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Effect of age on toxicokinetics among human volunteers exposed to propylene glycol methyl ether (PGME)

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Abstract (max 200 words)

Aging adults represent the fastest growing population segment in many countries. Physiological and metabolic changes in the aging process may alter how aging adults biologically respond to pollutants. In a controlled human toxicokinetic study (exposure chamber; $12m^3$), aging volunteers (n=10; >58 years) were exposed to propylene glycol monomethyl ether (PGME, CAS no. 107-98-2) at 50 ppm for 6 hours. The dose-dependent renal excretion of oxidative metabolites, conjugated and free PGME could potentially be altered by age. **Aims:** (1) Compare PGME toxicokinetic profiles between aging and young volunteers (20-25 years) and gender; (2) Test the predictive power of a compartmental toxicokinetic (TK) model developed for aging persons against urinary PGME concentrations found in this study. **Methods:** Urine samples were collected before, during, and after the exposure. Urinary PGME was quantified by capillary GC/FID. **Results**: Differences in urinary PGME profiles were not noted between genders but between age groups. Metabolic parameters had to be changed to fit the age adjusted TK model to the experimental results, implying a slower enzymatic pathway in the aging volunteers. For an appropriate exposure assessment, urinary total PGME should be quantified. **Conclusion**: Age is a factor that should be considered when biological limit values are developed.

Key words: Biological monitoring; Human; Age; 1-methoxy-2-propanol; propylene glycol monomethyl ether; PGME;

Introduction

Aging adults represent the fastest growing population segment in many countries. Consequently the average age of the working population will increase. In Switzerland, the percent of people older than 65 years will increase from today's 17% to 28% in 2060 (SFSO, 2010), which is the same trend seen in other industrialized countries (Toossi, 2009).

Current knowledge regarding physiological changes in the aging process is known from pharmaceutical research, where the risk for adverse drug reactions can be devastating, especially in elderly patients with comorbidity and polypharmacy. Corsonello et al. (2010) summarized the changes due to aging as follows: decrease in hepatic drug clearance, mainly due to reduced blood flow and hepatocyte mass, decrease in renal function due to sclerotic changes in the glomeruli, and changes in pharmacodynamics in the cardiovascular and nervous system. The most important pharmacokinetic change in old age includes a decrease in the excretory capacity of the kidney, more than the decline in the rate of hepatic drug metabolism (ElDesoky, 2007).

Regarding occupational health, younger (defined as those aged <25 years) and older workers (defined as those aged >55 years) could potentially respond to workplace exposures differently. Translating from pharmaceutical research among older individuals, we could expect aging workers to respond to workplace exposures similarly; resulting in a depressed clearance of the workplace pollutant. Implications may be that current exposure risk management programs do not sufficiently protect the aging workers from potential health hazards associated with workplace exposures.

Biological monitoring of chemical exposures is the measurement of the substances themselves, their metabolites and/or the induced biological effects in biological media (ex. urine and blood) to evaluate exposure compared to an appropriate reference value (BEI, BAT,

BLV). Biological monitoring programs are used to manage health risks in the workplace. However, these biological reference values are based on published exposure studies, and included both occupational and volunteer studies (ACGIH 2011); often with little or no information about the health of the workers and the age range selected. Large biological variabilities exist among individuals (Truchon et al., 2006), mainly due to absorption, distribution, metabolism, and elimination (ADME) processes (Sobus et al., 2010), thus collection times specified for the reference values might not be optimal for aging workers with a slower ADME. Consequently, this may lead to an under or overestimate of exposure depending on the substance measured.

To help interpretation of biomonitoring results, a toxicokinetic (TK) modeling approach may be used since it describes the fate of chemicals in the body. The impact of age on biological monitoring has been explored (Tomicic and Droz, 2009) by changing the physiological parameters due to age in an already developed TK model (Pierrehumbert et al., 2002). Overall changes due to age were 10–20%, but differences up to 50% were observed (Tomicic and Droz, 2009). These differences appeared to depend on the chemical and the exposure biomarker considered. The influence of age on industrial chemical toxicokinetics has not yet been determined and could be a source for inter-worker variability (Truchon et al., 2006).

In a previous human volunteer study, young (<25 years) men (n=10) and women (n=15) were exposed to three solvents (propylene glycol monomethyl ether (PGME, CAS no. 107-98-2), methyl ethyl ketone, methyl chloroform) separately, during six hours and at half their threshold limit values (Tomicic et al., 2011). Using an univariate analysis of variance adjusting for potential confounders (body mass index, body fat, genotype), the authors showed significant differences in the concentrations of the respective urinary exposure biomarkers among women, with an increase of more than 50% in metabolites concentrations (urinary trichloroethanol) and a decrease of up to 50% in unchanged substances

concentrations (urinary methyl ethyl ketone, urinary free PGME) for women under hormonal contraceptive regimen. The explanation provided by the authors (Tomicic et al., 2011) is an increase in the metabolic rate, as explained by the estrogen-regulated CYP2E1 (Kennedy, 2008). Actually, free estradiol level is lower in women under hormonal contraceptive than in women without hormonal contraceptive (Bjørnerem et al., 2004). Thus, the use of hormonal contraceptives like the combined contraceptive pill may increase CYP2E1 activity.

Consequently, the observed variabilities in toxicokinetics were not only due to physiological differences between genders but also an individual's hormone levels. These factors may lead to misinterpretation of biomonitoring results (Tomicic et al., 2011). Moreover, unmetabolized urinary fraction of PGME was found a suitable biomarker among the young volunteers (Tomicic et al., 2011), and can therefore be compared to the biological limit values collected at the end of the work shift. The biological limit value for urinary PGME is 20 mg/L in Switzerland (SUVA, 2011) and 15 mg/L in Germany (DFG, 2011).

In the exposure chamber study of young volunteers, PGME was chosen as the substance of interest because it is commonly used in industry and consumer products (Dentan et al., 2000) and the toxicokinetics are well understood. PGME is increasingly replacing other glycol ethers as it is assumed to be less toxic (Dentan et al., 2000) than ethylene glycol derived glycol ethers, which have been associated with depression of the central nervous system. Commercial PGME contains 95-98 % of α-isomer (secondary alcohol) and <5% of the β-isomer (primary alcohol). The α-isomer is eliminated through expired air or metabolized through the microsomal mixed function oxydase P450 and the metabolites (largely propylene glycol) are excreted with unchanged and conjugated PGME in urine (Miller, 1987). The β-isomer is eliminated through expired air or metabolized through alcohol dehydrogenase

(ADH) followed by aldehyde dehydrogenase (ALDH) to lactate and to pyruvate, then excreted with conjugated PGME in urine (Miller et al., 1986). Elimination kinetics is dose-dependent (0 order) (Kolloffel et al., 1996). At higher doses (5.1 g/day), the metabolic clearance is saturated, and half-lives increase (Speth et al., 1987).

Based on current knowledge, we hypothesized that (1) there would be a difference in PGME toxicokinetic profiles between young (20-25 years) and aging volunteers (58- 62 years) and (2) the toxicokinetic model developed for aging persons (Tomicic and Droz, 2009) would predict well the urinary PGME concentrations in aging volunteers. The present study was designed to test these hypothesis; we repeated the human study of Tomicic et al. (Tomicic et al., 2011), but only men and women over the age of 58 years were included. Our aims of this study were to (1) compare the PGME toxicokinetic profiles between the young (20-25 years) and aging volunteers (58- 62 years) exposed to PGME (50 ppm, 6 hours), and between genders, and (ii) test the predictive power of the toxicokinetic model adjusted with physiological parameters for aging persons (Tomicic and Droz, 2009).

