Hydrolysis Kinetics of Propylene Glycol Monomethyl Ether Acetate in Rats in Vivo and in Rat and Human Tissues in Vitro

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The kinetic equvalency of propylene glycol monomethyl ether (PGME), derived from propylene glycol monomethyl ether acetate (PGMEA), as well as the parent compound (PGME) following intravenous administration to Fischer 344 rats was evaluated. In addition, in vitro hydrolysis rates of PGMEA in blood and liver tissue from rats and humans were determined. The blood kinetics were determined following iv administration to rats of PGME and PGMEA of low [10 and 14.7 mg/kg body weight (bw)] or high (100 and 147 mg/kg) equimolar dosages of PGME and PGMEA, respectively. The blood time courses of PGME elimination for both dosages of both compounds were identical. Half-lives of PGMEA elimination following iv administration of 14.7 or 147 mg PGMEA/kg bw were calculated to be 1.6 and 2.3 min, respectively. Rat and human in vitro hydrolysis rates of PGMEA were determined by incubation of 5 or 50 μg PGMEA/ml in whole blood or liver homogenate. The rate of loss of PGMEA was more rapid in rat blood than in human blood, with hydrolysis half-lives of 36 and 34 min in human blood and 16 and 15 min in rat blood for the 5 and 50 μg/ml concentrations of PGMEA, respectively. In contrast the rate of loss of PGMEA in human and rat liver homogenate incubations was similar, 27–30 min and 34 min, respectively. These data demonstrate the rapid hydrolysis of PGMEA in vivo to its parent glycol ether, PGME and that, once hydrolyzed, the kinetics for PGME derived from PGMEA are identical to that for PGME. This study supports the use of the toxicological database on PGME as a surrogate for PGMEA.

Key Words: propylene glycol monomethyl ether; propylene glycol monomethyl ether acetate; rat metabolism; human metabolism; pharmacokinetics.

Propylene glycol monomethyl ether acetate (PGMEA) and propylene glycol monomethyl ether (PGME) are glycol ether solvents commonly used in surface coatings, cleaners, and inks (Boatman, 2001). An extensive toxicological database exists for PGME, and an understanding of the metabolism and hydrolysis kinetics of PGMEA would support the use of the extrapolation of PGME data to PGMEA, since long-term toxicity studies are lacking in the database for PGMEA (Boatman, 2001). An examination of the toxicological databases for PGME and PGMEA reveal that these two chemicals are essentially toxicologically equivalent, with the exception that lesions of the nasal mucosa are observed in inhalation studies with PGMEA and not with PGME. Equivalence is based on the assumption that PGMEA is rapidly and completely hydrolyzed to PGME and acetic acid in vivo and subsequently metabolized via common metabolic pathways.

The metabolism, disposition and excretion of PGMEA is similar to that of PGME, following either single oral or single 6 h inhalation exposure. Following gavage administration of 8.7 mmol per kg body weight (mmol/kg bw) of 14C-PGME, 56% of the administered dose was recovered in the expired air as 14CO2 and 24% recovered in the urine (Miller et al., 1983). Urinary radioactivity was identified predominately as metabolized PGME, with propylene glycol and the sulfate and glucuronide conjugates of PGME identified as the major metabolites. It was concluded that PGME is metabolized via microsomal O-demethylation as the initial metabolic step to form propylene glycol that is further metabolized to CO2. In the case of 14C-PGME, 24% of the administered gavage dose of 8.7 mmol/kg bw to Fischer 344 rats was recovered in the urine and 64% as expired 14CO2. Following a 6-h inhalation exposure to 3000 ppm of 14C-PGMEA, 26% of the recovered radioactivity was found in the urine and 53% recovered as expired 14CO2 (Miller et al., 1984). Propylene glycol, PGME, and the sulfate and glucuronide conjugates of PGME were identified as urinary metabolites following gavage, as well as after inhalation exposure to 14C-PGMEA. The urinary metabolic profile and disposition of 14C-PGMEA were nearly identical to that observed with 14C-PGME, indicating that PGMEA is rapidly hydrolyzed to PGME in vivo. Only PGME, and not PGMEA, was detected in urine and plasma of rats that were sacrificed immediately after a 6 h exposure to 3000 ppm 14C-PGMEA.

