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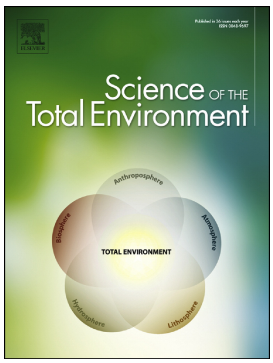

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Blood absorption toxicokinetics of glycol ethers after inhalation: a human controlled study

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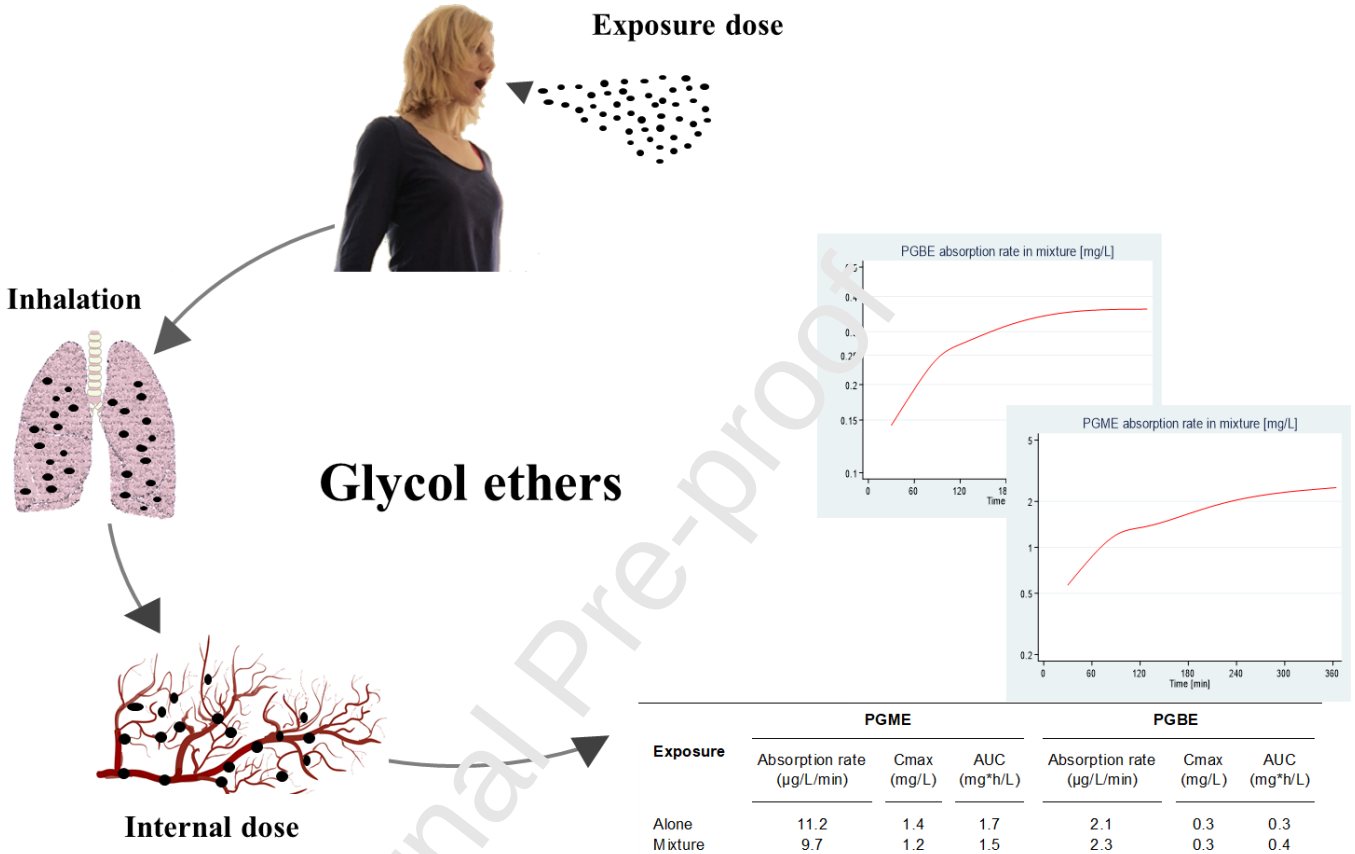
Key words

Blood absorption, internal dose, glycol ethers, toxicokinetics, human study, inhalation exposure

Highlights

- PGME and PGBE bioavailability in blood is immediate during inhalation exposure.
- Humans can absorb extensive amount of PGME in blood.
- Blood absorption rate controls PGME and PGBE internal doses during exposure.
- PGME and PGBE blood absorption rate is non-linear in humans.