METHODS

Chemicals

PGME (>98%) was obtained from Sigma-Aldrich (Buchs, Switzerland), tert-butoxy-2-propanol (CAS 57018-52-7, internal standard) from Fluka (Buchs, Switzerland), and distilled dichloromethane, methanol, heptane (CAS 142-82-5, internal standard), anhydrous sodium sulfate, hydrochloric acid, and sodium hydroxide from Merck (Darmstadt, Germany).

Study population

A total of ten volunteers (n=10) were included in the study; five men and five women. Exclusion criteria employed were the same as for the group of young volunteers in the previous study (Tomicic et al. 2011).

It was very difficult to recruit healthy volunteers between the ages 55-65 years who did not smoke, use medications, and drink more than 1-3 glasses of alcohol each day. More than a thousand pamphlets explaining the study were distributed in waiting rooms among local dentists, ophthalmologists, opticians, and in fitness centers in Lausanne, Switzerland. We also explained our study to groups of unemployed persons over the age of 50 years who were attending a job training seminar, and to persons attending evening university classes. The most successful recruitment, however, was from displaying the pamphlet on information boards in a large hospital (Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland). During the initial phone contact with a potential volunteer, the study was explained in detail and the use of medication, tobacco products, and alcohol consumption were used for screening.

Selected healthy volunteers underwent a medical examination by an occupational physician here in our institute (the Institute of Work and Health (IST, Lausanne, Switzerland), and included a health questionnaire, a general physical examination (a blood sample to analyze common liver enzymes, spirometry, and an electrocardiogram).

This human volunteer study was approved by the by the Clinical Research Ethics Committee of the Faculty of Biology and Medicine of the University of Lausanne, according to the Declaration of Helsinki, and all volunteers signed a written informed consent before participating in the study. The volunteers were reimbursed for their time and inconvenience CHF 200 and travel expenses.

Exposure conditions

We allowed maximum two volunteers in the exposure chamber (12 m^3) for each 6-hour exposure session, resulting in eight sessions. Each volunteer was checked by an IST physician just before entering and exiting the exposure chamber. The volunteers were considered at rest (0 Watts) during the exposure period.

The exposure chamber have been extensively described elsewhere (Tomicic et al., 2011). Briefly, air renewal rate was 12-15 per hours at ambient temperature (24 ˚C) with PGME air concentration fixed at 50 ppm and controlled by the software Lab VIEW (National Instruments Corporation, Texas, USA). The PGME air concentrations were continuously monitored with a portable FTIR (Fourier Transform Infrared Spectroscopy) gas analyzer (Gasmet TM DX4015, Gasmet Technologies Oy, Helsinki, Finland) and a stationary gas chromatograph (GC) (Perkin Elmer) with a flame ionization detector (FID). In addition, personal air concentrations were collected (air sampling rate of 100 ml/min) using activated charcoal to confirm the actual PGME exposures.

Urine collection

Urine samples were collected before the volunteers entered the exposure chamber and every 2 hours during exposures (in a solvent free area) for six hours. The urine was quantitatively collected for additional 20 hours by the volunteers themselves. The volunteers were instructed to continue collecting their urine in polyethylene bottles, note the collection time, and store the bottles in his/her refrigerator (4 ˚C) until bringing them to the laboratory the next day. The urine samples were immediately aliquoted after reception and stored frozen (-20 ˚C) until analysis.

Analytical methods

Urine analysis of PGME was performed according to the method of Tomicic and Berode (Tomicic and Berode, 2010). In brief, quantification of urinary PGME included a gas chromatograph (Agilent 6890, Agilent Technologies AG, Urdorf, Switzerland) equipped with a capillary column (CP-Sil 8 CB, 95% dimethylpolysiloxane polymer, 5% phenyl groups; 60 m×0.25 mm i.d., 1.00 μm film thickness Varian Chrompack Milian SA, Geneva, Switzerland), a multipurpose sampler (Gerstel AG, Sursee, Switzerland) operated in headspace mode, and a flame ionization detector. Total PGME was determined in urine samples (2 ml) after acidic hydrolysis (200 μl of 10 M hydrochloric acid), incubation at 100 °C for 16 h, and neutralization with 200 μl of 10 M sodium hydroxide. We did not adjust for creatinine due to the passive process of elimination in the kidney (Tomicic et al., 2011). We used the urinary creatinine concentrations to check for the completeness of urine collection (Edwards et al 1969), and if creatinine concentrations were within the normal range 0.3-3.0 g/L (WHO 1996) we deemed the urine sample complete.

The charcoal tubes were desorbed with dichloromethane:methanol (95:5) and analyzed with GC/FID as described above for the urine analysis using liquid injection (Varian 3800, Varian Chrompack Milian SA, Geneva, Switzerland).

Model description

We used the age-adjusted TK model developed by Tomicic and Droz (2009). This model incorporates physiological parameters such as body weight, cardiac output, blood flows, volumes of the central compartment and slowly perfused tissues, renal clearances and metabolic constants (Pierrehumbert et al., 2002). The age-adjusted physiological parameters were liver weight (- 8%), body fat (+ 30%), kidney weight (- 20%), renal blood flow (- 25%), hepatic blood flow (- 20%), urinary excretion rate (- 30%), and metabolic rate (- 8%). The

cardiac output was unchanged. The age parameters for metabolic rates were based on values found among young volunteers (Tomicic and Vernez, submitted 2011). The specific physiological and metabolic parameters used in the model are given in table 1. This model was used in comparison to our experimental results.

Statistical and simulation methods

We computed the geometric mean (GM) and geometric standard deviation (GSD) for the demographics of the study participants. Figures were made in Stata version 11.0 (Stata Corp L8, Texas, USA). The toxicokinetic model was run in Berkley Madonna software version 8.3.18 (University of California, USA).

We adjusted metabolic and physiologic parameters for curve-fitting. Occupational exposure scenarios at the current Swiss OEL for 8-hour/work-day and 5 days/week over four weeks were simulated using the obtained model. The physical workload was set at 50 W for 12 hours per day and at 0 W the remaining of the day.

Analysis of variance (ANOVA) was conducted to test whether age was a significant factor in the PGME toxicokinetic. Both increasing and decreasing toxicokinetic slopes were used as kinetics descriptors. Age, hormonal treatment, and gender were dependent variables and log increasing (0 -6 hrs) and log decreasing (6-16 hrs) were independent variables. Tests were performed for both free and total PGME.

RESULTS

A total of 11 volunteers, six women and five men, participated in our study. Table 2 summarizes the volunteer demographics. During the study, one of the volunteers was under menopausal hormonal treatment, which might alter the urinary excretions of PGME as shown in the study among younger volunteers (Tomicic et al., 2011). We therefore decided not to retain this volunteer for further analysis, reducing the number of female volunteers to five (total n=10).

The PGME air concentrations in the exposure chamber remained stable during all exposure sessions (49.6 ppm ± 3.70). The FTIR, GC/FID, and PGME absorbed onto the charcoal tubes collected using personal sampling for each session were not significantly different (*p*-value = 0.3).

We had a complete number of urine samples for all participants during the time in the exposure chamber (6 hours). After exposure ceased, the urine samples were collected *ad lib*. This resulted in missing urine sample collections for some of the 2-hour periods.

