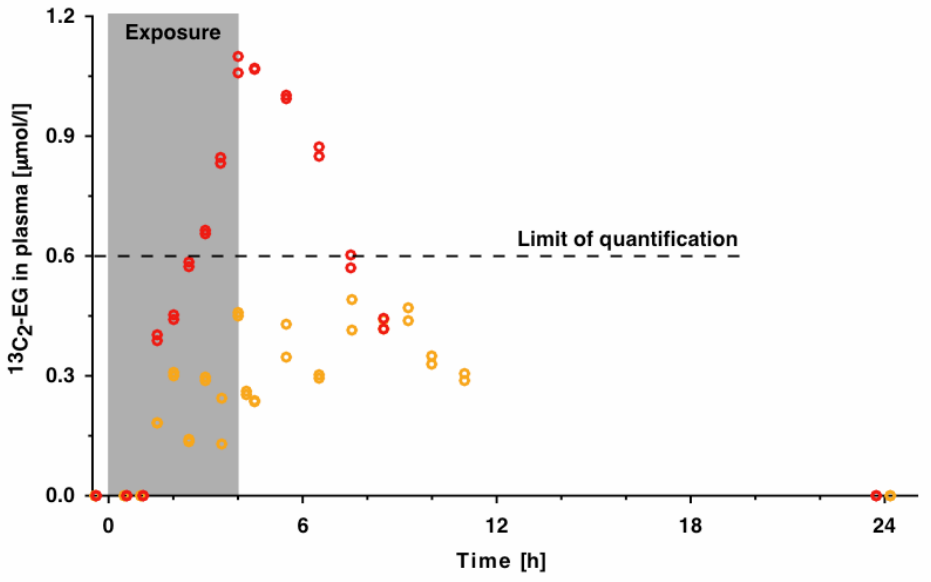

Figure 29: Concentration-time courses of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) in plasma during and after 4 h epidermal exposures to liquid $^{13}C_2$-EG (red: volunteer A; orange: volunteer D).

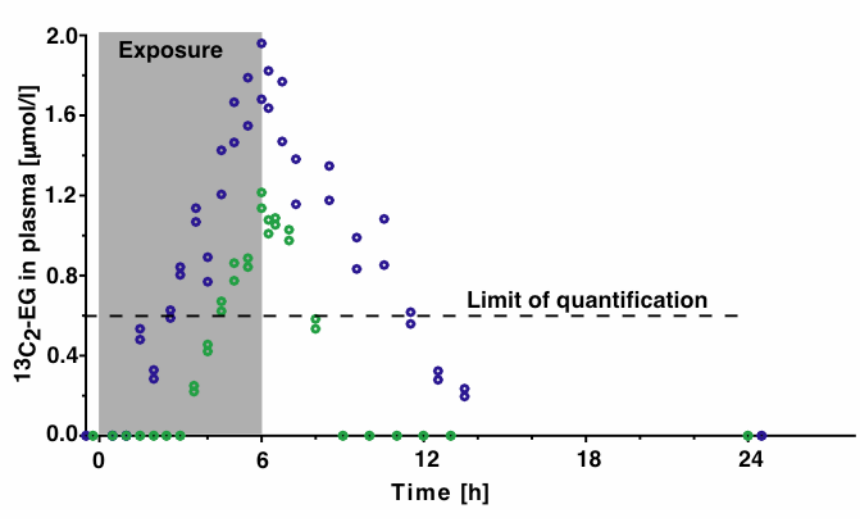

Figure 30: Concentration-time course of ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) in plasma during and after a 6 h dermal exposure to liquid $^{13}C_2$-EG (blue: volunteer B; green: volunteer C).

Consequently, the calculated $t_{1/2}$ represent a mixture of both transdermal uptake and elimination of $^{13}C_2$-EG. The AUC_0^∞ values (Table 14) were distinctly smaller than those obtained in the inhalation experiments (Table 8). The expected C_{ss} of $^{13}C_2$-EG in plasma (Table 14) were calculated using equation 22 (see 2.6, considering infinitely long epidermal exposure to pure $^{13}C_2$-EG. The required J values were taken from Table 13, the V_d from the inhalation experiments (Table 8) and the values of the apparent elimination rate constants were calculated by means of equation 7 (see 2.6) using the $t_{1/2}$ values (Table 14) determined in the epidermal application studies. The C_{ss} values are in agreement with the $^{13}C_2$-EG concentration-time courses measured in plasma (Figures 29 and 30).

Volunteer	**$t_{1/2}$ [h]**	**AUC_0^∞ [µmol/l·h]**	**C_{ss} [µmol/l]**
A	3.9	8.0	2.8
B	4.0	14.1	3.5
C	2.8	5.9	1.5
D	n.a.	n.a.	n.a.

Table 14: Calculated toxicokinetic parameters of ^{13}C-labelled ethylene glycol (EG) in volunteers A, B, and C following epidermal (skin area of 66 cm^2) exposure to liquid ^{13}C-labelled EG; half-life ($t_{1/2}$), area under curve over the time interval 0 to ∞ (AUC_0^∞) in plasma, the expected concentration at steady-state (C_{ss}) in plasma.
n.a.: not available.

The cumulative amounts of $^{13}C_2$-EG and unlabelled EG excreted in urine are shown in Figures 31 (volunteers A and D; exposure duration 4 h) and 32 (volunteers B and C; exposure duration 6 h).
The excretion of unlabelled EG increased almost linearly over time in volunteers A, C, and D (Figure 31, part I and Figure 32, part I). For an unknown reason, linearity was less pronounced in volunteer B (Figure 32, part I). The amounts of unlabelled EG

excreted within 24 h were between 16.6 and 35.0 µmol (Table 17) covering the same range as in the inhalation studies (Table 11).

The excretion of $^{13}C_2$-EG increased over time and reached a plateau after 24 h and 28 h (Figure 31, part II; exposure duration 4 h), and after 30 h (Figure 32, part II; exposure duration 6 h). The time to reach the plateau were distinctly longer than in the inhalation experiments (Figure 25), indicating that there was a $^{13}C_2$-EG uptake from skin even after the end of the epidermal exposures. The larger amounts of $^{13}C_2$-EG excreted by volunteers B and C correspond to the 1.5 times longer exposure time as compared to the volunteers A and D (Table 16). Only a small portion (5.3-11.5 %) of the transdermal $^{13}C_2$-EG-doses appeared in urine (Table 16). A similar observation was made in the inhalation experiments (Table 10).

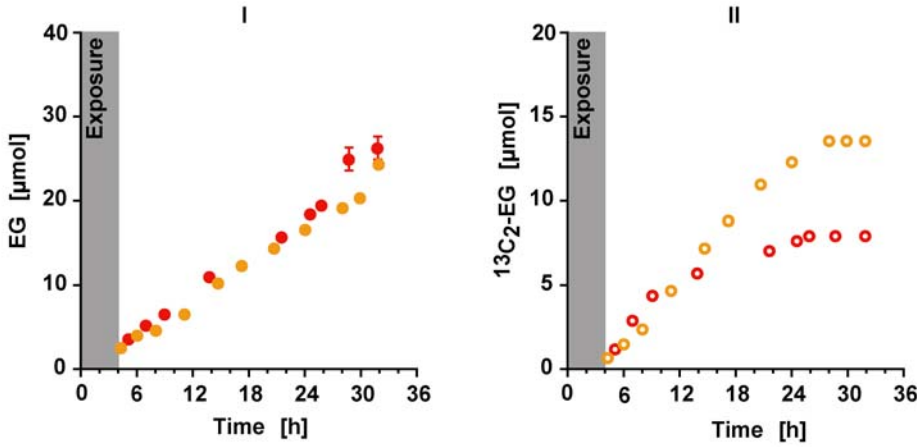

Figure 31: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) ethylene glycol (EG) determined in experiments with 4 h epidermal exposures to liquid $^{13}C_2$-EG (red: volunteer A; orange: volunteer D). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size.

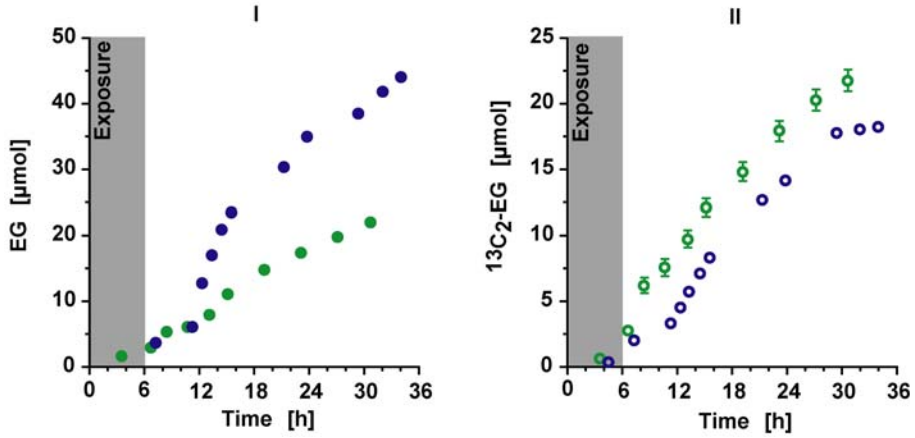

Figure 32: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) ethylene glycol (EG) during and after 6 h epidermal exposures to liquid $^{13}C_2$-EG (blue: volunteer B; green: volunteer C). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size.

3.2.3 Glycolic acid in urine

$^{13}C_2$-GA could be determined in urine but not in plasma (limit of quantification in plasma=0.8 µmol/l). The cumulative amounts of $^{13}C_2$-GA and unlabelled GA excreted in urine of the volunteers following 4 h and 6 h epidermal exposure to $^{13}C_2$-EG are given in Figures 33 (volunteers A and D) and 34 (volunteers B and C).

The excretion of unlabelled GA in urine of the volunteers increased almost linearly over the time (Figure 33, part I and Figure 34, part I). This was not unexpected, since the GA excretion is not related to the exposure but mostly to endogenous GA formation in the intermediary metabolic pathway and to GA intake via food. The average amount of unlabelled GA excreted within 24 h was 455 µmol (range: 196-695 µmol; Table 17). There was a considerable variation (3.5fold) among the volunteers. The results are similar to those obtained in the inhalation experiments (Table 11).

Only in volunteer A the $^{13}C_2$-GA concentration in urine after about 9 h was above the limit of detection (Figure 33, part II). The cumulative urinary excretion amounted to 0.87 µmol (Table 16), corresponding to 0.6 % of the $^{13}C_2$-EG dose taken up

(D_{dermal}=150 µmol; Table 13). This value (0.6 %; Table 16) is almost the same as that found in the urine of the volunteer after inhalation exposure (0.7 %; Table 10).