Graphical abstract



Exposure	PGME			PGBE		
	Absorption rate (µg/L/min)	Cmax (mg/L)	AUC (mg*h/L)	Absorption rate (µg/L/min)	Cmax (mg/L)	AUC (mg*h/L)
Alone	11.2	1.4	1.7	2.1	0.3	0.3
Mixture	9.7	1.2	1.5	2.3	0.3	0.4

Summary

Glycol ethers are organic solvents present in countless products for professional and domestic use. The main toxicological concerns are hematotoxicity, respiratory and reproductive toxicity. The general population can be exposed when using products containing one or several glycol ethers that evaporate or if sprayed, generate aerosols that can be inhaled. The rate at which glycol ethers enters blood following inhalation exposure are unknown in humans, and chemical risk assessors only rely on animal and *in vitro* toxicity studies. Propylene glycol monomethyl ether (PGME) and propylene glycol monobutyl ether (PGBE) are two examples of glycol ethers used worldwide. Our study aimed to provide human

toxicokinetic data after inhalation exposure of low PGME and PGBE concentrations tested alone or in mixture. Healthy participants (n = 28) were exposed to 35 ppm (131 mg/m^3) of PGME and 15 ppm (i.e., 83 mg/m^3) of PGBE for two or six hours. Blood was regularly collected during the exposure sessions. PGME and PGBE were immediately bioavailable in blood during exposure, and the mean absorption rates were up to 13 µg/L/min and 2.45 µg/L/min, respectively. Maximum mean blood concentration (Cmax) was 2.91 mg/L and 0.41 mg/L for PGME and PGBE. The cumulative internal doses over time (area under the curve, AUC) was 11 mg*h/L and 1.81 mg*h/L for PGME and PGBE. PGME and PGBE total blood uptake could possibly be higher in physically active individuals, such as workers. We recommend that glycol ethers present on the market undergo toxicological testing with the internal doses we found in our toxicokinetic study.

Introduction

The internal dose is the fraction of a chemical that is absorbed by the body and is available for interaction with biologically significant receptors following exposure [1–3]. The fraction of the chemical that reach blood can be called internal dose in blood or blood concentration. The internal dose is different than the exposure dose (i.e., administered or external dose) and likely more representative of the target organ concentration that may trigger an effect. To understand the toxicity of a chemical, the evolution of an effect is followed with time to provide a dose-response relationship. As it is difficult to measure the concentration of a chemical directly at the site of action, such as in brain or testis, in exposed individuals, a solution is to quantify the internal dose in blood [1]. Since the 1990's, the development of analytical method and laboratory techniques are capable of determining and quantifying low chemical doses in blood [4–7]. Despite these major technological advances, toxicological studies are still recording toxic effects in relation to the exposure, not the internal dose. This traditional protocol is commonly reported in studies with volatile organic solvents. These chemicals are widely distributed to highly vascularized

tissues such as the central nervous system, the reproductive glands and hematopoietic organs, and several are known to have depressant, reprotoxic and hematotoxic chemical effects [8–12].

Glycol ethers are amphiphilic organic solvents extensively used worldwide in domestic and professional products such as cleaning products, coating (e.g., paints and varnishes), inks, glues, cosmetics and pesticides [13–15]. Some of the main toxicological concerns with these are hematotoxicity, respiratory and reproductive toxicity [16–20]. Glycol ethers is a broad family of 80 chemicals facilitating the homogenous blending of ingredients with diverse solubility characteristics. Glycol ethers are divided into ethylene glycol (e-series) and propylene glycol (p-series) derivatives [21]. Propylene glycol monomethyl ether (PGME, CAS # 107-98-2) and propylene glycol monobutyl ether (PGBE, CAS # 5131-66-8) are among the five most used propylene glycol derivatives worldwide [22,23]. Workers and the general population are exposed to both aerosols and vapors generated during application of products containing these chemicals alone or in mixture. The greatest potential for exposure is during surface coating and cleaning [22,24,25]. Inhalation exposure is also possible during manufacture or production. Although inhalation is an important route of exposure for glycol ethers, toxicological data on PGME come mainly from oral studies in animals. Moreover, no human or animal toxicological results on PGBE have so far been published in peer-reviewed journals. No countries have established an occupational exposure limit (OEL) for PGBE [26]. The OEL is the maximum concentration of a chemical allowed in air for an occupational exposure of 8 hours, 5 working day over 40 years. In Switzerland the OEL for PGME is 100 ppm and is set on the threshold of human (eye) and upper respiratory tract (including nose and throat) irritation [27,28]. Few human inhalation studies have been performed with glycol ethers and even fewer have monitored the internal dose in blood before and as soon as the exposure started [27,29–33]. Kumagai et al. 1999 [34] and then Dévanthery et al. 2002 [29] mentioned the important role of air concentration on PGME blood concentration in humans. Despite this observation, the rate and extent at which PGME and PGBE enter the bloodstream, as well as the total internal dose in blood (AUC) are still unknown in humans exposed via the inhalation route.