Unmetabolized urinary fraction of PGME; urinary free, conjugated (free subtracted from total PGME), and total PGME in urine as a function of time after six hours of exposure to 187 mg/m^3 (50.9 ppm) PGME are depicted in figure 1 and 2. The concentration of PGME (free and total PGME) in urine rose rapidly, and did not reach an apparent plateau level during the six hours of exposure. The toxicokinetic profiles were similar between men and women, with slight differences in the maximum concentration and elimination rate.

Intriguingly, the free and total PGME urinary profiles seemed to overlap, which was not noted for young (<25 years) individuals (Tomicic et al., 2011). The ratio between free and

conjugated PGME was higher in the group >58 years compared to <25 years, signifying much less conjugation in the older group. The total quantities of PGME (free and conjugated) excreted during 6 hours were 2.8 mg for the young and 2.3 mg for the aging volunteers. The age-adjusted TK model (Tomicic and Droz, 2009) did predict well for total urinary PGME (Figure 1) but not for urinary free PGME in the aging group (Figure 2),

Consequently, it was necessary to perform curve-fitting for the urinary free PGME as the age-adjusted TK model fit was not satisfactory (Figure 2). The main toxicokinetic PGME pathway is metabolism through the enzymes O-demethylation via microsomal enzymes (pentoxyresorufin O-depentylase (PROD) activity) (Miller et al., 1986) and the rate best described with the Michaelis-Menten model, which was governed by the parameter V_{max1} in our model. V_{max1} represented the maximum rate achieved by the enzyme system at maximum PGME concentrations. A second parameter was used to improve curve-fitting and were either V_{max2}, describing the maximum rate of the conjugation step of PGME; K_{m1} the Michaels-Menten constants for PROD activity; K_{m2} the Michaels-Menten constants for the conjugation pathway; and the parameter governing the urinary excretion rate (k_{ur}), which is known to be reduced in older individuals. The curve-fitting for men and women >58 years resulted in V_{max1}=15.2 mg/h*kg$^{0.75}$ and V_{max1}=12.1 mg/h*kg$^{0.75}$, respectively, and V_{max2}=0.02 mg/h*kg$^{0.75}$ for both genders. The good fit achieved using V_{max2} as the second parameter indicated that the conjugation pathway was also affected. The curve-fitting achieved using V_{max1} and the parameter representing urinary excretion (k_{ur}) lead to equally good fit as adjusting with V_{max2}. Adjusting the K_{m1} and K_{m2} values did not result in a better curve-fitting than V_{max1} alone (data not shown). Compared to the group of young volunteers (<25 years), the aging group (>58 years) only achieved half the value for V_{max1} (V_{max1}=30 mg/h*kg$^{0.75}$ for young group), implying a slower metabolic pathway in the aging group.

These developed models were used to simulate occupational exposure scenarios at the current Swiss OEL for 8-hour/work-day and 5 days/week over four weeks. The physical workload was set at 50 W for 12 hours per day and at 0 W the remaining of the day. No build-up over the week or month was seen for the aging population (Figure 3).

The urinary excretion of both free and total PGME followed an exponential decay. It is known that PGME follows a 0-order kinetics. We therefore calculated time-specific half-lives for free, conjugated, and total PGME from the fitted curves starting from when exposure ceased (6 hours) to the end of the urine collections (24 hours). The calculated half-lives are given in Table 3.

To test for possible saturation, it was necessary to change the age-adjusted TK model (Tomicic and Droz, 2009), which had a simplified Michaels-Menten equation to incorporate the commonly known equation to allow for saturation in the model. Saturation of PGME metabolism did not occur at 50 ppm among our volunteers (i.e. linear elimination kinetics). In simulation runs with increasing inhalation exposures, the two models (one that allowed for saturation (our TK model) and one that did not (Tomicic et al., 2010)), predicted no saturation to occur below 100 ppm. The average total dose of PGME was calculated with PGME concentration (C), assumed respiratory minute volume (RMV), duration (D), and body weight (BW) on an "average" person, giving:

Dose = (C (mg/l) * RMV (L/min) * D (min)) / BW = 0.188 * 15 * 360 / 70 = 14.5 mg/kg bw.

Regarding the toxicological profiles in young and aging volunteers, age was found to be significant and accounted for about 50% of the total variance in the increasing slope of free

PGME (R^2=0.66, p<0.0001). No difference was detected in total PGME (as the dependent variable) or elimination rates. Although the kinetic curves exhibited some tendencies, the number of volunteers was limited and may explain the lack of significance.

DISCUSSION

In this study, we showed that PGME toxicokinetic profiles were similar among aging men and women (58-62 years). We also showed that the urinary PGME profiles were different for the group of young (20-25 years) and aging (58-62 years) volunteers exposed to PGME (50 ppm, 6 hours), which confirms our first hypothesis. The conjugation pathway was more pronounced in young men, implying a slower phase II enzymatic metabolism in both young women without OC and aging volunteers.

Conversely, the age-adjusted TK model (Tomicic and Droz, 2009) predicted well urinary total PGME concentrations in aging volunteers, but not urinary *free* PGME. These results show that adjusting for physiological parameters to accommodate changes in the body due to age was not sufficient to predict the PGME elimination in aging individuals. Adjusting the maximal metabolic rate (V_{max1}) increased the curve-fitting for both urinary total and *free* PGME. Results from curve-fitting parameters for the conjugation pathway (V_{max2}) or urinary excretion (k_{ur}) were equally efficient. Based on our data and model, it is difficult to choose which of these two parameters to use, nevertheless less conjugation was observed among the aging volunteers compared to the young, indicating that the conjugation pathway (V_{max2}) was affected.

Compared to the group of young men (<25 years), the aging men (>58 years) only achieved half the value for V_{max1} (V_{max1}=30 mg/h*$kg^{0.75}$ for young men), implying a slower enzymatic

pathway in the aging group, specially the PROD activity. PROD metabolizes the PGME α-isomer to propylene glycol. In a recent study (Yun et al., 2010), hepatic CYP isoform levels were measured in aging rats, and the authors found that the expressions of CYPs were regulated by age in an isoform-specific manner. More specifically, the hepatic expression of CYP1A2, 2B1, and 2E1 decreased markedly as the rats aged. This might explain the difference in V_{max1} seen between young and aging men (V_{max1} aging = 12-15 mg/h*kg$^{0.75}$ and V_{max1} young = 29 mg/h*kg$^{0.75}$). Alternatively, the difference in V_{max1} between young and aging men could be explained by a reduced ADH and ALDH production in aging men; ADH and ALDH being the two enzymes which metabolizes the PGME β-isomer. On the contrary, women have little or no change in ADH levels as they age, and this can explain the similar V_{max1} value for young women not under OC treatment (Tomicic et al., 2011) and aging women. It remains that the ADH levels in the aging groups were similar between genders, as supported with our data where no gender difference in V_{max} among aging individuals was observed (V_{max1} aging men = 15 mg/h*kg$^{0.75}$ and aging women = 12 mg/h*kg$^{0.75}$). However, since the commercial PGME product contains very small amounts of the β-isomer, the explanation that PROD activity diminishes with age is more plausible.