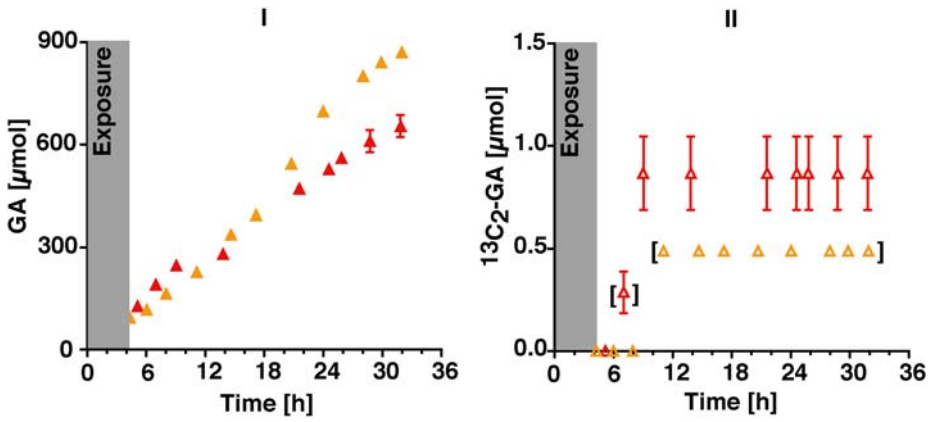

Figure 33: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) glycolic acid (GA) determined in experiments with 4 h epidermal exposures to liquid ^{13}C-labelled ethylene glycol (red: volunteer A; orange: volunteer D). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size. Values in brackets are only estimates because measured concentrations were below the limit of quantification (2.9 µmol/l).

For volunteers B and D the excreted amounts are given in brackets (Figure 33, part II; Figure 34, part II; Table 16) since the concentrations of $^{13}C_2$-GA in urine were below the limit of quantification (2.9 µmol/l). However, $^{13}C_2$-GA could be detected well after about 11 h following start of exposure. No $^{13}C_2$-GA could be detected in urine of volunteer C at all.

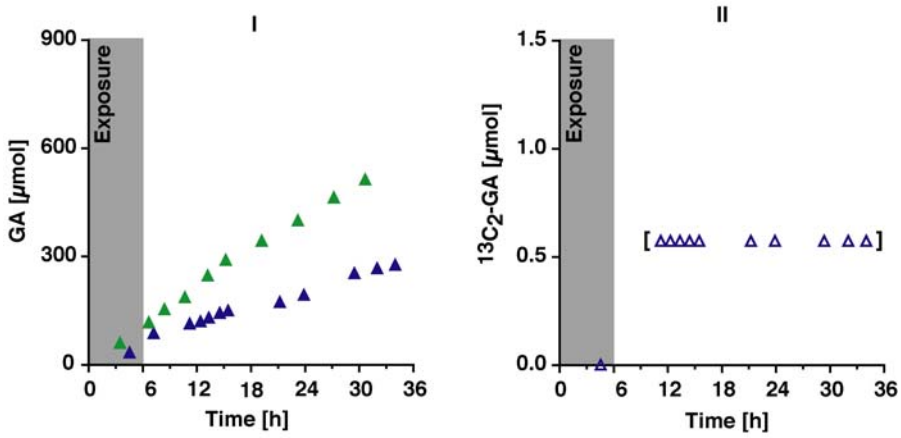

Figure 34: Cumulative urinary excretion of unlabelled (I) and ^{13}C-labelled (II) glycolic acid (GA) during and after 6 h epidermal exposures to liquid ^{13}C-labelled ethylene glycol (blue: volunteer B; green: volunteer C). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size. Values in brackets are calculated from determinations that were below the limit of quantification (2.9 µmol/l).

3.2.4 Oxalic acid in urine

No $^{13}C_2$-OA at all could be detected in the urine samples (the limit of quantification was 0.2 µmol/l). However, unlabelled OA could be quantified successfully. The cumulative urinary excretion of OA observed in the 4 h (volunteers A and D) and in the 6 h exposure experiments (volunteers B and C) are shown in Figures 35 and 36, respectively.

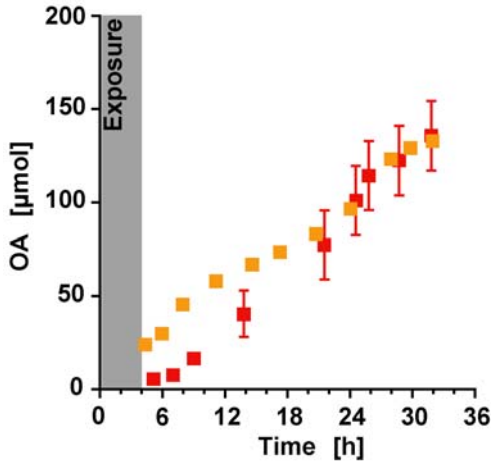

Figure 35: Cumulative urinary excretion of unlabelled oxalic acid (OA) after 4 h epidermal exposure to liquid ^{13}C-labelled ethylene glycol (red: volunteer A; orange: volunteer D). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size.

Similar to data obtained in the inhalation experiments, the excretion of unlabelled OA increased almost linearly over the time indicating that the unlabelled OA is related to the endogenous OA formation in the intermediary metabolism and to OA intake via food. The average amount of OA excreted within 24 h was about 99 µmol (range: 79-122 µmol; Table 17). There was only a 1.5fold variation among the volunteers. The results are similar to those obtained in the inhalation experiments (Table 11).

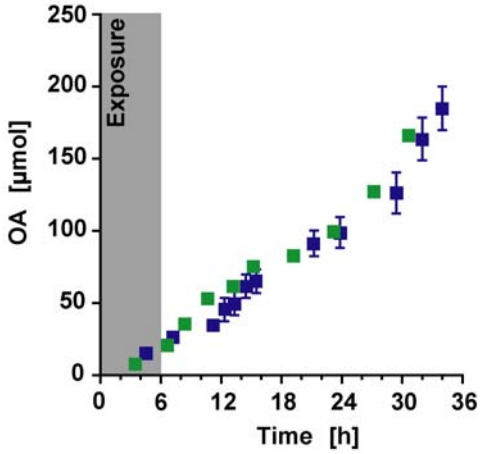

Figure 36: Cumulative urinary excretion of unlabelled oxalic acid (OA) during and after 6 h epidermal exposure to liquid ^{13}C-labelled ethylene glycol (blue: volunteer B; green: volunteer C). Symbols represent means±SD of three measurements per sample. Where SD is not plotted, it is smaller than the symbol size .

3.2.5 Comparative excretion of ethylene glycol and glycolic acid in urine

The total amounts of $^{13}C_2$-EG and $^{13}C_2$-GA excreted over 24 h in urine following dermal exposure to $^{13}C_2$-EG are shown in Table 16. On average, about 13 µmol $^{13}C_2$-EG and about 0.7 µmol $^{13}C_2$-GA were excreted. Table 16 presents also a comparison between these amounts and the doses of $^{13}C_2$-EG taken up transdermally. On average, about 8 and 0.4 % of the dose taken up was recovered in the urine as $^{13}C_2$-EG and $^{13}C_2$-GA, respectively. Therefore, on average only 8.4 % of the $^{13}C_2$-EG dose taken up via skin was excreted in urine. A similar result was obtained in the inhalation experiments (6.4 %; Table 10).

Volunteer	^{13}C-labelled compound in urine [µmol]		^{13}C-labelled compound [% of $^{13}C_2$-EG taken up]	
	$^{13}C_2$-EG	$^{13}C_2$-GA	$^{13}C_2$-EG	$^{13}C_2$-GA
A	7.89	0.87	5.3	0.6
B	14.1	[0.6]	7.5	[0.3]
C	17.9	n.a.	11.5	n.a.
D	12.3	[0.5]	n.a.	n.a.

Table 16: ^{13}C-labelled ethylene glycol ($^{13}C_2$-EG) and ^{13}C-labelled glycolic acid ($^{13}C_2$-GA) excreted in urine after epidermal exposures to liquid $^{13}C_2$-EG, given absolutely and as percentages of the doses of $^{13}C_2$-EG taken up. ^{13}C-labelled oxalic acid could not be detected in any of the samples. Values in brackets are calculated from determinations that were below the limit of quantification (2.9 µmol/l).
n.a.: not available.

The total amounts of endogenous, unlabelled EG, GA and OA excreted in urine within 24 h are shown in Table 17. On average 22, 455, and 99 µmol was excreted in urine as EG, GA, and OA, respectively. Also presented are the excreted ^{13}C-labelled compounds normalised for the excreted unlabelled ones. The amounts of ^{13}C-labelled EG and GA excreted in urine after an average dermal uptake of about 165 µmol $^{13}C_2$-EG are compared to the corresponding background levels (Table 17). Following dermal exposure to $^{13}C_2$-EG, on average 64 % of EG and 0.19 % of GA appeared in the urine as labelled compound if compared to the corresponding endogenous amounts excreted within 24 h. Obviously, the additional burden by the metabolite GA resulting from an average dermal uptake of 165 µmol EG is very low if compared with the unavoidable background.

Volunteer	Unlabelled compound in urine [µmol]			^{13}C-labelled compound [% of unlabelled background]	
	EG	GA	OA	EG	GA
A	18.4	515	122	42.8	0.17
B	35.0	196	99	40.2	[0.3]
C	18.1	414	79	98.9	n.a.
D	16.6	695	97	74.1	[0.1]

Table 17: Amounts of endogenous, unlabelled ethylene glycol (EG), glycolic acid (GA) and oxalic acid (OA) excreted in urine within 24 h. Comparatively, total amounts of urinary ^{13}C-labelled EG and ^{13}C-labelled GA, excreted after epidermal exposures to liquid $^{13}C_2$-EG, are presented as percentages of the corresponding endogenous compounds. ^{13}C-labelled oxalic acid could not be detected in any of the samples. Values in brackets are calculated from determinations that were below the limit of quantification (2.9 µmol/l).
n.a.: not available.

3.2.6 Extrapolation to workplace conditions

Based on the transdermal fluxes of $^{13}C_2$-EG (Table 13), an extrapolation can be done for a continuous epidermal exposure of both hands (skin area 910 cm^2, Health Canada 1995) to liquid EG over 8 h as it might occur at a workplace. Using the corresponding J values, the amounts of EG taken up (J·910 cm^2·8 h) are calculated to be 4100, 3500, and 2840 µmol for volunteers A, B, and C, respectively. The plasma concentration (C_8) of EG reached after 8 h under these epidermal exposure conditions can be calculated considering first order kinetics by the following equation:

$$[C_8 = \frac{J \cdot 910}{V_d \cdot k} \cdot (1 - e^{-k \cdot 8})]$$

The obtained concentrations are 30 µmol/l (volunteer A), 36 µmol/l (volunteer B), and 18 µmol/l (volunteer C) using the corresponding values for J, V_d, and $t_{1/2}$ of the three volunteers following epidermal application of $^{13}C_2$-EG. Similar values

(volunteer A: 31 µmol/l; volunteer B: 35 µmol/l; volunteer C: 22 µmol/l) were obtained by linearly extrapolating the $^{13}C_2$-EG concentrations in plasma determined at the end of the 8 h transdermal exposures (Figures 29 and 30) and considering that the transdermal uptake rate of EG (J·910 cm^2) through the skin area of both hands is 13.8 times larger (volunteer A: 519 µmol/h; volunteer B: 437 µmol/h; volunteer C: 355 µmol/h) than it was in the experiments with only 66 cm^2 exposed skin area. The AUC_0^∞ values of EG expected from epidermal exposure of both hands to liquid EG for 8 h are 13.8·8/4 times (volunteer A) and 13.8·8/6 times (volunteers B and C) of the values obtained after 4 h (volunteer A) and 6 h (volunteers B and C) epidermal exposures (Table 14), hence ranging between 109 and 259 µmol·h/l (AUC_0^∞: volunteer A: 221 µmol·h/l; volunteer B: 259 µmol·h/l; volunteer C: 109 µmol·h/l; volunteer D: not available).