The aim of our study was to provide human toxicokinetic data (i.e., blood absorption) for inhalation of low concentrations of PGME and PGBE alone and as a mixture. Healthy participants were exposed to PGME and/or PGBE in a controlled exposure chamber for 2-hours or 6-hours. Blood was collected before and during the exposure sessions to provide the absorption rate of PGME and PGBE, the maximum blood concentration (Cmax), and the cumulative internal dose in blood over time (area under the curve, AUC). Accounting for possible mixture effects were also possible. These toxicological data not only increase our understanding of the absorption rate of these major p-series derivatives into blood, but allow chemical risk assessors to rely on human data and no longer only on animal toxicity studies.

Material and methods

Chemicals

PGME and PGBE commercial products were used in this study. PGME (CAS # 107-98-2, ≥ 99%, CAS # 1589-47-5, < 0.5%), PGBE (CAS # 5131-66-8, ≥ 99%), the internal standard (IS) propylene glycol propyl ether (PGPE, CAS # 1569-01-3, 99%) were obtained from Sigma Aldrich (Buchs SG, Switzerland), sodium sulphate Na_2SO_4 (CAS 7757-82-6) from Merck (Darmstadt, Germany). are a mixture of two isomers.

Exposure design

Two exposure studies were run in a 12 m^3 exposure chamber: a Day study (6-h exposure) and a Task study (2-h exposure). The exposure chamber and the generation of solvents in air have been well described previously [29,35]. In the Day study, participants were exposed to a mixture of PGME and PGBE. In the task study, the participants were exposed to PGME, PGBE, and a mixture of PGME and PGBE. The participants wore their own clothes. Air concentration of PGME and PGBE was set to about half the Swiss OEL of PGME (i.e., 50 ppm) giving PGME concentration at 35 ppm (131 mg/m^3 with the corresponding conversion factor: 1 ppm = 3.75 mg/m^3 [36]) and PGBE 15 ppm (i.e., 83 mg/m^3 with the

corresponding conversion factor: 1 ppm = 5.5 mg/m^3 [36]). In the exposure chamber, PGME and PGBE air concentrations were continuously monitored in real-time with the software Lab VIEW (National Instruments Corporation, Texas, USA). In addition, air samples were collected during exposures and analyzed to confirm that the targeted exposure concentration was reached. The air samples were collected on charcoal tubes according to National Institute for Occupational Safety and Health Manual of Analytical Methods # 2554.

Healthy participants

Twenty-eight subjects (n = 11 in the Day study; n = 17 in the Task study), 10 women and 18 men, were participated in our study. Women and men participants were non-smokers, not under hormonal contraception regime and not occupationally exposed to PGME and PGBE. Prior to admission into the studies, each participant underwent a screening session to verify heath status based on anamnesis, blood analysis such as blood count, aspartate transaminase (AST), alanine transaminase (ALT), Gamma-glutamyltransferase (GGT), creatinine concentration, electrolyte levels, ECG, spirometry, and body mass index (BMI) calculation. A pregnancy test was given to the non-menopausal women prior to the exposure session. The results from the screening appointment were within clinical limits for all participants and the pregnancy tests were negative. The participants were asked to abstain from alcohol the day before exposure sessions to avoid any possible metabolic interference. The participants were reimbursed for their time and inconvenience. Each participant signed a written informed consent before being included in the study. The human ethical committee of canton Vaud (Commission cantonale d'éthique de la recherche sur l'être humain) approved these two human toxicokinetic studies (amendments to the PB_2017-00043 (343/14) project).

Blood sampling

A small intravenous catheter (Optiva® II) was inserted in the participant's forearm by a trained nurse. Blood was collected in glass tubes (Vacutainers® NH Heparine Sodium), as recommended in Borgatta et al. [37] to avoid blood concentration underestimation due to adsorption to the tube walls. Blood (10 mL) was collected before exposure to provide baseline values (t0). The exposure period started when the

participant entered the exposure chamber. In the Day study, blood was drawn at 3 min (t1) when possible, and systematically at 30 min (t2), 60 min (t3), 90 min (t4), 120 min (t5), 180 min (t6), 240 min (t7), 300 min (t8), and 360 min (t9) after exposure started. In the Task study, blood was drawn after 15 min (t1), 30 min (t2), 60 min (t3), and 120 min (t4) after exposure started.