PGMEA and PGME are essentially toxicologically equivalent, with the exception of nasal irritation observed in inhalation studies with PGMEA. Hydrolysis of PGMEA in nasal tissues has been demonstrated by Stott and McKenna (1985),
and inhalation of acetic acid vapor has been shown to cause similar lesions (Stott, unpublished data). A similar mode of action for lesions of the nasal mucosa has been demonstrated for vinyl acetate (Bogdanffy and Taylor, 1993; Kuykendall et al., 1993; Simon et al., 1985). Significantly, the nasal mucosa is not a target tissue for PGME despite the high absorption of both chemicals by this tissue (Stott and McKenna, 1984).

In order to examine the conversion of PGMEA to PGME quantitatively, in vitro hydrolysis rates of PGMEA were determined in blood and liver tissue from Fischer 344 rats. In addition, the rates of hydrolysis of PGMEA in human blood and liver tissue were generated to obtain biochemical constants for use in the development of a physiologically based pharmacokinetic (PB-PK) model for PGMEA and PGME. An in vivo study was conducted to determine the kinetics of PGME following iv administration, to demonstrate equivalency in kinetics of PGME when derived from in vivo hydrolysis of PGMEA or administered as PGME. Data obtained from the intravenous route of administration was needed to validate a future PB-PK model for PGMEA and PGME. Data generated from these studies would support the use of the extensive toxicological database on PGME as a surrogate for PGMEA, since long-term toxicity studies are lacking for PGMEA, and aid in the future development of a PB-PK model.

MATERIALS AND METHODS

Chemicals. PGMEA was obtained from The Dow Chemical Company, Midland, MI, with a purity of 99.3% as determined by area percent gas chromatography using a flame ionization detector (GC/FID). Infrared spectroscopy (IR) was used to confirm the structure. PGME was obtained from Aldrich, Milwaukee, WI, with a purity of 99.6% as determined by the methodology stated above and structurally confirmed by IR.

Animals. Male Fischer 344 rats, 10 weeks of age, were purchased from the Charles River Breeding Laboratory (Raleigh, NC) for noncannulated animals and from Hilltop Lab Animals, Inc. (Scottsdale, PA) for jugular vein cannulated animals. Care and husbandry of animals were in accordance with the Guide for the Care and Use of Laboratory Animals (1996). Animal use activities were approved by the Institutional Animal Care and Use Committee. Upon arrival in the laboratory, the animals were examined by a veterinarian and found to be in good health and were then acclimated to the laboratory environment and to glass Roth-type metabolism cages. Animal rooms were maintained at 21 ± 1°C and 40–70% relative humidity during 12-h light/dark cycles. Certified rodent chow (#5002, Purina Mills, Inc., St. Louis, MO) and municipal drinking water were provided ad libitum.

Study Design

Intravenous kinetics of PGME and PGMEA in the rat. Groups of four male rats/dose group implanted with indwelling jugular vein cannulae received a single low dose of 10 mg PGME/kg bw or 14.7 mg PGMEA/kg bw by intravenous administration via the jugular vein cannula, prepared in physiological saline, or a high dose of 100 mg PGME/kg bw or 147 mg PGMEA/kg bw.