The urinary excretion data can be also extrapolated for workplace conditions by considering the EG uptake resulting from continuous wetting of both hands over 8 h. It is expected that the exposure related excretion increases in proportion to the dose taken up. Consequently, the exposure related excretion should amount to 27.6 times the values determined after the 4 h exposures and 18.4 times those obtained after 6 h exposures (Table 16). The same increases are also expected in the relative urinary excretion data (Table 17). Consequently, the increases of the toxic metabolite GA over the urinary backgrounds are predicted to range from 2.8 to 5.5 % (volunteer A: 4.7 %; volunteer B: 5.5 %; volunteer D: 2.8 %). For EG, a clear increase (7.4-21fold) over the urinary background is expected (volunteer A: 12fold; volunteer B: 7.4fold; volunteer C: 18fold; and volunteer D: 21fold).

3.2.7 Comparison of inhalative and dermal exposure to ethylene glycol

Table 18 opposes the calculated EG concentrations in plasma and the urinary excretion data of EG and GA of the volunteers resulting from 8 h exposures to 10 ppm vaporous EG under workplace conditions to the corresponding data resulting from continuous wetting of both hands with pure liquid EG. Considering that the predicted urinary amounts of GA, following transdermal EG uptake by volunteers B

and D, are based on concentrations that were below the limit of quantification, it becomes evident from comparing mean values and standard deviations that equally long lasting inhalation exposure to 10 ppm vaporous EG under workplace conditions or transdermal exposure of both hands by pure liquid EG result in about the same body burdens by EG or GA. A further method to compare EG inhalation (10 ppm, 8 h, 50 W) with continous wetting of both hands with liquid EG for 8 h is based on the AUC_0^∞ of EG in plasma (Table 19).

Volunteer	Inhalation of vaporous EG*			Transdermal uptake of liquid EG*		
	EG in plasma at 8 h [µmol/l]	Total amount excreted in urine [µmol]		EG in plasma at 8 h [µmol/l]	Total amount excreted in urine [µmol]	
	EG	EG	GA	EG	EG	GA
A	23.0	310	34.3	30	218	24
B	27.5	325	32.5	36	259	[11]
C	17.0	134	24.7	18	329	n.a.
D	n.a.	118	33.0	n.a.	339	[14]
Mean±SD (n=3-4)	22.5±5.3	222±111	31.1±4.4	28±9.2	286±57.8	[16.3±6.8]

Table 18: Ethylene glycol (EG) concentrations in plasma and total amounts of EG and glycolic acid (GA) in urine.

***** Calculations consider the exposure of the volunteers to vaporous EG (workplace conditions: 10 ppm, 8 h, 50 W) or to liquid EG, wettening the skin area of both hands (910 cm^2, 8 h). Values in brackets are calculated from determinations that were below the limit of quantification (2.9 µmol/l).

n.a.: not available.

The obtained mean values are again similar and there is no statistical difference between them (paired t-test; confidence intervals 95 %). The mean values and the standard deviations obtained by the three methods of comparing the EG-burdens resulting from inhalation of vaporous EG (10 ppm, 8 h, 50 W) and from transdermal uptake of liquid EG through the surface of the hands (910 cm^2, 8 h) can be used to estimate the reliability of the skin uptake data by using a coefficient of variation (CV). By comparing the mean values of each method i, an EG workplace (50 W) exposure concentration (x_i; ppm) that is equivalent to the equally time-long wetting of both hands with liquid EG was calculated [x_i=10 ppm times the mean (m_{di}) from transdermal uptake divided by the corresponding mean (m_{ii}) from inhalation]. The corresponding standard deviation (SD_{xi}) was obtained using the standard deviations of the means (SD_{di} and SD_{ii}) according to:

$SD_{xi}=x_i\,[(SD_{ii}/m_{ii})^2+(SD_{di}/m_{di})^2]^{1/2}$ (Sachs, 2002).

Volunteer	**AUC_0^∞ of EG in plasma resulting from exposure to gaseous EG [µmol/l·h]**	**AUC_0^∞ of EG in plasma resulting from transdermal uptake of liquid EG [µmol/l·h]**
A	258	221
B	260	259
C	159	109
D	n.a.	n.a.
Mean±SD (n=3)	226±58	196±78

Table 19: AUC_0^∞ of ethylene glycol (EG) in plasma calculated for the exposure of volunteers to vaporous EG (10 ppm, 8 h, 50 W) or to liquid EG, wettening the skin area of both hands (910 cm^2, 8 h).
n.a.: not available.

The coefficient of variation (CV_i) of the method i was obtained by multiplying SD_{xi} with 100 followed by division with its x_i. Table 20 shows the x_i concentrations and

their CV_i values being equivalent to the transdermal uptake of EG through the surface of both hands within the same time frame. The agreement of the 3 methods is striking. The mean exposure concentrations that are equivalent to dipping both hands into liquid EG range from 8.7 ppm to 12.9 ppm. Also the CV values ranging from 40 % to 54 % of the corresponding exposure concentration are not too large considering that they were obtained from studies with 3-4 male volunteers differing considerably in age (31-56-years) and bw (57-96 kg).

Method i	**EG vapour concentration equivalent to wetting of both hands with liquid EG over equally long time [ppm]***	**Coefficient of variation [%]**
i corresponds to the EG concentration in plasma	12.4	40
i corresponds to the EG excretion in urine	12.9	54
i corresponds to the AUC_0^∞ of EG in plasma	8.7	47

Table 20: Concentration of vaporous ethylene glycol (EG) at workplace conditions (50 W) resulting in the same body burden as an equally long dermal exposure of both hands (skin area 910 cm^2) to liquid EG. The equivalent concentrations were calculated on the basis of the EG concentrations in plasma (n=3), the total urinary EG excretion (n=4), and the AUC_0^∞ of EG in plasma (n=3), respectively.

* Calculated from mean values.

4. Discussion

4.1 Exposure systems

4.1.1 Inhalation exposure

In order to carry out the inhalation experiments with vaporous $^{13}C_2$-EG, closed exposure conditions were chosen for two reasons. First, the exact amount of inhaled $^{13}C_2$-EG could easily be quantified. Second, the amount of the expensive $^{13}C_2$-EG could be minimized. Much higher amounts of $^{13}C_2$-EG would have been required if an open system containing $^{13}C_2$-EG vapours in a continuous airflow would have been used. With the closed inhalation system for vapours of low volatile liquids developed in this laboratory and first presented in by Carstens *et al*. (2003), a quasi-constant inhalation uptake could well be achieved due to the small ratio of the short time spans (15 min) between the moments of complete inhalation of defined amounts of $^{13}C_2$-EG vapour and the relative long half time of $^{13}C_2$-EG in plasma.
Shortly before each inhalation process, warming-up the injected liquid $^{13}C_2$-EG was required for rapid and complete evaporation. In order to avoid heat decomposition (starting at 350-400 °C; Herndon and Reid, 1928), the maximum gas temperature of 140 °C was never exceeded.

4.1.2 Epidermal exposure

For occlusive epidermal exposure of humans to liquids, “chambers” as the Hill Top, the Fin, the Duhring or the Agfa-Bayer chamber are commercially available. The widely used “Hill Top Chambers®” are moulded plastic semi shells with a double rim to obtain close and occlusive fit to the skin. The chamber contains a Webril® pad for holding the test substance. The contact area to the skin is limited by the chamber diameters of 19 or 25 mm (Hill Top Research, INC.). Other commercial chambers are even smaller, for instance the diameter of the extra large “Finn chamber®” is only 18 mm (product information: Allerderm Laboratoties). The occlusive exposure system presented here enabled to apply a defined amount of the liquid EG over several hours onto a much larger circumscribed skin area of 66 cm^2. The correct

determination of the exposed skin area was possible due to the barrier made out of the vacuum grease Glisseal® that is insoluble in water and EG (product information: Keller, Mannheim; confirmed by own measurements, not shown). This grease, the skin, and the covering polyethylene foil formed an elastic, non-cumbersome, and gastight vessel for the EG soaked gauze. Accurate knowledge of the exposed skin area was a most important precondition for the determination of the EG flux through the skin. Recently, a similar system was described by which the backs of the hands of 7 subjects were exposed for 2 h to the solvent N-methyl-2-pyrrolidone (Bader *et al*., 2005). The surfaces of the applied pads was 3.77 times smaller than those used in the present study and there was no barrier against leakage except medical adhesive tape used for fixing the aluminium foil sheet that covered the pads. Concentrations of N-methyl-2-pyrrolidone, measured in urine of the participants, scattered by an about 3 times larger range than those of urinary EG in the present study. A series of factors influence transdermal transport of chemicals: Their physicochemical properties, occlusive or open administration, hydration, temperature, and thickness of the stratum corneum, the most critical layer for transdermal absorption. Because the physiological properties of the skin are changing with age, transdermal uptake of chemicals is also age dependent (reviewed in Kennish and Reidenberg, 2005).

It is evident that the exposed skin surface has to have a defined area in order to obtain reliable results for calculating the flux across skin. Several authors studied transdermal absorption of liquids by dipping fingers or hands into them (Stewart and Dodd, 1964; Mraz and Nohova, 1992). Unfortunately, calculation of fluxes from such studies is less precise because the exposed skin area can only be estimated.

4.2 Ethylene glycol, glycolic acid, and oxalic acid in urine and plasma

4.2.1. Background values

Background amounts of EG, GA and OA in urine of the four volunteers excreted within 24 h were comparable with the amounts published earlier for two of the volunteers (Carstens *et al*., 2003).

Amounts of EG were between 16.5 and 38.7 µmol/24 h fitting well into the range from 0-80 µmol EG/24 h that was calculated by Carstens *et al*. (2003) from published

background ratios of EG to creatinine in non-exposed persons (mean±SD: 1.7±0.7 mmol/mol creatinine, n=10, Laitinen *et al*., 1995; mean±SD: 0.67±0.72 mmol/mol creatinine, n=41, Laitinen *et al*., 1997; range: 0-5 mmol/mol creatinine, n=64, Gérin *et al*., 1997; median: 0.31 mg/g creatinine, urinary EG concentrations range: 1.1-47 µmoll/l, n=16, Letzel *et al*., 2000). For their calculation, Carstens *et al*. (2003) took a normal creatinine excretion in a healthy man of 1.8 g/24 h, equivalent to 0.016 mol/24 h (Documenta Geigy, 1975) into account. From the data determined by these authors, considerably lower urinary EG concentration are to be expected than those of 270-1240 µmol/l reported by Wills *et al*. (1974). However, the latter authors used highly unspecific methods for EG determination in air, blood, and urine, not complying with contemporary analytical requirements.