Chemical analyses

Stock solutions for calibration curves (2 g/L) were prepared by diluting PGME and PGBE with deionized water (Milli-Q® Advantage A10) in glass flasks and stored at 4 °C for no longer than a month. Calibration curves were generated by diluting PGME and PGBE stock solutions in blood, and a minimum of six calibration points in the concentration range 0.04 mg/l to 5.0 mg/L. The limit of quantification (LOQ) was 0.04 mg/L and the limit of detection (LOD) was 0.01 mg/L. The internal standard (IS) stock solution with PGPE was prepared identical to the two analytes (PGME and PGBE) and then diluted 20-times. Blood samples were immediately stored at 4°C and analyzed within 42-hours. Blood samples (2 mL) were diluted with water (1:1) and transferred into injection headspace (HS) glass vials (20-mL) containing the internal standard PGPE (50µL). Coefficient of variation was less than 20% for all reported analysis.

The free form of PGME and PGBE was quantified with a gas chromatography (GC) system (Agilent Technologies AG, Urdorf, Switzerland) equipped with multipurpose sampler (MPS2 Gerstel AG, Sursee, Switzerland), solid phase microextraction (SPME) fiber (75µm Carboxen/PDMS Supelco, Buchs, Switzerland), capillary column (Optima FFAPplus 15m length, 320nm diameter, and film 0.50µm by Agilent J&W), and a flame ionization detector (FID, air flow of 300 mL/min, hydrogen flow of 30 mL/min, and 30 mL/min of makeup nitrogen).

HS vials containing blood samples were incubated at 90°C (10 min) and the fiber extracted (2 min) at the same temperature, then desorbed in the GC injector at 260°C for (2 min). The GC program was 40°C (1 min), increased to 120°C at 11.43°C/minute, and held for 1 min (total cycle time was 8.99 min). FID detector was set at 280 °C.

The area under the curve (AUC) and maximum concentration (Cmax) in blood

Cmax is the highest concentration of PGME and PGBE measured in blood within the exposure. AUC is a concentration–time parameter that is calculated from the blood concentration of PGME or PGBE at each sampling time. In our study, AUC was the sum of the individual areas after 2-h exposure (Task study) and 6-h exposure (Day study). The area under each time interval was calculated following the trapezoidal algorithm:

(1) $$\Delta AUC_{1-2} = \frac{C_{p_1}+C_{p_2}}{2} \times (t_2 - t_1)$$

Blood volume calculation

Total blood volume in participants was calculated with the body weight, body height, and gender based on Nadler equation [38,39].

Blood volume [L] for men:

(2) $$0.3669 \times (\text{height in m})^3 + (0.03219 \times \text{weight in kilograms}) + 0.6041$$

Blood volume [L] for women:

(3) $$0.3561 \times (\text{height in m})^3 + (0.03308 \times \text{weight in kilograms}) + 0.1833$$

Total blood uptake calculation

Total blood uptake is the total absorbed dose of PGME and PGBE reaching blood within a certain period of exposure. The total blood uptake is based on the cumulative internal doses in blood (the AUC) and was calculated as follows:

(4) $AUC_{6\text{-h}}$ (mg/L*h) at rest x blood volume (L) according to gender x 6 hours

Total respiratory uptake calculation

The total respiratory uptake is the total absorbed amount of PGME and PGBE entering lungs after a certain time of exposure. The total respiratory uptake was calculated for 6-h representative of a working

day activity. We used Groeseneken et al. [30] physiological parameters recorded in a study where human participants were exposed to glycol ethers via inhalation. These physiological parameters (mean) at rest were: pulmonary ventilation of 8 L/min, respiratory frequency of 12 times per minute, and heart rate of 62 beats per min. In our study, no distinction was made between women and men because no respiratory parameters were found in the published literature for women exposed to glycol ethers in an exposure chamber. The total respiratory uptake was calculated at rest and during physical activity.

Physical activity can be expressed in Watts in relation to the physical energy delivered per time [40]. We used the alveolar ventilation (L/h) provided by Csanády et al. [41] at two different levels of physical activity : 50 W and 100 W. The pulmonary absorption used in our calculation was 87%. This pulmonary absorption is defined as ratio between retained and inhaled amount of glycol ethers, and was found in the study by Stott and McKenna [42]. These authors studied the pulmonary absorption of PGME in intubated and ventilated rats, and reported that 87% of the inhaled PGME reached the alveoli.

Statistics

The initial (before exposure) respective blood concentration in PGBE and PGME were subtracted from the corresponding blood concentrations during exposure. When the initial blood concentration was lower than the LOD, it was set to LOD/2 i.e. 0.005. The thus normalized respective blood concentrations were log-transformed and analyzed using a mixed model with the subject ID as a random effect. In such mixed models, one considers a participant specific intercept which is assumed to follow a normal distribution. All the other independent factors (most notably the time course) were fixed effects.