All dose solutions prepared for iv administration were 90 to 101% of their targeted concentrations. At the time of dosing with PGME, mean body weights were 195 and 197 g for the low and high dose, respectively. The mean body weights at the time of dosing for PGMEA were 196 and 201, for the low and high doses, respectively. For targeted PGME doses of 10 or 100 mg/kg, the actual mean values were 10.72 or 99.57 mg/kg, respectively. For targeted PGMEA doses of 14.7 or 147 mg/kg, the actual mean values were 16.83 or 142.42 mg/kg, respectively (data not shown). Blood samples of about 0.1 ml each were drawn from the jugular cannulae, 5, 10, 15, 30, and 45 min, and 1, 2, 4, 6, 8, and 12 h post-dosing using a heparinized syringe. Following collection of each blood sample, approximately 0.1 ml of heparinized saline (10 units/ml) was used to clear the blood from the cannula and provide a heparin “lock.” Each blood sample was transferred to a tared glass vial containing internal standards in 400 µl acetonitrile. The samples were vortexed briefly, centrifuged, and the supernatant analyzed via positive ion chemical ionization (PCI)—gas chromatography (GC)/mass spectrometry (MS) for determination of PGMEA and PGME, as described below. Appropriate blood matrix spikes were prepared with each in vivo sample set and analyzed with the study samples to determine stability and recovery of analytes from blood.

In vitro hydrolysis. Two concentrations of PGMEA (5 and 50 µg/ml) were used in incubations with rat and human blood and also rat and human S-9 liver homogenate. Incubations were performed in triplicate. To follow the hydrolysis of PGMEA and formation of PGME, aliquots were taken at the following times: 1, 2, 4, 6, 8, 10, 15, 20, 30, and 45 min and 1, 2, 4, and 6 h. Liver S-9 homogenates were obtained from In Vitro Technologies (Baltimore, MD). Human S-9 fractions were received as a pooled sample derived from 15 individual liver samples. Liver S-9 from Fischer 344 rats was received as a pool from female and a pool from male rats. Upon receipt, rat liver S-9 was pooled equally from male and female lot numbers. Fischer 344 rat and human S-9 were 22 and 28 mg protein/ml, respectively. The protein yield of rat liver S-9 (In Vitro Technologies, personal communication) was in the range of 13–15 mg protein/g tissue.

Human blood samples were obtained from healthy human volunteers (three males and two females) by a medical ASCP certified technician. Samples were collected in glass tubes containing heparin to prevent clotting. The Dow Chemical Company’s Human Studies Review Board approved the portion of this study involving the use of human blood.

Assays of esterase activity were conducted at 37°C, utilizing PGMEA as a substrate. Incubation mixtures consisted of 0.1 M potassium phosphate (pH 7.4) buffer containing known concentrations of PGMEA (5 or 50 µg/ml) and an appropriate amount of whole blood or liver homogenate in one-dram vials. Substrate-buffer solutions were prepared just prior to incubation by adding an appropriate volume of a concentrated stock solution of PGMEA in saline to a known volume of buffer. Following designated periods of incubation, 1, 2, 4, 6, 8, 10, 15, 20, 30, and 45 min and 1, 2, 4, and 6 h, reactions were stopped by the addition of acetonitrile. The samples were subsequently analyzed for PGMEA content by PCI–GC/MS. This method provides a limit of quantitation for PGMEA and PGME of 0.3 µg/ml blood, which was sufficient to follow the hydrolysis PGME in the low-concentration incubations (5 µg/ml) through approximately four half-lives. Appropriate matrix spikes were prepared with each in vitro sample set and analyzed with the study samples to determine stability and recovery of the analytes from the in vitro matrices.

The in vitro half-life of PGMEA in the presence of blood and liver S-9 homogenates under the conditions of the assay was subsequently calculated.

Dose level justification. The in vitro hydrolysis of PGMEA in rat and human blood and liver homogenates was investigated at concentrations of 5 or 50 µg/ml. These concentrations are in the range of peak blood levels following dermal exposure of rats to 100 or 1000 mg PGMEA/kg bw (Sumner et al., 2000).