Amounts of GA in urine ranged from 88-695 µmol/24 h being consistent with the wide range from 75-1372 µmol/24 h published for healthy humans by several other groups (144-1220 µmol/24 h, Petrarulo *et al*., 1998; 291-907 µmol/24 h, n=15, Niederwieser *et al*., 1978; 75-508 µmol/24 h, n=12, Maeda-Nakai and Ichiyama, 2000; 205-1372 µmol/24 h, n=30, Yamaguchi and Ogawa, 1997; 260-790 µmol/24 h, number not given, Chalmers *et al*., 1984). Only in one publication, values smaller than 75 µmol/24 h have been reported (0-1400 µmol/24 h, male humans, Hagen *et al*., 1993,). Plasma concentrations of background GA, measured in the 4 volunteers during the inhalation experiments are given in Table 9. They ranged from 25.8 to 30.3 µmol/l (mean values) with a mean concentration of 28.6±5.20 µmol/l. Similar GA concentrations (6.6-32.9 µmol/l; number not given) were published by Chalmers *et al*. (1984). Other authors have reported lower GA background concentrations in plasma (3.6 µmol/l deproteinised serum, n=4, Schatowitz and Gerken, 1988; 4.51-12.2 µmol/l, n=14, Petrarulo *et al*., 1991; 1.4-7.5 µmol/l, n=39, Hagen *et al*., 1993; approximately 8 µmol/l, Maeda-Nakai and Ichiyama, 2000). However, in these studies, plasma was taken from fasting subjects. Furthermore, as was pointed out by Carstens (2003) who compared in his thesis published methods of EG, GA, and OA determinations, lower values could be expected when determining GA from plasma ultrafiltrate (Petrarulo *et al*., 1991; Hagen *et al*., 1993) because of a probable loss of protein bound GA during ultrafiltrate preparation.

Background OA excretion in urine was between 79 and 232 µmol/24 h fitting well into the range from 86-622 µmol/24 h published by other authors for healthy adults

(Chalmers *et al*., 1984; Hagen *et al*., 1993; Holmes *et al*., 1993; Keßler *et al*., 2002; Maeda-Nakai and Ichiyama, 2000; Marangella *et al*., 1992; Siener and Hesse, 2002; von Unruh *et al*., 1998; Wandzilak *et al*., 1991; Carstens *et al*., 2003). In a recent review on primary hyperoxaluria, an urine oxalate/creatinine ratio of ≤0.04 mol/mol was summarised for healthy adults (Milliner, 2005). This corresponds to a daily OA exretion of ≤640 µmol/24 h, considering a creatinine excretion of 0.016 mol/24 h. It has to be stressed that the OA excretion is also dependent on its dietary intake (about 80-150 mg/24 h or 0.9-1.7 mmol/24 h), although only a small fraction from the daily oxalate intake is absorbed from the intestinal tract (reviewed in Hoppe *et al*., 2003).

4.2.2 Inhalation kinetics

From two of the four volunteers, the kinetic data obtained after $^{13}C_2$-EG inhalation were already published by Carstens (2003) and Carstens *et al*. (2003). The additional data obtained in the present work in two further volunteers are in full agreement with the earlier ones. The $^{13}C_2$-EG doses inhaled were between 1340 and 1610 µmol. The same amounts are inhaled by a 70 kg person exposed over 4 h to 7.0-8.4 ppm EG under workplace conditions. None of the volunteers noticed any exposure related adverse effect. Inhaled $^{13}C_2$-EG was completely taken up by the volunteers; there was no exhalation of unchanged EG. This finding is in agreement with the very high blood:air partition coefficient of almost 18000 (Corley *et al*., 2005). The volume of distribution of $^{13}C_2$-EG (related to its concentration in plasma) was on average 0.87 l per kg bw (range: 0.78-0.91 l per kg bw). It agrees with the physicochemical properties of EG, the blood:saline, and various tissue:blood partition coefficients that had the value of about 1, except the somewhat smaller fat:blood partition coefficient that was about 0.6 (Corley *et al*., 2005). Related to the EG concentration in blood, similar volumes of distribution of 0.54-0.56 l/kg bw were published by Jacobsen *et al*. (1988), of 0.5-0.8 l/kg bw by Eder *et al*. (1998), and of 0.83 l/kg bw by Peterson *et al*., (1981). Elimination of $^{13}C_2$-EG was according to first order with the half-lives between 1.6 and 2.6 h. Only a small percentage (3.0-9.3 %) of the inhaled compound was excreted as unchanged EG within 24 h after start of the exposure. For untreated persons, longer half-lives of up to 10 h and a higher urinary recovery are published (Reif, 1950; Peterson *et al*., 1981; Eder *et al*., 1998; Jacobsen *et al*., 1988; Moreau *et*

al., 1998). However, these values were determined either following voluntary intake of high doses (5, 10, and 12 ml; Reif, 1950) or in EG intoxicated patients showing EG blood concentrations in the millimolar range. In contrast, the present study was carried out at micromolar EG concentrations in plasma. It is highly probable that metabolism of EG was saturated at the high concentrations resulting in longer half-lives. Corresponding findings were obtained in rats (Marshall, 1982; Frantz *et al*., 1996c; Pottenger *et al*., 2001) and in mice (Frantz *et al*., 1996a).

Half-lives of metabolically formed $^{13}C_2$-GA, determined in plasma following $^{13}C_2$-EG inhalation, was between 1.8 and 2.9 h, in agreement with the data of Carstens *et al*., (2003). The half-life of a rapidly eliminated metabolite cannot be shorter than that of its parent compound. From the findings that half-lives of $^{13}C_2$-EG and $^{13}C_2$-GA were very similar, it follows that the elimination of $^{13}C_2$-GA was limited by its formation from $^{13}C_2$-EG. Only 9-24 % of $^{13}C_2$-EG in plasma was detected as $^{13}C_2$-GA. These findings are not surprising considering that GA is only a secondary metabolite of EG (Figure 1).

Corresponding to their short half-lives, urinary excretion of $^{13}C_2$-EG and $^{13}C_2$-GA was completed 24 h after starting the exposure to $^{13}C_2$-EG. Of the inhaled $^{13}C_2$-EG doses 3.0-9.3 % ($^{13}C_2$-EG) and 0.59-0.92 % ($^{13}C_2$-GA) were excreted in urine. The data are in agreement with those published earlier for only two of the volunteers (Carstens *et al*., 2003). Urinary excretion of $^{13}C_2$-OA differed considerably among the volunteers. In the two volunteers investigated by Carstens *et al*. (2003), urinary $^{13}C_2$-OA excretions were completed 22 and 28 h after starting the exposure. In urine of the two other volunteers exposed by inhalation in the present study, $^{13}C_2$-OA was no more measurable after about 12 h following the beginning of the exposures. Also the percentage of the dose of $^{13}C_2$-EG, which was detected as urinary $^{13}C_2$-OA, diverged drastically although being very small (0.02-0.28 %). However, the inter-individual variation in metabolite formation was expected to increase with the numbers of metabolic steps involved. Similar percentages were determined in female rats following $^{13}C_2$-EG administration by gavage. Of the EG dose administered (10 mg/kg bw), 14.6 % were excreted in urine as $^{13}C_2$-EG, 1.4 % as $^{13}C_2$-GA, and 0.4 % as $^{13}C_2$-OA (Pottenger *et al*., 2001). Obviously, EG underlies considerable intermediate metabolism, especially at the micromolar range. This conclusion is also supported by data of Marshall and Cheng (1983) and of Frantz *et al*. (1996c) who

found that about 60 and 40 %, respectively, of $^{14}C_2$-EG administered to rats were exhaled as $^{14}CO_2$ within 4 days post exposure.

4.2.3 Dermal uptake

The outermost layer of the epidermis, the stratum corneum, is biphasic made up of stacks of protein-filled dead corneocytes surrounded by lipid bilayer-membranes. It represents the hydrophobic-hydrophilic lipid-protein partitioned diffusion-limiting barrier for the percutaneous penetration of epidermal applied xenobiotics (reviewed in Wills, 1969; Scheuplein and Blank, 1971; Madison, 2003; Kennish and Reidenberg, 2005). This barrier is not absolute to water and hydrophilic compounds. Gaseous, amphiphilic compounds consisting of small and non charged molecules show the highest rates of diffusion through the epidermis (summarised in Wills, 1969). Considering also the small octanol:water partition coefficient of EG (log K_{ow}=-1.36; USEPA, 1988), a small permeability constant (K_p) was expected for liquid EG. K_p values can differ by orders of magnitude, depending on the physicochemical properties of the substances. For instance, the vapour of the amphiphilic chemical dimethyl formamide has a K_p of 51 cm/h for the rat skin, which means that 2.5 times more dimethyl formamide is taken up through the skin than is inhaled, when rats are exposed to vaporous dimethyl formamide (Filser *et al*., unpublished). In contrast, for liquid EG, a K_p in the range of only $2.2 \cdot 10^{-5}$-$3.2 \cdot 10^{-5}$ cm/h (n=3) was calculated from the present volunteer study. It agrees excellently with results obtained *in-vitro* using excised human skin: K_p values for the transport of liquid EG through human skin *in-vitro* were $1.2 \cdot 10^{-5}$ and $1.4 \cdot 10^{-5}$ cm/h with undiluted and with 50 % aqueous EG, respectively (Sun *et al*., 1995). Corresponding K_p values of $3.1 \cdot 10^{-5}$, $1.2 \cdot 10^{-5}$, and $1.8 \cdot 10^{-5}$ cm/h can be calculated from the amounts of undiluted and 50 and 10 % aqueous EG passing within 8 h through a area of 0.64 cm^2 hydrated excised human skin (Korinth *et al*., 2003). These values demonstrate that the amount of EG diffusing through the skin is indeed proportional to the concentration of EG in the aqueous liquid that is wetting the skin. The value of the K_p for liquid EG ($1.0 \cdot 10^{-4}$ cm/h) obtained by Lodén (1986) who used also human skin *in-vitro*, is 3.1 to 4.5 times

larger than the one determined *in-vivo*. Considering the experimental difference between the *in-vivo* and *in-vitro* measurements, a difference of less than one order of magnitude should not be interpreted as contradictory. Although the *in-vivo* K_p value was determined only in three volunteers, it is concluded to be generally valid, considering its similarity with the *in-vitro* obtained K_p values.

4.3 Is exposure to ethylene glycol under workplace conditions dangerous to health?

From comparison of the AUCs of EG resulting from inhalation exposure to vaporous EG and from epidermal exposure to liquid EG, the present work demonstrates that continuous epidermal exposure of both hands to liquid EG over 8 h is equivalent with inhalation exposure to 10 ppm vaporous EG over 8 h at workplace conditions (50 W), where a worker of 74.8±16 kg bw inhales daily EG amounts of 4138±585 µmol (257±36.3 mg) EG (n=4). This result agrees excellently with the amount of 247 mg EG, as calculated by Carstens *et al.* (2003) for a 70 kg reference man from the inhalation data obtained with only two of the four volunteers. In an early study, a volunteer ingested 5.5, 11, and 13.2 g liquid EG on days 1, 22, and 106. Although these at-once ingested bolus doses were 21, 43, and even 51 times higher than those taken up within in 8 h under workplace exposure to 10 ppm EG, the author did neither detect any crystalls of calcium oxalate in urine nor did he remark any health effect (Reif, 1950). Toxic effects from EG in humans were described from acute poisoning from oral intake of high doses. In chronic exposures to low EG doses as they occur at workplaces, no toxic effects have been detected (Laitinen *et al.*, 1995; Gérin *et al*., 1997).