In the joint analysis of the two studies with PGME and PGBE in mixture, the effect of time since exposure was modelled using spline functions of time with knots set at 30, 50, 80, 120, 220 and 360 minutes after beginning of exposure. These knots followed closely the design time points and allowed a flexible modelling of the time course. Only a few blood samples were available before 30 minutes of exposure in the Day study, therefore these were excluded. The basic model with only the spline estimates therefore only seven parameters, based on 169 observations. The possible effects of the co-variates (task

vs day study and age class) were subsequently fitted as interactions with the first spline component and tested using standard Wald tests.

The comparison of respectively PGME and PGBE alone and in mixture was only performed within the task study. When analyzing these data, only two knots were considered at 40 and 100. The basic model with only the spline estimates and the indicator of mixture included therefore only five parameters, based on 102 observations. Again, the comparison was based on a Wald test of the interaction between the first spline component and the indicator of alone *vs* in mixture. Furthermore, AUC, Cmax and absorption rates of respectively PGME and PGBE alone and in mixture were compared using a T test within the task study.

All statistical analyses were done using Stata version 16 (StataCorp LLC College Station, Tx).

Results

Table 1 summarizes the participant demographics for the Task (2-h exposure) and the Day (6-h exposure) studies. The PGME and PGBE air concentrations in the exposure chamber was stable during all exposure sessions.

Table 1: demographics of study participants. In the Day study, 11 participants (n) were exposed to a mixture of PGME and PGBE (131 mg/m^3 and 83 mg/m^3, respectively). In the task study, 17 participants (n) were exposed to PGME (131 mg/m^3), PGBE (83 mg/m^3) and a mixture of PGME and PGBE (131 mg/m^3 and 83 mg/m^3, respectively). Body mass index is BMI, body weight is BW, body height is BH, and blood volume is BV.

Participant [n]	Age [years]	BW [kg]	BH [cm]	BMI [kg/m^2]	BV [L]
Day study : 6 hours of exposure					
Women (4)	60 +/- 8	61 +/- 10	166 +/- 4	22 +/- 3	3.8
Men (7)	40 +/- 18	76 +/- 5	175 +/- 7	25 +/- 2	5
Total (11)	47 +/- 19	71 +/- 10	172 +/- 8	24 +/- 3	4.4
Task study : 2 hours of exposure					
Women (6)	34 +/- 14	65 +/- 9	167 +/- 9	24 +/- 3	4

Men (11)	29	+/-	13	76	+/-	12	178	+/-	10	24	+/-	3	5.1
Total (17)	31	+/-	13	73	+/-	12	174	+/-	11	24	+/-	3	4.55

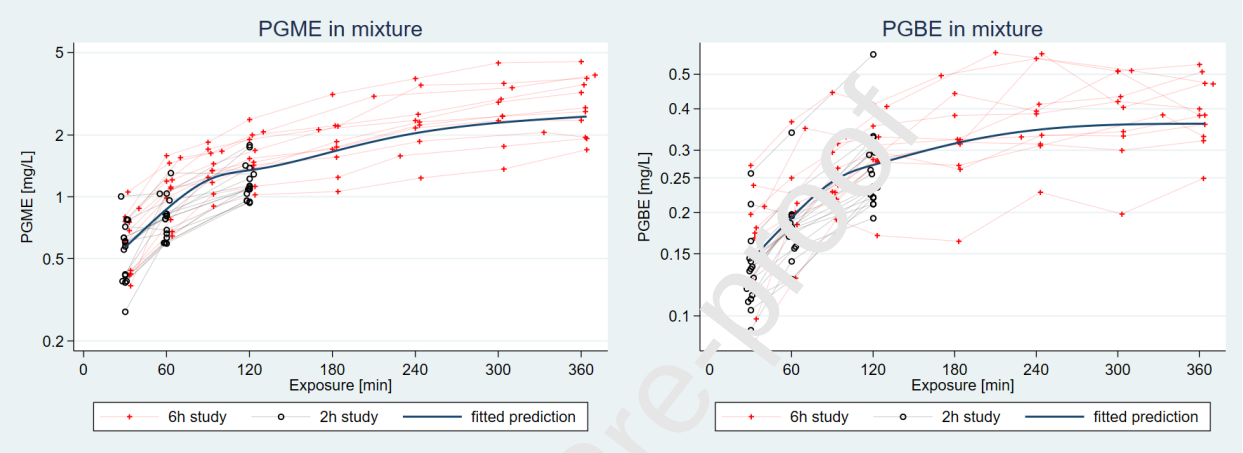


Figure 1: individual blood concentration-time curves of PGME and PGBE in mixture. The Task study = 2-h exposure (17 participants). The Day study = 6-h exposure (11 participants). The results are shown as the spline-fitted evolution since the beginning of exposure. Note that the units of the y axes are not identical.