In young volunteers, the men had twice the maximal metabolic rate compared to young women (V_{max1}; young men = 30 mg/h*kg$^{0.75}$ and young women = 15 mg/h*kg$^{0.75}$), but similar to young women under hormonal treatment (OC). Hormones may change the phase I and II hepatic metabolizing enzymes (Kennedy, 2008), particularly when hormonal fluctuations are most pronounced. This might explain the differences between young women taking or not taking OC (Tomicic et al., 2011), and the non-differences seen between aging men and women where hormonal interference on biotransformation of PGME is low. Metabolizing enzyme expression and activity may be effected by sex steroids, and may alter the PGME

phase II metabolism as they are also glucuronidated. A large cross-sectional study exploring testosterone and estradiol levels in an aging population, showed total and free estradiol to be *negatively* associated with age in postmenopausal women (50-59 years, n=379) and *positively* associated with age in aging men (50-59 years, n=467) (Bjørnerem et al., 2004). The mean concentration of total estradiol in men was about twice that of postmenopausal women, and four times less compared to pre-menopausal women (25-39 years, n=120). There is not a clear cut or obvious pattern of how testosterone, estrogen and progesterone could alter PGME clearance. The estrogenic component of OC has shown to increase phase II drug metabolism via conjugation and glucuronidation (Kennedy, 2008). Estrogen levels in men increase with age while decrease for women, making them more similar in estrogen levels compared to women and men of younger ages but also prone to less glucuronidation resulting in more urinary excretion of free PGME. This might explain why the toxicokinetic model for the young only worked for the total PGME in the aging. We did not ask about the volunteers' menopausal status. The activities of phase II metabolic enzymes during this hormonal status were therefore not assessed.

The phase II metabolic enzyme pathway might not be the only explanation as physiological parameters in the aging body also play an important role in excretion. Reduction of the renal function with age affects a substance excretion rate, but also the urinary flow rate. In the model, the assumption was that renal functions are reduced by 30 % from young to aging volunteers. We found reduction in urinary flow rate from the young to the aging volunteers to be greater than 30% (52% in men and 41% in women) by comparing total urine volumes excreted after 6 hours. We obtained higher concentrations of free PGME in aging compared to young men, while similar values between the two groups for total PGME. The urinary excretion rate is not the same for free and conjugated PGME, and therefore a decrease in renal

function cannot explain the age difference in the ratio between free and conjugated PGME, but could explain the longer apparent half-life we observed (Table 3). Other physiological changes during aging that implicate toxicokinetics have been reviewed (Ginsberg et al 2005). For instance body mass indices (BMI) increase with age and result in longer half-lives by sequestering lipophilic substances in body fat. In our study, the volunteers BMIs were similar in both age groups, and are probably not a major player. Likewise, decreased plasma protein binding capacity can lead to increased proteinuria, which gives higher urinary concentration of the parent compound (Masoro and Snyder, 2001 referenced in Ginsberg et al 2005). Free PGME concentration was indeed higher than the conjugated PGME in aging individuals, and this could potentially be an alternate explanation. We did not measure protein content in the urine samples and cannot assess if proteinuria was common among our study participants. Other possible explanations are the diminished hepatic blood flow (included in the model) and bile flow (Zeeh and Platt 2002) seen in aging individuals. More conjugated PGME through bile excretion could be possible; however, since the amount excreted in urine among aging and young was similar (2.8 and 2.3 mg in young and aging volunteers, respectively) this is not very likely. The fitness of the model was enhanced by adjusting the Michaelis-Menten maximum rate to 1/10 of the V_{max2} for the young. A recent review suggests that no age related changes in phase II metabolism occur (Mitchell et al 2011); however, this might be due to few studies conducted and that were performed in the 1970s. Age have also been demonstrated to affects elimination capabilities through glucuronidation by disturbing the balance between glucuronidation and deglucuronidation which vary with age, tissue, and substrate (Borghoff and Birnbaum 1985; Birnbaum 1991). Furthermore, alternate explanations exist in that the conjugation step itself is not slower, but that the hepatic blood flow is less than what was assumed in the model, resulting in less PGME reaching the liver among aging versus young volunteers. Another explanation is reduced glucuronide secretion

in the kidneys in aging individuals (Dickinson et al. 1993), which would also lead to higher urinary free PGME as observed in our volunteers. These factors were not assessed in our study, and should be further explored in the future. As pointed out by Ginsberg and colleagues (2005), multiple factors may combine in aging individuals to decrease hepatic clearance of xenobiotics.

The factor of saturation of the metabolic pathway was not observed among the aging volunteers at 50 ppm for 6 hrs at a dose of 14.5 mg/kg bw, giving 1 mg/day for a 70 kg person. This was in agreement with other human studies that found saturation of metabolic clearance to occur approximately at 7 mg/day (Speth et al., 1987). Apparent elimination half-lives in humans have been assessed experimentally in clinical-, occupational-, and toxicokinetic studies. In patients, Speth et al. (1987) found a PGME half-life before saturation of metabolic clearance (1st order kinetics) of 2.3 hours following an intravenous (IV) administration. Elimination PGME half-lives were longer and followed a 0-order kinetics after metabolic saturation. The following apparent eliminations have been reported in the literature: >3 hours when doses (IV) were above 12 g/day (Speth et al 1987); ~ 4 hours after repeated high doses (20.7 or 41.4 g doses given 2–3 times per day) (oral administration) (Yu et al., 1985); and 2.8 hours after a dose of 8.64 g (rectal administration) (n=10) (Kolloffel et al., 1996).

In occupational settings, PGME apparent half-life was 1st order and estimated to 4.4 hours among highly exposed breakhose manufacturing workers (n=3, all men) after four days of PGME exposures (20 ppm) (Hubner et al 1992). The age of the workers were not reported. Three toxicokinetic studies exposing volunteers in an exposure chamber reported half-lives of <2.0 hours (n=6: two women and four men between 22-44 years, 8 hours, 100 ppm) (Jones et al., 1997); 1.5 hours (n=4; two women and two men between 26-45 years, 4 hours, 100 ppm);

and 3.5 hours (n=6; all men between 23-31 years, 6 hours, at 15, 50, and 95 ppm) (Devanthery et al., 2002). Only *free* PGME was determined in two of the toxicokinetic studies (Brooke et al., 1998; Jones et al., 1997), and may therefore not be directly comparable to Devanthery et al. (Devanthery et al., 2002) and our study. The apparent half-lives of 4.5 hours obtained in our study are somewhat longer than those seen for unsaturated metabolic pathway in patients (2.3 hours; (Speth et al., 1987)); however, they are not directly comparable as the administration pathways were different; inhalation (this study) and IV (Speth et al., 1987). The apparent PGME half-lives we found among aging volunteers were very similar to what has been reported previously for workers (4.4 hours; (Hubner et al., 1992)). The discordance between half-lives at lower concentrations (i.e. not saturated metabolic pathway) may be explained by the study participants' ages; we observed a shorter PGME half-life among young (<25 years) volunteers with 2.2 and 3.2 hours in men and women, respectively, compared to the aging (>58 years) volunteers with 4.5 hours for both genders. The difference in apparent PGME half-life due to age could be explained by a slower metabolism in the aging volunteers as previously described, while the difference due to gender could be attributed to the competing hormonal enzymatic action.

Half-lives may increase among individuals with impaired bladder emptying caused by urethral obstruction (benign prostatic hyperplasia and urethral stricture, shy bladder syndrome) or detrusor muscle areflexia or impared centricity (neurologic diseases damaging the nerves that innervate the bladder, detrusor muscle damage, anticholinergic drugs and reduced sensory deficit common in diabetic neuropathy patients) (Yoshimura and Chancellor, 2004).