Developmental and kidney toxicity of EG result from its metabolites GA and OA. The present results demonstrate, that exposure to 10 ppm EG (8 h, 50 W) leads to a GA concentration in plasma of 5.25±1.70 µmol/l. Considering the background GA concentration in plasma of 28.8±5.20 µmol/l, it becomes evident that the maximum increase of GA resulting from exposure to 10 ppm EG under workplace conditions is not higher that the SD of the mean background GA concentration. Comparing the urinary excretion data, similar results were obtained. Inhalation exposure to EG (10 ppm, 8 h, 50 W) was shown in this work to result in total amounts in urine of

31.1±4.35 µmol GA and 3.79±4.12 µmol OA. Considering the corresponding daily (24 h) urinary excretion of background GA (312±191 µmol; Table 11) and background OA (186±49.2 µmol; Table 11), it becomes again evident that inhalation exposure to EG (10 ppm, 8 h, 50 W) leads only to a very small increases in the metabolites GA and OA (on average 10 % GA and 2 % OA) if compared to their background burden. Because both GA and OA burdens remain in their backgroud ranges, when EG exposure is maintained under MAK-conditions or if epidermal exposure by liquid EG does not exceed the area of 910 cm^2 and a daily exposure length of 8 h, it has to be deduced that there is no rational basis for concern about nephrotoxic or developmental effects from such exposure conditions.

This conclusion is also supported by data obtained in rats. According to the data presented in this work, an 8 h exposure of workers to 10 ppm EG could lead to a maximum increase in the GA plasma concentration of 6.8 µmol/l. With the highest background GA concentration of 32.9 µmol/l, reported by Chalmers *et al*. (1984), this would summate to 40 µmol/l. Considering that in rats the lowest NOEL for EG-induced nephrotoxicity was 80 mg/kg bw/day (Blood, 1965) leading to a maximum GA concentration of 144 µmol/l blood (linear extrapolation from the maximum GA blood concentration of 271 µmol/l reached after a dose of 150 mg EG/kg bw; Pottenger *et al*., 2001), and that the NOEL for developmental toxicity in rats was 500 mg/kg bw/day (Neeper-Bradley *et al*., 1995) resulting in a maximum GA concentration in blood of 1723 µmol/l (Pottenger *et al*. 2001), it follows in agreement with Carstens *et al*. (2003) that GA- and OA-induced nephrotoxic or developmental toxic effects resulting from human exposure to 10 ppm EG ("MAK-value") and from continuously wetting of both hands with liquid EG over 8 h are highly unlikely.

The German "MAK-value" of 10 ppm of EG mist is found to have irritating effects on the mucosa without knowledge of the internal burden by the kidney and developmental toxicity producing metabolites GA and OA. From both onsets, comparison of the exposure related burdens by the toxicologically active metabolites GA and OA with the corresponding backgrounds in humans or with the effective burdens in rats, it follows concordantly that there is no risk for kidney damage or teratogenic effects if workers are exposed to EG vapours according to the German "MAK-value" and to liquid EG wettingof both hands during the workshift.

5 Summary

Ethylene glycol (EG), a liquid with a low vapour pressure (0.092 mm Hg at 25 °C), is mainly used for antifreeze formulations, de-icing of airplanes, and in the production of polyethylene terephthalate (PET) and polyester fibers or films. Occupational exposure of workers and of the general population to EG mostly occurs through dermal uptake and inhalation of vapours and mists during handling of EG-containing solutions and through dermal contact with products such as antifreeze solutions, respectively. Additionally, food or beverages can be contaminated by trace amounts of EG migrating from the package material PET or regenerated cellulose film. The EG metabolites are glycolic acid (GA) and oxalic acid (OA). EG, GA, and OA are also produced endogenously in the intermediary metabolism and are natural constituents of food.

Embryotoxic and nephrotoxic effects of EG at high doses have been ascribed to its metabolites GA and OA. There are only very few data available on human body burden by inhaled EG and its toxic metabolites under exposure conditions as might occur at the workplace and no data are published on dermal uptake of humans exposed to liquid EG. Based on only limited information, the Commission for the Investigation of Health and Hazards of Chemical Compounds in the Work Area established a maximum workplace concentration ("MAK-value") for a daily eight-hour exposure (40 h per week) to EG of 26 mg/m^3 (10 ppm).

The "MAK-value" for EG is founded only on the irritating effects observed in a volunteers study. The MAK documentation states, if exposure conditions comply with the "MAK-values", there is no reason to fear a risk of damage to the embryo or fetus. However, no data on the systemic burdens by EG, GA, and OA in EG exposed humans were available to verify this statement. Consequently, for validating the current "MAK-value", it was the aim of this work to quantify in volunteers the burdens by EG, GA and OA resulting from uptake of EG following inhalation and epidermal exposure. In order to establish a risk evaluation, the body burdens were compared with those representing the unavoidable background. For differentiating background from exposure-derived substances, exogenous EG was administrated as the ^{13}C-labelled compound ($^{13}C_2$-EG).

In the present study, two volunteers inhaled $^{13}C_2$-EG vapour within 4 h. Previous results, obtained with another two volunteers (Carstens *et al*., 2003), are also presented. In addition, applying a newly developed method, four volunteers were occlusively exposed up to 6 h to liquid $^{13}C_2$-EG (0.8 ml), applied to 66 cm^2 skin.

After inhalation of $^{13}C_2$-EG and during and after application of liquid $^{13}C_2$-EG onto skin, unlabelled and labelled EG and GA were measured from plasma and unlabelled and labelled EG, GA, and OA from urine of the volunteers. For quantitative analysis, previous developed mass selective and sensitive methods (GC/MSD) were used.

For the determination of EG and $^{13}C_2$-EG, samples were derivatised with n-butylboronic acid. Propylene glycol and 1,3-propandiol served as internal standards. For the determination of GA and $^{13}C_2$-GA, samples were derivatised using SILA (99 % *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide and 1 % of *tert*-butyldimethylchlorosilane). Internal standards were [$^2H_6$]succinic acid ($^2H_6$-SA) and 2-hydroxyisovaleric acid. To detemine OA and $^{13}C_2$-OA, samples were silylated with SILA. Unlabelled SA and $^2H_6$-SA served as internal standards.

The amounts of inhaled $^{13}C_2$-EG (1340-1610 µmol) correspond to inhalation exposures (4 h; alveolar ventilation 20 l/min) ranging from 7.0 to 8.4 ppm vapour concentration. Maximum plasma concentrations ranged from 10.1 to 14.8 µmol/l of $^{13}C_2$-EG and from 0.9 to 2.6 µmol/l of $^{13}C_2$-GA. The average amounts of $^{13}C_2$-EG, $^{13}C_2$-GA, and $^{13}C_2$-OA excreted in urine were 78, 11 and 1.3 µmol, respectively, corresponding on average to 5.5 % of $^{13}C_2$-EG, 0.76 % of $^{13}C_2$-GA, and 0.1 % of $^{13}C_2$-OA of the inhaled dose of $^{13}C_2$-EG. Endogenous, unlabelled EG was not found in plasma (limit of quantification of 7.6 µmol/l). For endogenous GA, the average plasma concentrations were between 25.8 and 30.3 µmol/l. On average, 24.5, 312 and 186 µmol of endogenous EG, GA, and OA respectively, were excreted in urine. Following 4 h inhalation exposure to $^{13}C_2$-EG, 359 % of EG, 5.7 % of GA, and 0.75 % of OA (mean values), given as percentages of the corresponding endogenous compounds, appeared in the urine as labelled compounds.

Following epidermal application of $^{13}C_2$-EG, the amount of $^{13}C_2$-EG taken up was between 150 and 189 µmol. The maximum plasma concentrations of $^{13}C_2$-EG were 1.1-2.0 µmol/l. $^{13}C_2$-GA could not be quantified in plasma after epidermal exposure to $^{13}C_2$-EG. The average amounts of $^{13}C_2$-EG and $^{13}C_2$-GA excreted in urine were 13 µmol and 0.7 µmol, respectively, corresponding on average to 8 % of $^{13}C_2$-EG and

0.4 % of $^{13}C_2$-GA, of the dose taken up. $^{13}C_2$-OA could not be detected in any urine sample. On average, 22, 455 and 99 µmol of endogenous unlabelled EG, GA and OA, respectively, were excreted in urine. About 64 % of EG and 0.19 % of GA, given as percentages of the corresponding endogenous compounds, were excreted in the urine as labelled compounds.

By extrapolating the inhalation exposure to workplace conditions (8 h, 50 W) and by extrapolating dermal exposure to liquid EG to a skin area of both hands (910 cm^2), it can be concluded from comparing the EG concentrations in plasma and the urinary excretion of EG and GA, that the equally long lasting inhalation exposure to 10 ppm vaporous EG under workplace conditions and the epidermal exposure of both hands to pure liquid EG result in about the same body burdens by EG or GA.

The present results demonstrate, that exposure to 10 ppm EG (8 h, 50 W) leads to a GA concentration in plasma is of 5.25±1.70 µmol/l. By taking into account the background GA concentration in plasma of 28.8±5.20 µmol/l, it becomes evident that the maximum increase of GA resulting from exposure to 10 ppm EG under workplace conditions is not higher than the SD of the background GA concentration.

Considering the corresponding daily urinary excretion of background GA (312±191µmol) and background OA (186±49.2 µmol), it becomes again evident that inhalation exposure to EG (10 ppm, 8 h, 50 W) leads only to a very small increase in the metabolites GA and OA (on average 10 % GA and 2 % OA) if compared to their background burden. Therefore, it has to be deduced that there is no risk for GA- and OA-induced nephrotoxic or developmental effects resulting from human exposure to 10 ppm vaporous EG ("MAK-value") under workplace conditions (8 h, 50 W) and from continuously wetting of both hands with liquid EG over 8 h.