Figure 1 summarizes PGME and PGBE blood concentration-time curves of each participant in the Day and the Task studies with the chemicals tested in mixture, and the plot of the analyzed and the fitted values predicting the absorption rates. All samples below LOD (24/28 for PGME and 28/28 for PGBE) were at t0 (i.e., before exposure). PGME and PGBE entered blood quickly, after 3 min of exposure, irrespective of exposure time and mixture. The absorption rates of PGME and PGBE in mixture showed no statistically significant difference between the Day and the Task studies within the first two hours. After two hours, PGME and PGBE blood concentrations still rose but at a slower rate, indicative of a non-linear trend in the molecular absorption. The rise continued until the end of the exposure (no steady state) in any participants exposed to PGME, irrespective of exposure conditions (i.e., in mixture or alone, 2-h or 6-h of exposure). In the Day study, seven participants had a PGBE concentration that was still

increasing at the end of the exposure (one just 2 min after the participant left the exposure chamber). Four participants showed a PGBE concentration in blood higher between 210 and 330 min than at 360 min.

In the Task study, the absorption rates were not significantly different when PGME and PGBE were tested alone *versus* in mixture (Figure 2). In the Task study, a participant had 0.36 mg/L of PGME in blood before entering the exposure chamber (t0) and a lower adjusted PGME blood concentration.

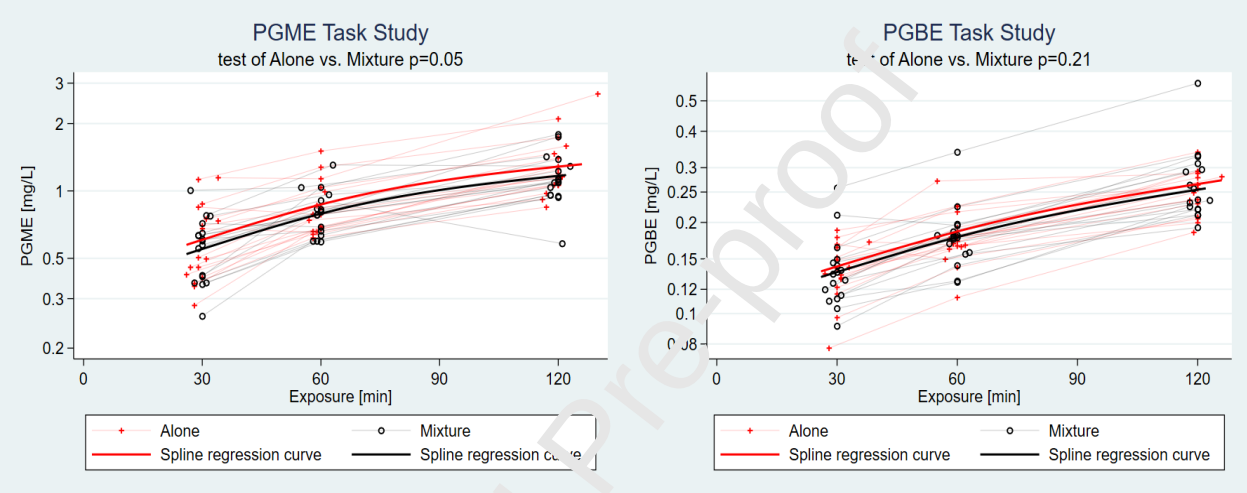


Figure 2: individual blood concentration-time curves of PGME and PGBE in the Task study when the chemical was tested alone *versus* in mixture. Note that the units of the y axes are not identical.

Table 2 depicts PGME and PGBE blood absorption rates, the maximum blood concentration (Cmax) and the total amount of PGME and PGBE that was absorbed in blood during the exposure (i.e., area under the concentration curve, AUC). PGME Cmax were observed at the end of the exposure in the Task and the Day studies (Figure 1 and Table 2). AUC was higher in the Day study than in the Task. The reason is that the AUC is computed over 6 hours as compared to 2 hours for the task study. No difference in absorption rates, AUC or Cmax was observed for PGME or PGBE stratified by mixture, age, sex, BMI or exposure session dates (data not shown).

Table 2: PGME and PGBE blood results after 2-h and 6-h exposure in the Task (N = 17) and Day (N = 11) studies, respectively. PGME and PGBE were inhaled alone and in mixture. The table presents the absorption rates, maximum blood concentrations (Cmax) and cumulative internal doses over time (total AUC for 2-h and 6-h). Nd = not determined.