Simulation of exposures to 100 ppm (occupational exposure limit for PGME in Switzerland) for one work-week (5 days of exposure followed by 2 days of no exposure) for one month,

did not show any significant build-up of PGME in the body over time (Figure 3). This is supported by previous published studies among breakhose manufacturing workers where no difference in concentrations was seen after four days of PGME exposures (Hubner et al., 1992). However some accumulation of internal PGME dose in the body was observed using our model, we therefore recommend that biological monitoring is performed at the end of the work-week post-shift. It is also necessary to consider the half-life differences among young and aging workers as this will play a roll if the PGME exposures occur in the beginning of a work-shift or later. For example, assessing PGME exposures post-shift in men who have exposures (100 ppm) only early in the shift (8-9 am) will result in low exposures (12.5 ppm) in young men since three half-lives ($T_{1/2}$=2.2 hours) will have passed, while in aging men exposures will be higher (15.7 ppm) because only 1.5 half-lives ($T_{1/2}$=4.5 hours) will have occurred. The biological monitoring should be performed by determining the total (both free and conjugated) PGME in urine as this eliminates gender (or hormonal) and age differences.

One limitation of our study was assumptions used in our model. We considered a PGME retention rate of 90% (Stott and McKenna, 1984), PGME clearance mainly through metabolism (90%), and the rest by renal excretion of the parent compound. Previously developed PBPK models for PGME, were based on rat and mouse studies, hence have slightly different model estimates for PGME clearance, such as 56-63% through metabolism and 11-25% through the minor pathway, than our model (Corley et al., 1996; Corley et al., 2004; Kirman et al., 2005). However, human metabolic clearance of PGME has shown to be similar to the rabbit (Yu and Sawchuk, 1987) with 85.8-97.6% of total clearance at lower doses, and have been supported by another human study (Speth et al., 1987).

Conclusion

This is the first study describing PGME inhalation toxicokinetic profiles in aging (>58 years) human volunteers. Results show a difference in urinary profiles according to age, where aging volunteers excrete higher concentration of free PGME compared to conjugated PGME than the young (<25 years) group in a previous study. By adjusting the maximum rates of PGME in the toxicokinetic model, we achieved a good fit, indicating slower phase I and II metabolism with age. Several age-related physiological explanations can be offered for the less conjugation seen among aging individuals such as changed balance between glucuronidation and de-glucuronidation (Borghoff and Biernbaum, 1985), decreased plasma protein binding capacity (Masoro and Snyder, 2001 referenced in Ginsberg et al. 2005) and reduced secretion of glucuronides (Dickinson et al 1994), all giving higher urinary concentrations of the parent compound. Another explanation is that the hepatic blood flow was less than assumed in the model, resulting in less PGME reaching the liver, consequently less conjugation among aging versus young volunteers. Regarding the TK model, changing physiological parameters to reflect changes in age was not sufficient in predicting free PGME; however, it predicted well urinary total PGME for both young and aging individuals. TK models are useful, but metabolic and physiological parameters should be amended according to age. Gender differences in toxicokinetic profiles observed among the young volunteers were not observed among the aging. Consequently, for biological monitoring of men and women exposed to PGME, we recommend quantifying total PGME in urine. This would eliminate differences in conjugated and free PGME seen between young and aging, and genders. PGME apparent half-life in aging volunteers exposed to PGME at 50 ppm for six hours was 4.5 hours and followed 1st order kinetics, i.e. saturation in the PGME metabolism did not occur. Age is a factor that should be considered when biological limit values are developed because both physiological and metabolic changes due to age may play a significant role in type and amount of substance excreted, and its apparent half-life.

The authors declare that there are no conflicts of interest.

References

ACGIH 2011. Threshold limit values for chemical substances and physical agents and biological exposure indices. 2011 TLVs® and BEIs®. Publication #0111. Cincinnati, OH: American Conference of Governmental Industrial Hygienists. ISBN: 978-1-607260-28-8.

Bjørnerem, Å., Straume, B., Midtby, M., Fønnebø, V., Sundsfjord, J., Svartberg, J., Acharya, G., Øian, P., Rosvold Berntsen, G.K., 2004. Endogenous Sex Hormones in Relation to Age, Sex, Lifestyle Factors, and Chronic Diseases in a General Population: The Tromsø Study. J Clin Endocrin Met 89, 6039-6047.

Brooke, I., Cocker, J., Delic, J.I., Payne, M., Jones, K., Gregg, N.C., Dyne, D., 1998. Dermal uptake of solvents from the vapour phase: an experimental study in humans. Ann Occup Hyg 42, 531-540.

Borghoff, S., Birnbaum, L., 1985. Age-related changes in glucuronidation and deglucuronidation in liver, small intestine, lung, and kidney of male Fischer rats. Drug Met Disp 13, 62-67.

Birnbaum, L., 1991. Pharmacokinetic basis f age-related changes in sensitivity to toxicants. Annu Rev Pharmacol 31, 101-128.

Corley, R.A., Crissman, J.W., Redmond, J.M., McGuirk, R.J., Cieszlak, F.S., Stott, W.T., 1996. Adaptive metabolic and pathologic changes following chronic inhalaation of propylene glycol monomethyl ether in rats and mice. Occup Hyg 2, 319-328.

Corley, R.A., Gies, R.A., Wu, H., Weitz, K.K., 2004. Development of a physiologically based pharmacokinetic model for propylene glycol monomethyl ether and its acetate in rats and humans. Toxicol Lett 156, 193-213.

Corsonello, A., Pedone, C., Incalzi, R.A., 2010. Age-related pharmacokinetic and pharmacodynamic changes and related risk of adverse drug reactions. Curr Med Chem 17, 571-584.

Dentan, A., Devanthery, A., de Peyer, J.E., Droz, P.O., 2000. Propylene glycol monomethyl ether (PGME) exposure. 2. Identification of products containing PGME, their importance and their use in Switzerland. Int Arch Occup Environ Health 73, 349-351.

Devanthery, A., Berode, M., Droz, P.-O., 2002. Propylene glycol monomethyl ether occupational exposure. 3. Exposure of human volunteers. Int Arch Occup Environ Health 75, 203-208.

Devanthéry, A., 2003.Toxico-cinétique et surveillance biologique du propylène glycol monométhyl éther. Etude sur des volontaires. Unpublished PhD thesis. Federal Polytechnic School of Lausanne, Switzerland.

DFG, D.F., 2011. List of MAK and BAT values 2011. Wiley-VCH, Weinheim.

Dickinson R.G., King A.R, McKinnon G.E., Hooper W.D., Eadie M.J., Herkes G.K., 1993. Studies on the renal excretion of the acyl glucuronide, phenolic glucuronide and sulphate conjugates of diflunisal. Br J Clin Pharmac 35, 609-613.

Edwards O.M., Bayliss R.I., Millen S., 1969. Urinary creatinine excretion as an index of the completeness of 24-hour urine collections. Lancet 2, 1165–1166.

ElDesoky, E.S., 2007. Pharmacokinetic-pharmacodynamic crisis in the elderly. Am J Ther 14, 488-498.

Ginsberg G., Hattis D., Russ A., Sonawane B., 2005. Pharmacokinetic and pharmacodynamic factors that can affect sensitivity to neurotoxic sequelae in elderly individuals. Environ Health Perspect 113, 1243-1249.

Hubner, B., Lehnert, G., Schaller, K.H., Welte, D., Angerer, J., 1992. Chronic occupational exposure to organic solvents. XV. Glycol ether exposure during the manufacture of brakehoses. Int Arch Occup Environ Health 64, 261-264.

Johanson, G., Dynésius B., 1988. Liquid/air partition coefficients of six commonly used glycol ethers. Br J Ind Med 45(8), 561–564.