6 Abbreviations

ACN	Acetonitrile
ADH	Alcohol dehydrogenase
AUC	Area under curve
bw	Body weight
CNS	Central nervous system
C_{ss}	Concentration at steady state
CV	Coefficient of variation
CYP450	Cytochrome P-450
DABCO	1,4-Diazabicyclo[2.2.2]octane
EI	Electron impact
EG	Ethylene glycol
eV	Electron volt
GA	Glycolic acid
GAO	Glycolic acid oxidase
GC	Gas chromatograph
GD_s	Gestation days
HCl	Hydrochloric acid
HIVA	2-Hydroxyisovaleric acid
ID	Internal diameter
i.v.	Intravenous
J	Flux
k	Elimination rate constant
K_p	Permeability constant
LDH	Lactate dehydrogenase
$M^{+\bullet}$	Molecular ion
m/z	Mass-to-charge ratio
MAK	Maximum workplace concentration
MLD	Minimum lethal doses
MSD	Mass selective detector
MTBSTFA	*N*-*tert*-Butyldimethylsilyl-*N*-methyltrifluoracetamid
NaCl	Sodium chloride

NADH	Nicotinamide adenine dinucleotide hydrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
NOEL	No-Observed-Effect Level
OA	Oxalic acid
p.c.	Percutaneous
1,3-PD	1,3-Propandiol
PET	Polyethylene terephthalate
PG	Propylenglycol (1,2-Propandiol)
p.o.	Peroral
ppm	Parts per million
Q_{alv}	Alveolar ventilation
rpm	Revolution per minute
SA	Succinic acid
SD	Standard deviation
SILA	SILA mixture containing MTBSTFA with 1% TBDMSCl
SIM	Single ion monitoring
$t_{1/2}$	Half-life
TBDMS	*tert*-Butyldimethylsilyl-
TBDMSCl	*tert*-Butyldimethylsilylchloride
V_d	Volume of distribution
W	Watt

7 Literature

Abdelghani, A.A.; Anderson, A.C.; Khoury, G.A.; Chang, S.N.:
Fate of ethylene glycol in the environment
Prepared for the Louisiana Department of Transportation and Development
New Orleans, LA, Tulane University, 125 (1990)

Allerderm Laboratoties:
http://www.truetest.com/physician/finnchamber.htm

Åstrand, I.:
Effects of physical exercise on uptake, distribution and elimination of vapors in man
in: Modeling of Inhalation Exposure to Vapors: Uptake, Distribution and Elimination
(V. Fiserova-Bergerova, editor)
CRC Press, Boca Raton, second edition, 107-130, 1983

ATSDR, Agency for Toxic Substances and Disease Registry:
Toxicological Profile for Ethylene Glycol and Propylene Glycol
Atlanta, 1-249 (1997)

Bader, M.; Keener, A.S.; Wrbitzky, R.:
Dermal absorption and urinary elimination of N-methyl-2-pyrrolidone
International Archives of Occupational and Environmental Health, 78, 673-676
(2005)

Blood, F.R.:
Chronic toxicity of ethylene glycol in the rat
Food and Cosmetics Toxicology, 3, 229-234 (1965)

Bronstein, I.N.; Semendjajew, K.A.:
Taschenbuch der Mathematik
Harri Deutsch, Frankfurt/Main, sixteenth edition, 337-338, 1976

Budavari, S.; O'Neil, M.J.; Smith, A.; Heckelman, P.E.; Kinneary, J.F. (editors):
An encyclopedia of chemicals, drugs, and biologicals
in: The Merck Index
Merck & Co., Inc., Whitehouse Station, twelfth edition, 3849, 1996

Carney, E.W.:
An integrated perspective on the developmental toxicity of ethylene glycol
Reproductive Toxicology, 8, 99-113 (1994)

Carney, E.W.; Freshour, N.L.; Dittenber, D.A.; Dryzga, M.D.:
Ethylene glycol developmental toxicity: unraveling the roles of glycolic acid and
metabolic acidosis
Toxicological Sciences, 50, 117-126 (1999)

Carstens, J.:
Bestimmung von Ethylenglykol, Glykolsäure und Oxalsäure aus biologischen Proben mittels Gaschromatographie und massenselektiver Detektion-Untersuchungen am Ethyleneglykol-exponierten Probanden
Dissertation, http://mediatum.ub.tum.de/mediatum (2003)

Carstens, J.; Csanády, G.A.; Faller, T.H.; Filser, J.G.:
Human inhalation exposure to ethylene glycol
Archives of Toxicology, 77, 425-432 (2003)

Caravati, E.M.; Erdman, A.R.; Christianson, G.; Manoguerra, A.S.; Booze, L.L.; Woolf, A.D.; Olson, K.R.; Chyka, P.A.; Scharman, E.J.; Wax, P.M.; Keyes, D.C.; Troutman, W.G.:
Ethylene glycol exposure: an evidence-based consensus guideline for out-of-hospital management
Clinical Toxicology, 43, 327-345 (2005)

Cavender, F.L.; Sowinski, E.J.:
Glycols
in: Patty´s Toxicology (G.D. Clayton; F.E. Clayton, editors)
John Wiley & Sons, Inc., New York, fourth edition, 4645-4719, 1994

Cavender, F.L.; Sowinski, E.J.:
Glycols
in: Patty´s Toxicology (E. Bingham; B. Cohrssen; C.H. Powell, editors)
John Wiley & Sons, Inc., New York, fifth edition, 1-71, 2001

Chalmers, R.A.; Tracey, B.M.; Mistry, J.; Griffiths, K.D.; Green, A.; Winterborn, M.H.:
L-Glyceric aciduria (primary hyperoxaluria type 2) in siblings in two unrelated families
Journal of Inherited Metabolic Disease, 7, 133-134 (1984)

Chen, Z.; Ye, Z.; Zeng, L.; Yang, W.:
Clinical investigation on gastric oxalate absorption
Chinese Medical Journal, 116, 1749-1751 (2003)

Chou, J.Y.; Richardson, K.E.:
The effect of pyrazole on ethylene glycol toxicity and metabolism in the rat
Toxicology and Applied Pharmacology, 43, 33-44 (1978)

Clejan, L.A.; Cederbaum, A.I.:
Structural determinants for alcohol substrates to be oxidized to formaldehyde by rat liver microsomes
Archives of Biochemistry and Biophysics, 298, 105-113 (1992)

Corley, R.A.; Bartels, M.J.; Carney, E.W.; Weitz, K.K.; Soelberg, J.J.; Gies, R.A.; Thrall, K.D.:
Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic acid, in rats and humans
Toxicological Sciences, 85, 476-490 (2005)

Corley, R.A.; McMartin, K.E.:
Incorporation of therapeutic interventions in physiologically based pharmacokinetic modeling of human clinical case reports of accidental or intentional overdosing with ethylene glycol
Toxicological Sciences, 85, 491-501 (2005)

Cruzan, G.; Corley, R.A.; Hard, G.C.; Mertens, J.J.; McMartin, K.E.; Snellings,W.M.; Gingell, R.; Deyo, J.A.:
Subchronic toxicity of ethylene glycol in Wistar and F-344 rats related to metabolism and clearance of metabolites
Toxicological Sciences, 81, 502-511 (2004)

Dawson, T.A.:
Ethylene glycol sensitivity
Contact Dermatitis, 2, 233 (1976)

De Duve, C.; Baudhuin, P.:
Peroxisomes (microbodies and related particles)
Physiological Reviews, 46, 323-357 (1966)

DePass, L.R.; Garman, R.H.; Woodside, M.D.; Giddens, W.E.; Maronpot, R.R.; Weil, C.S.:
Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice
Fundamental and Applied Toxicology, 7, 547-565 (1986)

DFG, Deutsche Forschungsgemeinschaft:
Ethylenglykol
in: Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten (H. Greim, editor)
Senatskommision zur Prüfung gesundheitsschädlicher Arbeitsstoffe, VCH-Verlagsgesellschaft, Weinheim, 1-22, 1991

DFG, Deutsche Forschungsgemeinschaft:
Arbeitsleistung
in: Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten (H. Greim, editor)
Wiley-VCH, Weinheim, 2001

Documenta Geigy:
Wissenschaftliche Tabellen
Georg Thieme, Stuttgart, seventh edition, 657-661, 1975

Driver, J.; Tardiff, R.G.; Sedik, L.; Wester, R.C.; Maibach, H.I.:
In vitro percutaneous absorbtion of [^{14}C] ethylene glycol
Journal of Exposure Analysis and Environmental Epidemiology, 3, 277-284 (1993)

Duez, P.; Kumps, A.; Mardens, Y.:
GC-MS profiling of urinary organic acids evaluated as a quantitative method
Clinical Chemistry, 42, 1609-1615 (1996)

Duncan, R.J.; Tipton, K.F.:
The oxidation and reduction of glyoxylate by lactic dehydrogenase
European Journal of Biochemistry, 11, 58-61 (1969)

DuPont glycolic acid:
http://www.dupont.com/glycolicacid/applications/index.html (2005)

Eder, A.F.; McGrath, C.M.; Dowdy, Y.G.; Tomaszewski, J.E.; Rosenberg, F.M.; Wilson, R.B.; Wolf, B.A.; Shaw, L.M.:
Ethylene glycol poisoning: toxicokinetic and analytical factors affecting laboratory diagnosis
Clinical Chemistry, 44, 168-177 (1998)

Filser, J.G.; Denk, B.; Törnqvist, M.; Kessler, W.; Ehrenberg, L.:
Pharmacokinetics of ethylene in man; body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene
Archives of Toxicology, 66, 157-163 (1992)

Filser, J.G.; Kreuzer, P.E.; Greim, H.; Bolt, H.M.:
New scientific arguments for regulation of ethylene oxide residues in skin-care products
Archives of Toxicology, 68, 401-405 (1994)

Filser, J.G.:
Toxikokinetik
in: Toxikologie (H. Greim; E. Deml, editors)
VCH-Verlagsgesellschaft, Weinheim, first edition, 13-40, 1996

Frantz, S.W.; Beskitt, J.L.; Grosse, C.M.; Tallant, M.J.; Dietz, F.K.; Ballantyne, B.:
Pharmacokinetics of ethylene glycol I. Plasma disposition after single intravenous, peroral, or percutaneous doses in female Sprague-Dawley rats and CD-1 mice
Drug Metabolism and Disposition, 24, 911-921 (1996a)

Frantz, S.W.; Beskitt, J.L.; Grosse, C.M.; Tallant, M.J.; Dietz, F.K.; Ballantyne, B.:
Pharmacokinetics of ethylene glycol II. Tissue distribution, dose-dependent elimination, and identification of urinary metabolites following single intravenous, peroral or percutaneous doses in female Sprague-Dawley rats and CD-1 mice
Xenobiotica, 26, 1195-1220 (1996b)

Frantz, S.W.; Beskitt, J.L.; Tallant, M.J.; Zourelias, L.A.; Ballantyne, B.:
Pharmacokinetics of ethylene glycol III. Plasma disposition and metabolic fate after single intravenous, peroral or percutaneous doses in the male Sprague-Dawley rat
Xenobiotica, 26, 515-539 (1996c)

Gérin, M.; Patrice, S.; Bégin, D.; Goldberg, M.S.; Vyskocil, A.; Adib, G.; Drolet, D.; Viau, C.:
A study of ethylene glycol exposure and kidney function of aircraft de-icing workers
International Archives of Occupational and Environmental Health, 69, 255-265 (1997)

Gessner, P.K.; Parke, D.V.; Williams, R.T.:
Studies in detoxification. 86. The metabolism of ^{14}C-labelled ethylene glycol
The Biochemical Journal, 79, 482-489 (1961)

Green, M.L.; Hatch, M.; Freel, R.W.:
Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats
American Journal of Physiology. Renal Physiology, 289, 536-543 (2005)

Guo, C.; McMartin, K.E.:
The cytotoxicity of oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation
Toxicology, 208, 347-355 (2005)

Gupta, S.C.; Dekker, E.E.:
Malyl-CoA formation in the NAD^+-, CoASH-, and α-ketoglutarate dehydrogenase-dependent oxidation of 2-keto-4-hydroxyglutarate. Possible coupled role of this reaction with 2-keto-4-hydroxyglutarate aldolase activity in a pyruvate-catalyzed cyclic oxidation of glyoxylate.
The Journal of Biological Chemistry, 259, 10012-10019 (1984)