Study (molecule)	N	Exposure (h)	PGME						PGBE					
			Absorption rate (µg/L/min)		Cmax (mg/L)		AUC (mg*h/L)		Absorption rate (µg/L/min)		Cmax (mg/L)		AUC (mg*h/L)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Task (alone)	17	2	11.7	3.58	1.38	0.47	1.72	0.57	2.11	0.34	0.26	0.04	0.34	0.06
Task (mixture)	17	2	9.74	2.47	1.19	0.31	1.53	0.32	2.28	0.74	0.28	0.09	0.35	0.1
Day (mixture)	11	2	13.00	3.25	nd	nd	nd	nd	2.1[illegible]	0.54	nd	nd	nd	nd
Day (mixture)	11	6	7.84	2.57	2.91	0.93	11.07	3.61	1.10	0.26	0.41	0.10	1.81	0.52
Task & Day (all)	45	2	11.06	3.30	nd	nd	nd	nd	2.26	0.57	nd	nd	nd	nd

Total respiratory and blood uptakes after 6-h of exposure

After 6-h of exposure, the mean pulmonary ventilation of the participants was 483 L/h and the mean breathing volume 2.901 L (or 2.9 m^3). The total respiratory and blood uptake are summarized in Table 3. For PGME, the total respiratory uptake was close to the blood uptake (i.e. internal blood dose) and higher for PGBE. Since blood uptakes were calculated from blood samples, no total uptake was provided at 50 W and 100 W.

Table 3: internal exposure in 11 participants exposed 6-h to 131 mg/m^3 of PGME and 83 mg/m^3 of PGBE. Total blood uptake is calculated from measured blood concentrations, and total respiratory uptake from the concentrations of PGME and PGBE in air. The dose per body weight is also for 6-h exposure and calculated from either total blood uptake ([§]) or total respiratory uptake ([§§])

		Total blood uptake (mg)			Total respiratory uptake (mg)			Dose (mg/kg bw)			
		at rest	50 W	100 W	at rest	50 W	100 W	at rest [§]	at rest [§§]	50 W[§§]	100 W[§§]
PGME	94	2	-	-	331	800.1	1374.5	4.3	4.9	11.8	20.2

PGBE	4 8.1	-	-	209.5	506.9	870.9	0.7	3.1	7.5	12.8

Discussion

PGME and PGBE concentrations in blood

Blood profile during inhalation exposure showed fast PGME and PGBE absorption rates, indicative of an immediate bioavailability of these solvents in blood. Both glycol ethers were measured in blood after 3-min exposure. The absorption rate in blood was not influenced by the inhalation of PGME and PGBE in mixture, suggesting that pulmonary diffusion (i.e., no active transporters) was likely the primary absorption mechanism into blood during inhalation exposure. PGME internal doses in blood were still increasing just before the participants left the exposure chamber. These internal doses are related to the inhalation exposure only, as dermal uptake of PGME vapors is non-significant [29,43]. Workers that are typically exposed for longer periods and higher air concentrations, such as 8.5 hours per day and up to 100 ppm (OEL of Switzerland), likely have higher internal doses than in our study. A reason explaining the increasing internal dose of PGME may be the blood:air partition coefficient. This coefficient determines blood affinity for a gas. The higher the blood:air partition coefficient the more gas is absorbed in blood and thus, the higher is the internal dose [29,44]. PGME blood:air partition coefficient is 12,383 meaning that at equilibrium PGME concentration in blood is 12,383 times higher than in lungs [45]. Therefore, PGME can extensively be absorbed in blood. This absorption is also dependent on the air concentration and the respiratory rate, indicative that in active people the internal doses in blood would be much greater than those found in our participant exposed to PGME at rest. PGBE blood:air partition coefficient is unknown. After six hours of exposure, our result showed that PGBE blood concentration

was still increasing in seven participants and decreasing in three, excluding a steady state (i.e., the rate of elimination did not match the rate of absorption). Since the physicochemical properties of PGBE are close to those of PGME, we can assume blood:air partition coefficient, air concentration, and respiratory rate to also influence the PGBE internal dose in blood. Since PGME, and possibly PGBE, can extensively be absorbed into blood, an occupational health question arises on the time required between two work shifts to eliminate these chemicals from blood. Studies on half-lives of glycol ethers in blood are highly encouraged.