Jones, K., Dyne, D., Cocker, J., Wilson, H.K., 1997. A biological monitoring study of 1-methoxy-2-propanol: analytical method development and a human volunteer study. Sci Total Environ 199, 23-30.

Kennedy, M.J., 2008. Hormonal Regulation of Hepatic Drug Metabolizing Enzyme Activity During Adolescence. Clin Pharmacol Ther 84, 662-673.

Kirman, C.R., Sweeney, L.M., Corley, R.A., Gargas, M.L., 2005. Using physiologically-based pharmacokinetic modeling to address nonlinear kinetics and changes in rodent physiology and metabolism due to aging and adaptation in deriving reference values for propylene glycol methyl ehter and propylene glycol methyl ether acetate. Risk analysis 25, 271-284.

Kolloffel, W.J., Weekers, L.E., Goldhoorn, P.B., 1996. Pharmacokinetics of propylene glycol after rectal administration. Pharm World Sci 18, 109-113.

Laparé, S., Tardif, R., Brodeur, J., 1995. Effect of various exposure scenarios on the biological monitoring of organic solvents in alveolar air II. 1-1-1-trichloroethane and trichloroethylene. Int Arch Occup Environ Health 67, 375-394.

Miller, R.R., 1987. Metabolism and disposition of glycol ethers. Drug Metab Rev 18, 1-22.

Miller, R.R., Langvardt, P.W., Calhoun, L.L., Yahrmarkt, M.A., 1986. Metabolism and disposition of propylene glycol monomethyl ether (PGME) beta isomer in male rats. Toxicol Appl Pharmacol 83, 170-177.

Mitchell, S.J., Kane, A.E., Hilmer, S.N., 2011. Age-related changes in the hepatic pharmacology and toxicology of paracetamol. Curr Gerontol Geriatr Res. 2011;2011:624156. Epub 2011 May 11.

Pierrehumbert, G., Droz, P.-O., Tardif, R., Charest-Tardif, G., Truchon, G., 2002. Impact of human variability on the biological monitoring of exposure to toluene, phenol, lead, and mercury: II. Compartmental based toxicokinetic modelling. Toxicol Lett 134, 165-175.

SFSO, 2010. Les scénarios de l'évolution de la population de la Suisse 2010-2060. Swiss Federal Statistical Office, Available at http://www.bfs.admin.ch/bfs/portal/fr/index/news/publikationen.Document.132793.pdf.

Sobus, J.R., Pleil, J.D., McClean, M.D., Herrick, R.F., Rappaport, S.M., 2010. Biomarker variance component estimation for exposure surrogate selection and toxicokinetic inference. Toxicol Lett 199, 247-253.

Speth, P., Vree, T.B., Neilen, N.F.M., de Mulder, P.H.M., Newell, D.R., Gore, M.E., de Pauw, B.E., 1987. Propylene glycol pharmacokinetics and effects after intravenous infusion in humans. Ther Drug Mon 9, 255-258.

Stott, W.T., McKenna, M.J., 1984. The comparative absorption and excretion of chemical vapors by the upper, lower, and intact respiratory tract of rats. Fund appl toxicol 4, 594-602.

SUVA, S.N.A.I.F., 2011. Valeurs limites d'exposition aux postes de travail 2011. SUVA, Lucerne.

Thomas, R.S., Bigelow, P.L., Keefe, T.J., Yang R.S.H., 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. Am Ind Hyg Assoc J 57(1), 23-32.

Tomicic, C., Berode, M., Oppliger, A., Castella, V., Leyvraz, F., Praz-Christinaz, S.-M., Danuser, B., 2011. Sex differences in urinary levels of several biological indicators of exposure: a human volunteer study. Toxicol Lett 202, 218-225.

Tomicic, C., Berode, M., 2010. Sensitive headspace gas chromatography analysis of free and conjugated 1-methoxy-2-propanol in urine. Anal Bioanal Chem 396, 2709-2714.

Tomicic, C., Droz, P.-O., 2009. Age differences in biological monitoring of chemical exposure: a tentative description using a toxicokinetic model. Int Arch Occup Environ Health 82, 669-676.

Tomicic, C., Vernez, D., submitted 2011. Sex differences in urinary levels of several biological indicators of exposure: a simulation study using a general compartmental based toxicokinetic model. Int Arch Occup Environ Health.

Toossi, M., 2009. Employment outlook: 2008-18: labor force projections to 2018: older workers staying more active. Mon Labor Rev 132, 30-51.

Truchon, G., Tardif, R., Droz, P.-O., Charest-Tardif, G., Pierrehumbert, G., 2006. Biological exposure indicators: quantification of biological variability using toxicokinetic modeling. J Occup Environ Hyg 3, 137-143.

World Health Organization. Biological Monitoring of Chemical Exposure in the Workplace. Geneva: World Health Organization; 1996.

Yoshimura, N., Chancellor, M.B., 2004. Differential diagnosis and treatment of impaired bladder emptying. Rev Urol 6, S24-31.

Yu, D.K., Elmquist, W.F., Sawchuk, R.J., 1985. Pharmacokinetics of propylene glycol in humans during multiple dosing regimens. J pharma sci 74, 876-879.

Yu, D.K., Sawchuk, R.J., 1987. Pharmacokinetics of propylene glycol in the rabbit. J Pharmacokinetics Biophama 15, 453-471.

Yun, K.U., Oh, S.J., Oh, J.M., Kang, K.W., Myung, C.-S., Song, G.Y., Kim, B.-H., Kim, S.K., 2010. Age-related changes in hepatic expression and activity of cytochrome P450 in male rats. Arch Toxicol 84, 939-946.

Zeeh, J., Platt, D., 2002. The aging liver: structural and functional changes and their consequences for drug treatment in old age Gerontol 48,121–127.

- PGME toxicokinetic profiles differed between age groups (>58 years vs <25 years)
- Gender differences in PGME profiles were not observed among aging volunteers
- For an appropriate exposure assessment, urinary *total* PGME should be quantified
- Biological limit values and toxicokinetic models should include age as a factor

Table 1 Parameters used in the initial model >58 years old

Parameters [units]	Symbol	Value	Reference
Body weight [kg]	BW	Men: 76.0; Women 53.7	Experimental data
Body height [cm]	BH	Men: 174; Women: 165	Experimental data
Cardiac output [l/(h*kg$^{0.7}$)]	Q_c	18.0/30.8	Thomas et al. (1996)
Alveolar ventilation [l/(h*kg$^{0.7}$)]	V_{alv}	18.0/2.1951*Q_c	Thomas et al. (1996)
Urinary excretion rate [ml/(h*kg$^{0.82}$)]	k_{ur}	1.294	Laparé et al. (1995)
Molecular weight [g/mol]	MW	90.12	
Threshold limit value [mg/m3]	TLV	360	
Exposure concentration - modeled [mg/m3]	C_{exp}	180	
Exposure concentration - actual [mg/m^3]	C_{exp}	188	
Pulmonary retention [-]	R_{pulm}	0.9	Devanthéry (2003)
Fraction of cardiac output in peripheral compartment [-]	BF1	0.21/0.13	Thomas et al. (1996)
Blood/air partition coefficient [-]	P_{blood_air}	12383	Johanson et al. (1988)
Central/air partition coefficient [-]	P_{c_blood}	12280	Johanson et al. (1988)
Michaelis-Menten maximum rate for metabolism [mg/(h*kg$^{0.75}$]	Vmax1	27.6	Corley et al. (2005), fitted data
Michaelis-Menten constant [mg/l]	Km1	30	Corley et al. (2005), fitted data
Michaelis-Menten maximum rate for glucoronidation [mg/(h*kg$^{0.75}$]	Vmax2	0.18	Corley et al. (2005), fitted data
Michaelis-Menten constant [mg/l]	Km2	95	Corley et al. (2005), fitted data
Urinary excretion rate for conjugated PGME [h-1]	kconj	0.14	Devanthéry (2003), fitted data