For investigation of the kinetics of PGMEA and PGME in blood following intravenous administration to rats, doses of 10 or 14.7 and 100 or 147 mg/kg bw were used, respectively. The high dose of 100 mg PGME/kg bw was selected based on a study by Ferrala et al. (1992) and from earlier probe data (Stott et al., 1982). In the Ferrala study, PGME was given intravenously at a dose level of 90 or 450 mg/kg. The concentrations of PGME and PGMEA used in a probe study were 10 and 50 mg/kg, respectively.
**Chemical Analysis.** Blood samples (approximately 100 μl each) were obtained via a jugular vein cannula from each dosed rat at specified time points post-dosing. No absorption of either chemical occurred by the cannula material. The blood was immediately placed into a tared vial containing D_2^-PGME and D_2^-PGMEA (~50 ppm each) in 400 μl of acetonitrile. The vials were vortexed briefly (~15–30 s), weighed to obtain an accurate blood weight, followed by centrifugation (10 min at 3000 rpm). The acetonitrile/water extract was transferred to a GC vial for analysis. Initially, samples were analyzed by EI–GC/MS, however, the analysis was subsequently changed to positive ion chemical ionization PCI–GC/MS in order to monitor ions that are unique to the analytes, PGME and PGMEA. Matrix standards of PGME and PGMEA were prepared in extra, pooled control human and rat blood. Aliquots of blood were added to 400 μl of 50 ppm each D_2^-PGME and D_2^-PGMEA in acetonitrile and fortified with PGME and PGMEA to obtain matrix standards with final concentrations of 0.3 to 200 μg/ml PGME and PGMEA/ml blood. Also prepared were matrix standards containing rat or human blood only, internal standards only, and PGMEA only. All standards were vortex mixed, centrifuged, and analyzed in the same manner as the samples. With each set of blood incubations, spikes were prepared in control rat and human blood at concentrations of 1, 3, and 30 μg/ml and were prepared in the same manner as the matrix standards.

**Blood incubations.** Control blood was obtained from five human volunteers (three males and two females, 5–10 ml each), and equal volumes (3 ml) from each sample were pooled. Two 3-ml aliquots of the pooled blood were transferred to 20-ml vials that were subsequently fortified with 330 μl of 0.05 or 0.50 mg/ml PGMEA in saline. The final concentrations were approximately 5 or 50 μg/ml PGMEA in blood. The fortified blood samples were maintained at 37°C, and at appropriate times, 100-μl aliquots were removed and extracted in 400 μl acetonitrile containing internal standards D_2^-PGME and D_2^-PGMEA (~25 ppm each). After vortexing, weighing, and centrifuging, as above, the acetonitrile/water extracts were transferred to GC vials containing approximately 200 mg anhydrous Na_2SO_4 (mixed briefly, dried approximately 5 min). The dried extracts were then transferred to limited-volume GC vials for analysis by PCI–GC/MS. Control rat blood was obtained via cardiac puncture from two male Fischer 344 rats. Approximately equal volumes of each were pooled, and two 3-ml aliquots were removed to 20-ml vials. These were fortified with PGMEA at 5 and 50 μg/g, and 100-μl aliquots taken at specified times, while maintaining temperature of the fortified samples at 37°C. The timed samples were extracted, dried, and analyzed in the same manner as the human samples. This experimental procedure was conducted on three separate days, resulting in triplicate incubations for each dose level (5 or 50 μg PGMEA/g blood) and for each matrix (human or rat) with fresh blood collected each day.

**Liver homogenate incubations.** Pooled control human and rat liver homogenates were diluted 10-fold in a 0.1 M sodium phosphate buffer (pH 7.4). Two 3-ml aliquots each of the human and rat diluted samples were placed into 20-ml vials and fortified with 330 μl of 0.05 or 0.50 mg/ml PGMEA in saline to obtain final concentrations of approximately 5 or 50 μg/ml PGMEA in liver homogenate/buffer. While maintaining the temperature of the incubation samples at 37°C, aliquots (100 μl) were removed at specified times and extracted using the same procedure as described for the blood incubations. The incubations with the diluted liver homogenates were performed in triplicate (three separate days). Matrix standards of PGME and PGMEA were prepared in extra control, diluted liver homogenate in the same manner as with the blood samples. With each set of liver homogenate incubations, spikes were prepared in diluted control rat and human liver homogenate at concentrations of 1, 3, and 30 μg/ml. These were prepared in the same manner as the matrix standards.