Hagen, L.; Walker, V.R.; Sutton, R.A.L.:
Plasma and urinary oxalate and glycolate in healthy subjects
Clinical Chemistry, 39, 134-138 (1993)

Halabe, A.; Shor, R.; Wong, N.L.M.; Sutton, R.A.L.:
Effect of vitamin D_3 on the conversion of ethylene glycol to glycolate and oxalate in ethylene glycol-fed rats
Clinica Chimica Acta, 330, 135-139 (2003)

Harris, K.S.; Richardson, K.E.:
Glycolate in the diet and its conversion to urinary oxalate in the rat
Investigative Urology, 18, 106-109 (1980)

Health Canada: Investigating Human Exposure to Contaminants in the Environment in: A Handbook for Exposure Calculations
Published by authority of the Minster of National Health and Welfare, Ontario, 1-60, 1995

Herndon, L.R.; Reid, E.E.:
The decomposition of organic compounds at high temperatures and pressures
Journal of the American Chemical Society, 50, 3066-3073 (1928)

Hess, R.; Bartels, M.J.; Pottenger, L.H.:
Ethylene glycol: an estimate of tolerable levels of exposure based on a review of animal and human data
Archives of Toxicology, 78, 671-680 (2004)

Hill Top Research, INC.:
http://www.hill-top.com/nonflash/chambers/chambers.html

Hindson, C.; Ratcliffe, G.:
Ethylene glycol in glass lens cutting.
Contact Dermatitis, 1, 386-387 (1975)

Holmes, R.P.; Goodman, H.O.; Hart, L.J.; Assimos, D.G.:
Relationship of protein intake to urinary oxalate and glycolate excretion
Kidney International, 44, 366-372 (1993)

Hoppe, B.; Leumann, E.; von Unruh, G.; Laube, N.; Hesse, A.:
Diagnostic and therapeutic approaches in patients with secondary hyperoxaluria
Frontiers in Bioscience, 8, 437-443 (2003)

IPCS, International Programme on Chemical Safety:
Ethylene glycol: Environmental Aspects
World Health Organisation, Geneva (2000)

IPCS, International Programme on Chemical Safety:
Ethylene glycol: Human Health Aspects
World Health Organisation, Geneva (2002)

Jacobsen, D.; Ostby, N.; Bredesen, J.E.:
Studies on ethylene glycol poisoning
Acta Medica Scandinavica, 212, 11-15 (1982)

Jacobsen, D.; Ovrebo, S.; Ostborg, J.; Sejersted, O.M.:
Glycolate causes the acidosis in ethylene glycol poisoning and is effectively removed by hemodialysis
Acta Medica Scandinavica, 216, 409-416 (1984)

Jacobsen, D.; McMartin, K.E.:
Methanol and ethylene glycol poisonings. Mechanism of toxicity, clinical course, diagnosis and treatment
Medical Toxicology, 1, 309-334 (1986)

Jacobsen, D.; Hewlett, T.P.; Webb, R.; Brown, S.T.; Ordinario, A.T.; McMartin, K.E.:
Ethylene glycol intoxication: evaluation of kinetics and crystalluria
The American Journal of Medicine, 84, 145-152 (1988)

Jones, J.M.; Morrell, J.C.; Gould, S.J.:
Identification and characterization of HAOX1, HAOX2, and HAOX3, three human peroxisomal 2-hydroxy acid oxidases
The Journal of Biological Chemistry, 275, 12590-12597 (2000)

Karlson, P.:
Kurzes Lehrbuch der Biochemie für Mediziner und Naturwissenschaftler
Georg Thieme, Stuttgart, ninth edition, 1974

Keller, Mannheim:
http://www.keller-mannheim.de

Kennish, L.; Reidenberg, B.:
A review of the effect of occlusive dressings on lamellar bodies in the stratum corneum and relevance to transdermal absorption
Dermatology Online Journal, 11, 1-9 (2005)

Keßler, T.; Jansen, B.; Hesse, A.:
Effect of blackcurrant-, cranberry- and plum juice consumption on risk factors associated with kidney stone formation
European Journal of Clinical Nutrition, 56, 1020-1023 (2002)

Klug, S.; Mercker, H.-J.; Jäckh, R.:
Effects of ethylene glycol and metabolites on in vitro development of rat embryos during organogenesis
Toxicology in Vitro, 15, 635-642 (2001)

Korinth, G.; Geh, S.; Schaller, K.H.; Drexler, H.:
In vitro evaluation of the efficacy of skin barrier creams and protective gloves on percutaneous absorption of industrial solvents
International Archives of Occupational and Environmental Health, 76, 382-386 (2003)

Kukielka, E.; Cederbaum, A.I.:
Oxidation of ethylene glycol to formaldehyde by rat liver microsomes. Role of cytochrome P-450 and reactive oxygen species
Drug Metabolism and Disposition, 19, 1108-1115 (1991)

Kukielka, E.; Cederbaum, A.I.:
Increased oxidation of ethylene glycol to formaldehyde by microsomes after ethanol treatment: role of oxygen radicals and cytochrome P450
Toxicology Letters, 78, 9-15 (1995)

Laitinen, J.; Liesivuori, J.; Savolainen, H.:
Exposure to glycols and their renal effects in motor servicing workers
Occupational Medicine, 45, 259-262 (1995)

Laitinen, J.; Liesivuori, J.; Savolainen, H.:
Biological monitoring of occupational exposure to 1-methoxy-2-propanol
Journal of Chromatography. B, Biomedical Sciences and Applications, 694, 93-98 (1997)

LaKind, J.S.; McKenna, E.A.; Hubner, R.P.; Tardiff, R.G.:
A review of the comparative mammalian toxicity for ethylene glycol and propylene glycol
Critical Reviews in Toxicology, 29, 331-365 (1999)

Letzel, S.; Gündel, J.; Schaller, K.H.; Angerer, J.:
Biomonitoring von Glykol-belasteten Personen - Kapillargaschromatographische Bestimmung von Ethylenglykol und 1,2-Propylenglykol im Harn
Arbeitsmedizin Sozialmedizin Umweltmedizin, 35, 160-162 (2000)

Little, J.L.:
Artifacts in trimethylsilyl derivatization reactions and ways to avoid them
Journal of Chromatography. A, 844, 1-22 (1999)

Lodén, M.:
The in vitro permeability of human skin to benzene, ethylene glycol, formaldehyde, and n-hexane
Acta Pharmacologica et Toxicologica, 58, 382-389 (1986)

Loeppky, R.N.; Goelzer, P.:
Microsome-mediated oxidation of *N*-nitrosodiethanolamine (NDELA), a bident carcinogen
Chemical Research in Toxicology, 15, 457-469 (2002)

Madison, K.C.:
Barrier function of the skin: "la raison d'etre" of the epidermis
The Journal of Investigative Dermatology, 121, 231-241 (2003)

Maeda-Nakai, E.; Ichiyama, A.:
A spectrophotometric method for the determination of glycolate in urine and plasma with glycolate oxidase
Journal of Biochemistry, 127, 279-287 (2000)

Marangella, M.; Petrarulo, M.; Vitale, C.; Cosseddu, D.; Linari, F.:
Plasma and urine glycolate assays for differentiating the hyperoxaluria syndromes
The Journal of Urology, 148, 986-989 (1992)

Marshall, T.C.:
Dose-dependent disposition of ethylene glycol in the rat after intravenous administration
Journal of Toxicology and Environmental Health, 10, 397-409 (1982)

Marshall, T.C.; Cheng, Y.S.:
Deposition and fate of inhaled ethylene glycol vapor and condensation aerosol in the rat
Fundamental and Applied Toxicology, 3, 175-181 (1983)

McChesney, E.W.; Golberg, L.; Parekh, C.K.; Russell, J.C.; Min, B.H.:
Reappraisal of the toxicology of ethylene glycol. II. Metabolism studies in laboratory animals
Food and Cosmetics Toxicology, 9, 21-38 (1971)

McChesney, E.W.; Golberg, L.; Harris, E.S.:
Reappraisal of the toxicology of ethylene glycol. IV. The metabolism of labelled glycollic and glyoxylic acid in the rhesus monkey
Food and Cosmetics Toxicology, 10, 655-670 (1972)

McDonald, T.O.; Roberts, M.D.; Borgmann, A.R.:
Ocular toxicity of ethylene chlorohydrin and ethylene glycol in rabbit eyes
Toxicology and Applied Pharmacology, 21, 143-150 (1972)

McDonald, T.O.; Kasten, K.; Hervey, R.; Gregg, S.; Borgmann, A.R.; Murchison, T.:
Acute ocular toxicity of ethylene oxide, ethylene glycol, and ethylene chlorohydrin
Bulletin of the Parenteral Drug Association, 27, 153-164 (1973)

McGroarty, E.; Tolbert, N.E.:
Enzymes in peroxisomes
The Journal of Histochemistry and Cytochemistry, 21, 949-954 (1973)

McMartin, K.E., Wallace, K.B.:
Calcium oxalate monohydrate, a metabolite of ethylene glycol, is toxic for rat renal mitochondrial function
Toxicological Sciences, 84, 195-200 (2005)

McWhinney, B. C.; Nagel, S.L.; Cowley, D.M.; Brown, J.M.; Chalmers, R.A.:
Two-carbon oxalogenesis compared in recurrent calcium oxalate stone formers and normal subjects
Clinical Chemistry, 33, 1118-1120 (1987)

Milliner, D.S.:
The primary hyperoxalurias an algorithm for diagnosis
American Journal of Nephrology, 25, 154-160 (2005)

Moreau, C.L.; Kerns, W. 2nd.; Tomaszewski, C.A.; McMartin, K.E.; Rose, S.R.; Ford, M.D.; Brent, J.:
Glycolate kinetics and hemodialysis clearance in ethylene glycol poisoning. META Study Group
Journal of Toxicology. Clinical Toxicology, 36, 659-666 (1998)

Morris, H.J.; Nelson, A.A.; Calvery, H.O.:
Observations on the chronic toxicities of propylene glycol, ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl-ether and diethylene glycol mono-ethyl-ether
The Journal of Pharmacology and Experimental Therapeutics, 74, 266-273 (1942)

Mraz, J.; Nohova, H.:
Percutaneous absorption of N,N-dimethylformamide in humans
International Archives of Occupational and Environmental Health, 64, 79-83 (1992)

Neeper-Bradley, T.L.; Tyl, R.W.; Fisher, L.C.; Kubena, M.F.; Vrbanic, M.A.; Losco, P.E.:
Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice
Fundamental and Applied Toxicology, 27, 121-130 (1995)

NICNAS, National Industrial Chemicals Notification and Assessment Scheme:
Glycolic acid-Priority Existing Chemical Assessment Report No. 12
National Occupational Health and Safety Commission, Sydney (2000)

Niederwieser, A.; Matasovic, A.; Leumann, E.P.:
Glycolic acid in urine. A colorimetric method with values in normal adult controls and in patients with primary hyperoxaluria
Clinica Chimica Acta, 89, 13-23 (1978)

NIOSH , National Institute for Occupational Safety and Health:
US Department of Health and Human services, Public Health Service, Centers for Disease Control, Rockville (1990)