Table 2 Demographics of study participants (five women and five men)

	Age (years)		Height (m)		Weight (kg)		BMI	
	Women	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**
Min Max	52 – 62	58 - 61	1.60 – 1.74	1.67 – 1.81	41 - 75	66 - 84	16 – 24.8	20.2 – 28.3
GM (GSD)	58.5 (3.4)	59.2 (1.0)	1.65 (0.04)	1.74 (0.05)	52.9 (9.9)	75.8 (6.0)	19.5 (2.6)	24.9 (2.9)
AM (SD)	58.6 (3.6)	59.2 (1.1)	1.65 (0.05)	1.74 (0.06)	53.7 (10.6)	76.0 (6.7)	19.6 (2.8)	25.1 (3.2)

Table 3 Age and gender specific PGME half-lives for urinary free, conjugated, and total PGME (6-24 hours) based on modeled data

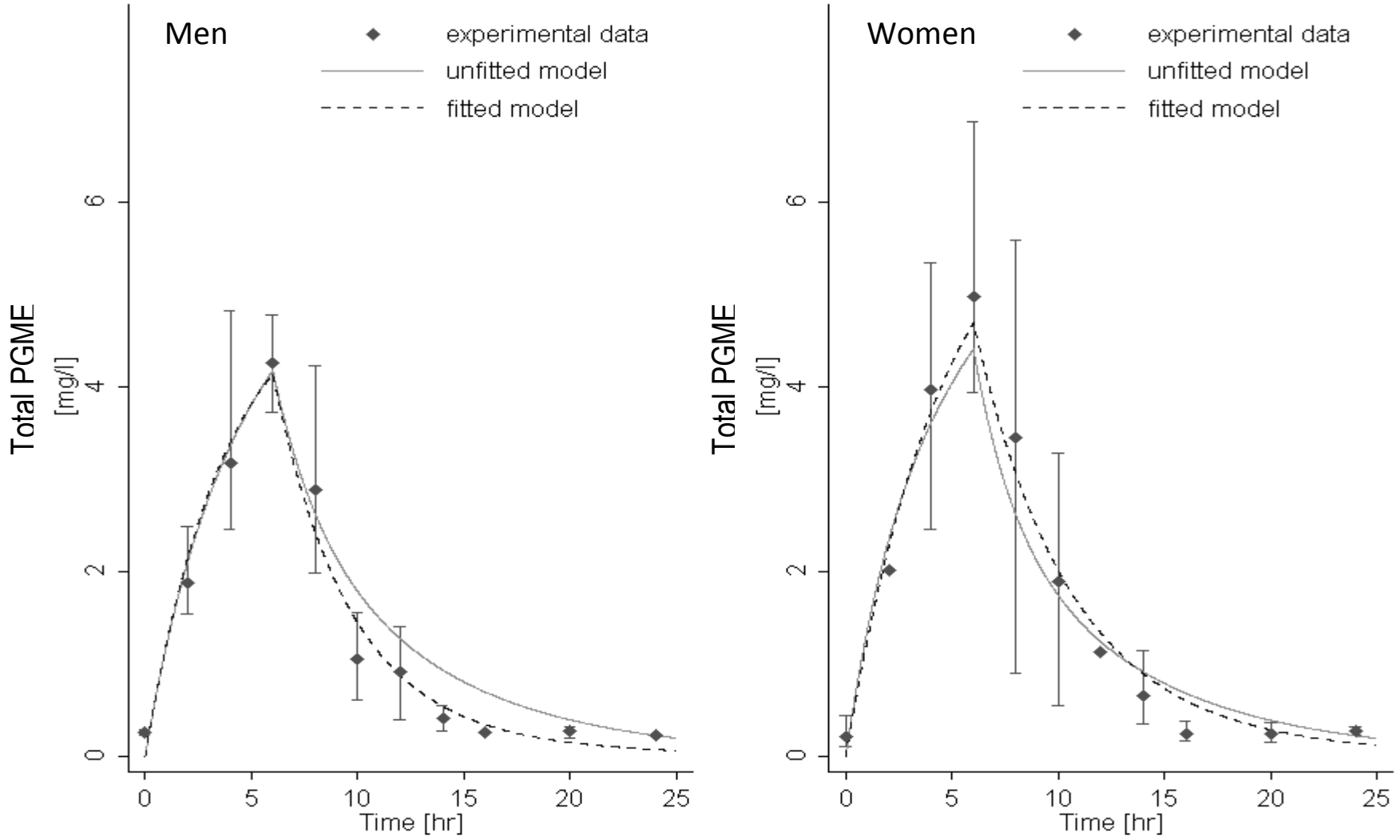
Age	Gender	Hormone treatment	N	PGME half-life (hours)			Ratio PGME
				Free	Conjugated[2)]	Total	free/conjugated
>58 years	Men	No	5	1.4	5.7	4.5	4.1
	Women	No	5	1.2	5.5	4.5	4.6
<25 years[1)]	Men	No	10	1.4	4.0	2.2	2.9
	Women with OC	Yes	10	1.2	3.8	3.2	3.2
	Women without OC	no	5	1.7	3.8	3.2	2.2