The non-linear trend increases observed in PGME and PGBE blood concentration after 2-h exposure may be explained by toxicokinetic parameters, such as the metabolism, elimination and absorption. For instance, the metabolism and urinary excretion can decrease PGME and PGBE internal dose in blood. Human toxicokinetic studies have reported the presence of PGME and its main metabolite in urine after 2 hours of exposure [29,32,46], confirming the metabolism and urinary excretion have already started at this time. No published results indicate that PGME metabolism and elimination begin before 2-h of exposure in humans, and no data exist for PGBE nor for PGME and PGBE in mixtures. However, it is known that gas uptake is not dominated by metabolism for chemicals with very high blood:air partition coefficients [47]. The constant increase of the internal doses and the absence of steady state observed in our study confirmed that absorption was the main toxicokinetic parameters influencing PGME and PGBE internal doses in blood. Although PGME and PGBE absorption rates in blood were high, our results also showed that these rates slowed down with exposure-time. After 6-h of exposure, PGME and PGBE absorption rates were almost halved compared to 2-h, suggesting an effect that likely decrease the diffusion of these solvents from the alveoli into blood. A diffusion effect at the pulmonary level would also explain the differences found between the total respiratory and blood uptake results. Indeed, 209 mg of PGBE had theoretically reached lungs and only 48 mg were effectively found in blood. Also, the individual who had already PGME in blood before entering the exposures chamber showed a less rapid increase of the internal doses than the other participants. This individual was likely exposed to PGME at

some point at home or work. Although from a single participant, this result also suggests that recent exposure to PGME induces a noticeable diffusion response of this solvent in blood. PGME is known to be an upper respiratory tract irritant [27,28] but to the best of our knowledge, no study has been performed at the alveolar level, and none exists at all for PGBE. Growing evidences correlate deleterious effects of cleaning products, including those with propylene glycol derivatives, on the respiratory system, especially in professional cleaners. Although no causal links have been established, supporting evidences indicate an increasing risk of asthma [48–50], chronic bronchitis [51], rhinitis, cough, and shortness of breath in professional cleaners [52]. Human toxicological studies are required to understand a causal link between pulmonary effects and propylene glycol derivatives exposure, after repeated exposures.

Other toxic effect have been reported with PGME. For instance, developmental and reproductive effects, such as delay in pubertal, vaginal opening, prepuce separation, and other reproductive effects (i.e., estrous cycles, pup body weights, survival and litter size), were observed in rats exposed to PGME via the inhalation route [13,53]. The dose-effect relationship was calculated based on air concentration. The no observable adverse effect level (NOAEL) in offspring was 1000 ppm and corresponded to an exposure dose of 1325 mg/kg bw, whereas the no observable effect level (NOEL) for parental toxicity was 300 ppm (396 mg/kg bw). In other studies, an increased incidence of kidney cortical-epithelial tumor and hepatocellular adenoma were observed in rats exposed by inhalation to PGME 6-h a day, 5 days a week for 2 years, with a NOAEL reported by the authors at 300 ppm [13,54,55]. Chemicals inducing renal tumors via α2microglobulin accumulation are essentially considered non-genotoxic carcinogens. Neurotoxic and hepatotoxic effects were reported in rats exposed orally to PGME and the lowest observed adverse effect level (LOAEL) was 460 mg/kg per day (Stenger et al. 1972 in [21]). These results are only a magnitude order higher than the total respiratory uptake calculated for PGME in our study. Indeed, our results showed that the total human uptake per body weight (bw) during a moderate work activity (100 W [56,57]) was 20.2 mg/kg bw of PGME after 6-h of inhalation exposure. Any increase in the workload induces a higher respiratory rate with significant increase in blood absorption of organic

solvents with a blood:air partition coefficient higher than 6 [40,58,59]. Therefore, questions arise not only about the total internal dose in active workers exposed 8 hours a day, 5 days a week, for several years, but also on the absence of internal dose results in toxicological studies more generally. For these reasons, we recommend future studies based on internal doses in blood rather than exposure.

Conclusion

Blood can absorb an extensive quantity of PGME and PGBE that is immediately bioavailable for highly vascularized tissues where they may induce toxic effects. Physically active people likely have a higher blood concentration than resting individuals exposed to these solvents. In Switzerland, the OEL for PGME (i.e, 368 mg/m^3 or 100 ppm) was set on the basis of eye and upper respiratory tract irritation, and is expected to be protective for potential systemic effects on workers. PGBE has no OEL. Owing to the lack of reported information on PGBE toxicity, internal doses of PGBE and PGME, and the possible pulmonary effects (diffusion effect) of both chemicals, we consider that an OEL for PGBE should be set and re-assessed for PGME based on internal concentrations. Besides, when extrapolating to humans a dose-response relationship observed in animals, assuming that the response level at a given blood concentration is the same in both species, it seems highly preferable to compare equivalent internal doses rather than the exposure concentration in air. Nowadays, blood sampling are common and easy to perform. Therefore, internal dose monitoring in studies where toxic effects are assessed should not pose any problem anymore.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- PGME and PGBE bioavailability in blood is immediate during inhalation exposure.
- Humans can absorb extensive amount of PGME in blood.
- Blood absorption rate controls PGME and PGBE internal doses during exposure.
- PGME and PGBE blood absorption rate is non-linear in humans.