NTP, National Toxicology Program:
Toxicology and carcinogenesis studies of ethylene glycol in B6C3Fl mice.
U.S. Department of Health and Human Services, Public Health
Service, National Institutes of Health, Washington, DC (1993)

NTP, National Toxicology Program:
NTP-CERHR expert panel report on the reproductive and developmental toxicity of ethylene glycol
National toxicity program-center for the evaluation of risks to human reproduction
Research Triangle Park (2004)

Ogawa, Y.; Takahashi, S.; Kitagawa, R.:
Oxalate content in common Japanese foods
Hinyokika kiyo. Acta Urologica Japonica, 30, 305-310 (1984)

Percy, R.:
A report for transport Canada on ethylene glycol exposure levels at Thunder Bay airport
Health and Welfare Canada, Medical Services Branch, Ottawa, 3 (1992)

Peterson, C.D.; Collins, A.J.; Himes, J.M.; Bullock, M.L.; Keane, W.F.:
Ethylene glycol poisoning: pharmacokinetics during therapy with ethanol and hemodialysis
The New England Journal of Medicine, 304, 21-23 (1981)

Petrarulo, M.; Marangella, M.; Linari, F.:
High-performance liquid chromatographic determination of plasma glycolic acid in healthy subjects and in cases of hyperoxaluria syndromes
Clinica Chimica Acta, 196, 17-26 (1991)

Petrarulo, M.; Vitale, C.; Facchini, P.; Marangella, M.:
Biochemical approach to diagnosis and differentiation of primary hyperoxalurias: an update
Journal of Nephrology, 11, 23-28 (1998)

Poldelski, V.; Johnson, A.; Wright, S.; Rosa, V.D.; Zager, R.A.:
Ethylene glycol-mediated tubular injury: identification of critical metabolites and injury pathways
American Journal of Kidney Diseases, 38, 339-348 (2001)

Pottenger, L.H.; Carney, E.W.; Bartels, M.J.:
Dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites in pregnant (GD10) and nonpregnant Sprague-Dawley rats following oral administration of ethylene glycol
Toxicological Sciences, 62, 10-19 (2001)

Price, C.J.; Kimmel, C.A.; Tyl, R.W.; Marr, M.C.:
The developmental toxicity of ethylene glycol in rats and mice
Toxicology and Applied Pharmacology, 81, 113-127 (1985)

Rajagopal, G.; Ramakrishnan, S.:
Hepatic metabolism of ethylene glycol (EG) and its relevance to ethanol as antidote in EG toxicity
Indian Journal of Pharmacology, 26, 108-111 (1994)

Reif, G.:
Selbstversuche mit Äthylenglykol
Pharmazie, 6, 276-278 (1950)

Richardson, K.E.; Tolbert, N.E.:
Oxidation of glyoxylic acid to oxalic acid by glycolic acid oxidase
The Journal of Biological Chemistry, 236, 1280-1284 (1961)

Richardson, K.E.:
The effect of partial hepatectomy on the toxicity of ethylene glycol, glycolic acid, glyoxylic acid and glycine
Toxicology and Applied Pharmacology, 24, 530-538 (1973)

Romano, M.; Cerra, M.:
The action of crystalline lactate dehydrogenase from rabbit muscle on glyoxylate
Biochimica et Biophysica Acta, 177, 421-426 (1969)

Rowsell, E.V.; Al-Naama, M.M.; Rowsell, K.V.:
Glycine metabolism in rat kidney cortex slices
The Biochemical Journal, 204, 313-321 (1982)

Rzepecki, J.; Stasiak, M.; Kolacinski, Z.:
Clinical symptomatology and laboratory diagnosis of 75 cases of ethylene glycol poisonings
Polski Merkuriusz Lekarski, 5, 74-79 (1998)

Sachs, L.:
Angewandte Statistik: Anwendung statistischer Methoden
Springer, Berlin, tenth edition, 130-140, 2002

Sanz, P.; Reig, R.:
Clinical and pathological findings in fatal plant oxalosis
The American Journal of Forensic Medicine and Pathology, 13, 342-345 (1992)

Sawaki, S.; Hattori, N.; Morikawa, N.; Yamada, K.:
Oxidation and reduction of glyoxylate by lactae dehydrogenase
The Journal of Vitaminology, 13, 93-97 (1967)

Scalley, R.D.; Ferguson, D.R.; Piccaro, J.C.; Smart, M.L.; Archie, T.E.:
Treatment of ethylene glycol poisoning
American Family Physician, 66, 807-812 (2002)

Schatowitz, B.; Gercken, G.:
Simultaneous determination of C_2-C_{22} non-esterified fatty acids and other metabolically relevant carboxylic acids in biological material by gas chromatography of their benzyl esters
Journal of Chromatography, 425, 257-268 (1988)

Scheuplein, R.J.; Blank, I.H.:
Permeability of the skin
Physiological Reviews, 51, 702-747 (1971)

Schlossberg, M.A.; Richert, D.A.; Bloom, R.J.; Westerfeld, W.W.:
Isolation and identification of 5-hydroxy-4-ketovaleric acid as
a product of alpha-ketoglutarate: glyoxylate carboligase
Biochemistry, 7, 333-337 (1968)

Siener, R.; Hesse, A.:
The effect of different diets on urine composition and the risk of calcium oxalate crystallisation in healthy subjects
European Urology, 42, 289-296 (2002)

Silbergeld, S.; Carter, H.E.:
Toxicity of glycolic acid in male and female rats
Archives of Biochemistry and Biophysics, 84, 183-187 (1959)

Stewart, R. D.; Dodd, H.C.:
Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin
American Industrial Hygiene Association Journal, 25, 439-446 (1964)

Sun, J.D.; Frantz, S.W.; Beskitt, J.L.:
In vitro skin penetration of ethylene glycol using excised skin from mice and humans
Journal of Toxicology. Cutaneuos and Ocular Toxicology, 14, 273-286 (1995)

Sykowski, P.:
Ethylene glycol toxicity
American Journal of Ophthalmology, 34, 1599-1600 (1951)

Thews, G.; Mutschler, E.; Vaupel, P.:
Anatomie, Physiologie, Pathophysiologie des Menschen
Wissenschaftliche Verlagsgesellschaft, Stuttgart, fourth edition, 358-356, 1991

Thornalley, P.J.:
Glutathione-dependent detoxification of alpha-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors
Chemico-Biological Interactions, 111, 137-151 (1998)

The Merck index:
an encyclopedia of chemicals, drugs, and biologicals
(Budavari, S.; O'Neil, M.J.; Smith, A.; Heckelman, P.E.; Kinneary, J.F., editors).
Merck & CO, Whitehouse Station, twelfth edition, 3849, 1996

Törnqvist, M.; Osterman-Golkar, S.; Kautiainen, A.; Jensen, S.; Farmer, P.B.; Ehrenberg, L.:
Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin
Carcinogenesis, 7, 1519-1521 (1986)

Troisi, F.M.:
Chronic intoxication by ethylene glycol vapour
British Journal of Industrial Medicine, 7, 65-69 (1950)

Tyl, R.W.; Price, C.J.; Marr, M.C.; Myers, C.B.; Seely, J.C.; Heindel, J.J.; Schwetz, B.A.:
Developmental toxicity of ethylene glycol by gavage in New Zealand white rabbits
Fundamental and Applied Toxicology, 20, 402-412 (1993)

Tyl, R.W.; Ballantyne, B.; Fisher, L.C.; Fait, D.L.; Savine, T.A.; Dodd, D.E.; Klonne, D.R.; Pritts, I.M.:
Evaluation of the developmental toxicity of ethylene glycol aerosol in the CD rat and CD-1 mouse by whole-body exposure
Fundamental and Applied Toxicology, 24, 57-75 (1995a)

Tyl, R.W.; Ballantyne, B.; Fisher, L.C.; Fait, D.L.; Dodd, D.E.; Klonne, D.R.; Pritts, I.M.; Losco, P.E.:
Evaluation of the developmental toxicity of ethylene glycol aerosol in CD-1 mice by nose-only exposure
Fundamental and Applied Toxicology, 27, 49-62 (1995b)

Tyl, R.W.; Fisher, L.C.; Kubena, M.F.; Vrbanic, M.A.; Losco, P.E.:
Assessment of the developmental toxicity of ethylene glycol applied cutaneously to CD-1 mice
Fundamental and Applied Toxicology, 27, 155-166 (1995c)

USEPA, U.S. Environmental Protection Agency:
Health Effects Assessment for Ethylene Glycol. EPA/600/8-88/038. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development
Cincinnati, OH. (1988)

von Unruh, G.E.; Langer, M.A.W.; Paar, D.W.; Hesse, A.:
Mass spectrometric-selected ion monitoring assay for an oxalate absorption test applying [13C2]oxalate
Journal of Chromatography. B, Biomedical Sciences and Applications, 716, 343-349 (1998)

von Wartburg, J.-P.; Bethune, J.L.; Vallee, B.L.:
Human liver-alcohol dehydrogenase. Kinetic and physicochemical properties
Biochemistry, 3, 1775-1782 (1964)

Wagner, F.W.; Burger, A.R.; Vallee, B.L.:
Kinetic properties of human liver alcohol dehydrogenase: oxidation of alcohols by class I isoenzymes
Biochemistry, 22, 1857-1863 (1983)

Wandzilak, T.R.; Hagen, L.E.; Hughes, H.; Sutton, R.A.L.; Smith, L.H.; Williams, H.E.:
Quantitation of glycolate in urine by ion-chromatography
Kidney International, 39, 765-770 (1991)

Wiener, H.L.; Richardson, K.E.:
The metabolism and toxicity of ethylene glycol
Research Communications in Substance Abuse, 9, 77-87 (1988)

Wiley, J.F. 2nd:
Novel therapies for ethylene glycol intoxication
Current Opinion in Pediatrics, 11, 269-273 (1999)

Williams, H.E.; Wandzilak, T.R.:
Oxalate synthesis, transport and the hyperoxaluric syndromes
The Journal of Urology, 141, 742-749 (1989)

Wills, J.H.;
Percutaneous absorption
in: Advances in biology of skin. Pharmacology and the skin (W. Montagana; E.J. Van Scott; R.B. Stoughon, editors)
The Oregon Regional Primat Research Center, twelfth volume, 169-176, 1969

Wills, J.H.; Coulston, F.; Harris, E.S.; McChesney, E.W.; Russell, J.C.; Serrone, D.M.:
Inhalation of aerosolized ethylene glycol by man
Clinical Toxicology, 7, 463-476 (1974)

Yamaguchi, K.; Ogawa, Y.:
Determination of urinary glycolate by ion chromatography: clinical and experimental implication
The japanese journal of urology, 88, 984-991 (1997)

Yanagawa, M.; Maeda-Nakai, E.; Yamakawa, N.; Yamamoto, I.; Kawamura, J.; Tada, S.; Ichiyama, A.:
The formation of oxalate from glycolate in rat and human liver
Biochimica et Biophysica Acta, 1036, 24-33 (1990)

Yin, L.; Liu, C.; Shih, L.; Po, K.:
A study on the teratogenic action of ethylene glycol in rats
Chinese Journal of Preventive Medicine, 20, 289-290 (1986)