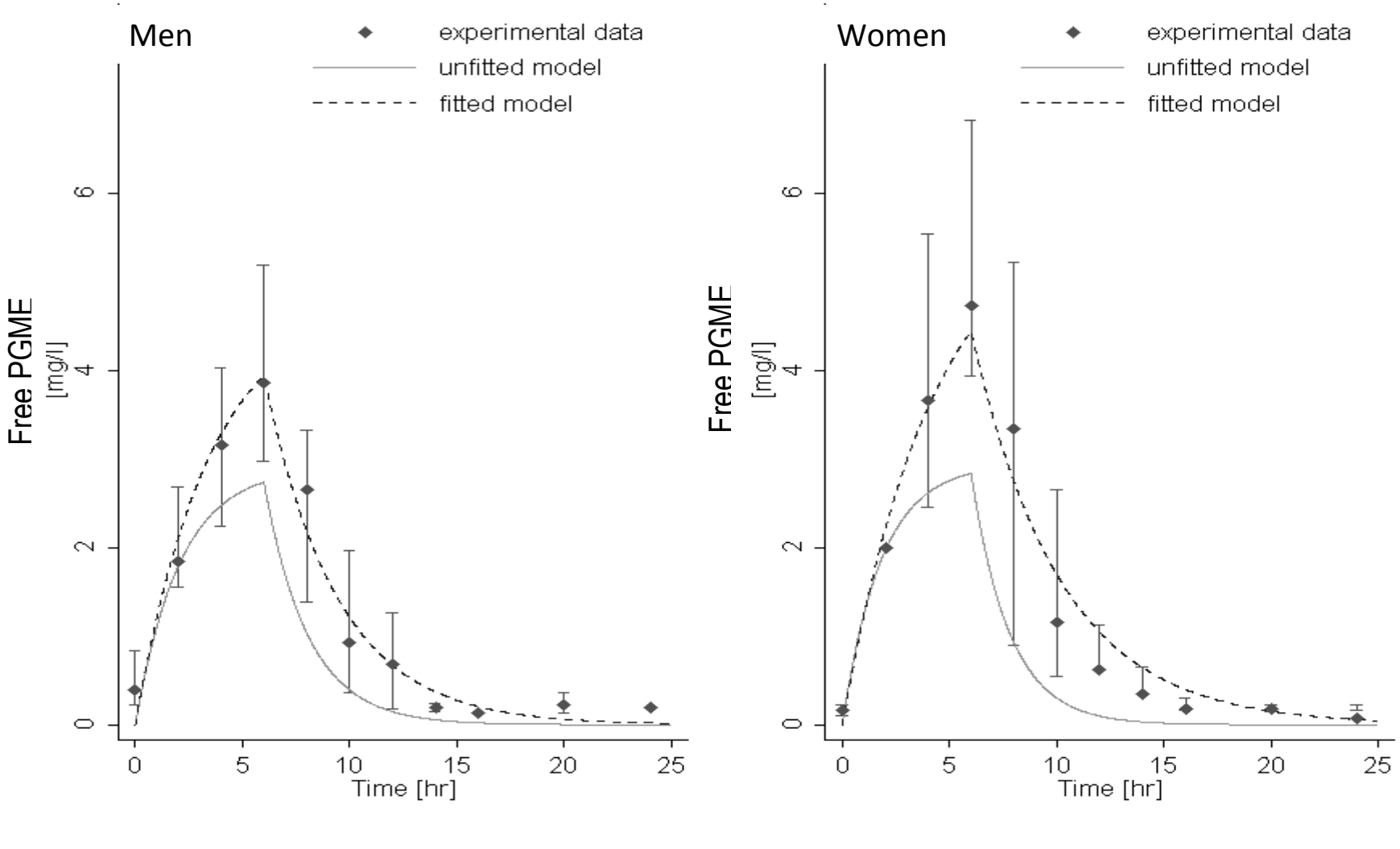
1) Data from the study of Tomicic et al. 2011
2) Conjugated fraction was not measured but calculated: total PGME-free PGME = Conjugated PGME

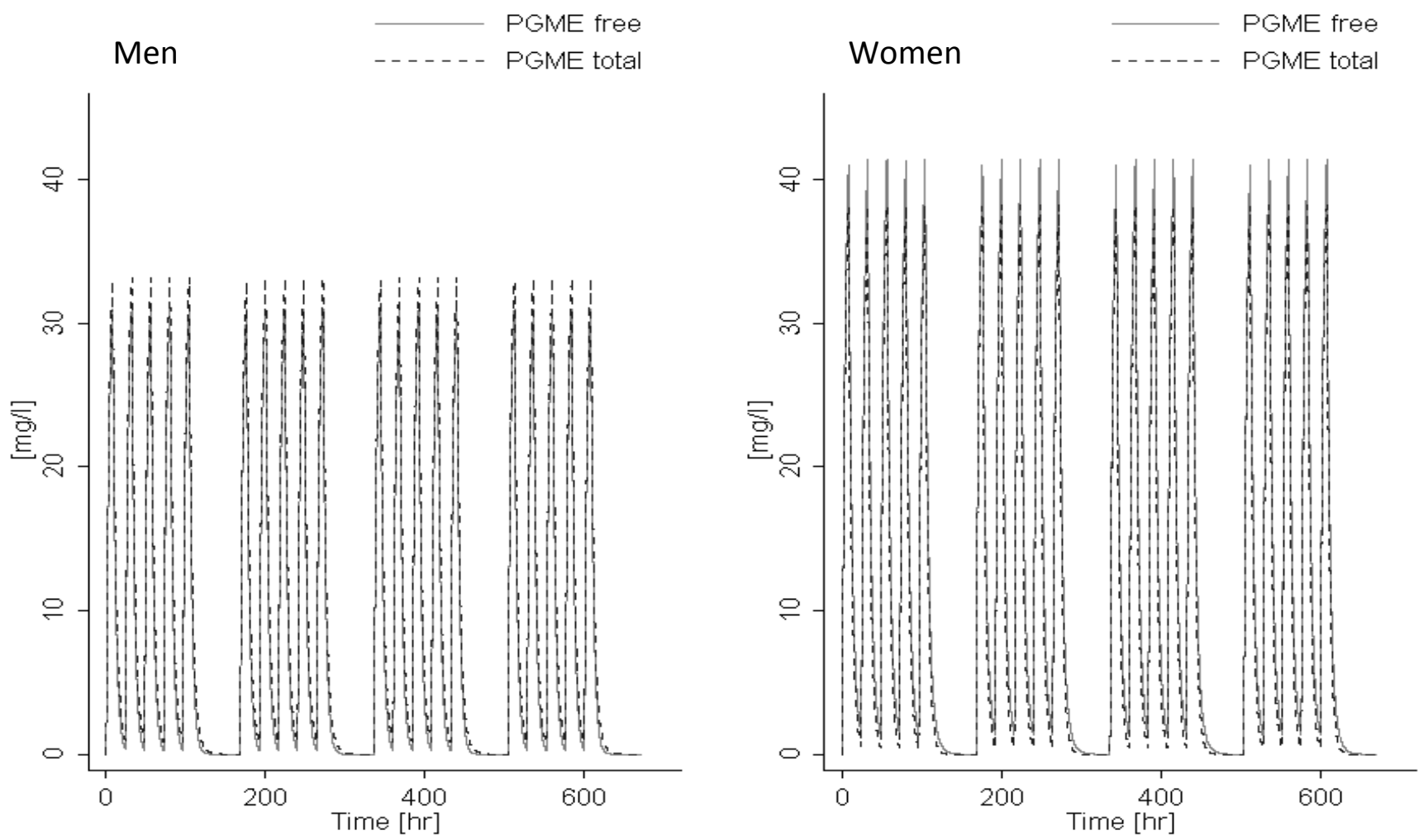
Figure 1 Urinary total PGME concentrations (arithmetic mean and ±SD) among aging volunteers (>58 years, n=10) by gender (men on the left and women on the right) with the model developed for the young volunteers (<25 years, n=20)(Tomicic and Vernez, submitted 2011)(black) and the fitted model (dotted)

Figure 2 Urinary free PGME concentrations (arithmetic mean and ±SD) among aging volunteers (>58 years) by gender (men on the left and women on the right) with the model developed for the young volunteers (<25 years, n=20) (Tomicic and Vernez, submitted 2011)(black) and the fitted model (dotted)

Figure 3 Simulating PGME exposure (100 ppm) during one month of work (5 days with and 2 days without exposure for four weeks) for aging volunteers by gender (men on the left and women on the right)


Men
experimental data
unfitted model
fitted model
Total PGME
[mg/l]
0
2
4
6
0
5
10
15
20
25
Time [hr]
Women
experimental data
unfitted model
fitted model
Total PGME
[mg/l]
0
2
4
6
0
5
10
15
20
25
Time [hr]


Men
experimental data
unfitted model
fitted model
Free PGME
[mg/l]
0
2
4
6
0
5
10
15
20
25
Time [hr]
Women
experimental data
unfitted model
fitted model
Free PGME
[mg/l]
0
2
4
6
0
5
10
15
20
25
Time [hr]


Men
PGME free
PGME total
[mg/l]
0
10
20
30
40
0
200
400
600
Time [hr]
Women
PGME free
PGME total
[mg/l]
0
10
20
30
40
0
200
400
600
Time [hr]