**Control matrix incubations.** Incubations at 5 and 50 μg PMGEA/g were performed in buffer and in diluted, boiled rat and human liver homogenate. The phosphate buffer was used unchanged, while the pooled rat and human liver homogenates were boiled for several min to inactivate them, cooled slightly, and then diluted 10-fold in buffer. The incubations in control matrices were performed in the same manner as the liver homogenate incubations, with the exception that only one incubation was performed for each concentration and matrix. Matrix standards of PGME and PGMEA were prepared in extra control matrix in the same manner as the blood samples. With each control sample incubation, spikes were prepared in the same matrix at concentrations of 1, 3, and 30 μg/ml, using the same procedure as the matrix standards.

**Analysis.** Quantitation was performed using ions at m/z 73 for PGME and m/z 73 or m/z 133 for PGMEA. The ions used for internal standards D_2^-PGME and D_2^-PGMEA were m/z 83 and m/z 143, respectively. Peak areas were corrected for isotopic crossover as determined by analysis of “internal standard only” or “analyte only” matrix standards. Quantitation standards were prepared in control matrix for each experiment. Bracketed linear regression curves were determined, for each analyte, in low and high concentration ranges (0.3–10 μg/ml and 3–100 μg/ml, or 30–400 μg/ml for high dose iv blood extracts). For all in vitro experiments, reported sample concentrations were corrected for spike recovery. Also, for each in vitro experiment, the target PGMEA concentration was calculated using sample weights and standard concentration. Since these targets varied for each experiment and in order to allow comparison between replicates, sample concentration results were normalized to the common targets of 5 and 50 μg/g PGMEA.

**Representative analysis conditions: Positive ion chemical ionization (PCI)–GC/MS.** The extracts were analyzed by GC/MS using an HP 5999x instrument (Agilent Technologies, Palo Alto, CA). Separations were achieved on a J&W DB-Wax capillary column (60 m × 0.25 mm i.d. × 0.5-μm film; J&W Scientific, Folsom, CA). Helium at 18 psi head pressure was used as the carrier gas. The GC temperature program consisted of an initial 1 min hold at 40°C followed by a ramp to 180°C at 10°C/min and to 250°C at 25°C/min (2 min hold at 250°C). The injector and transfer line temperatures were maintained at 200°C and 240°C, respectively. A 2-μl injection was made and a double groove-neck deactivated liner was used in the injection port. The mass spectrometer was operated in the chemical ionization mode using methane as the reagent gas, monitoring positive ions at m/z 73, 83 (PGME and D_2^-PGME, respectively) and 73 and 133, 143 (PGMEA –2 ions, and D_2^-PGMEA, respectively) at 50 mscion scan. The source was held at 250°C and the electron multiplier was operated at 2400–2900 V.

**Good laboratory practices.** This study was conducted in accordance with the EPA Toxic Substances Control Act Good Laboratory Practice Standards (U.S. EPA, Final Rule) and the Organisation For Economic Co-Operation and Development (OECD, 1997) Principles of Good Laboratory Practice.

**Calculations.** All data-based calculations were conducted using Microsoft Excel with full precision (Microsoft Corporation, 1998). Descriptive statistics were calculated mean ± SD for all sets of data collected during the study. The in vivo concentration time course of PGME and PGMEA in blood was described by a noncompartmental model using the method of residuals and linear regression to estimate the half-life (PK Solutions, Summit Research Services, Montrose, CO). PK Solutions was also used to estimate rates of hydrolysis for PGMEA in the in vitro studies.

**RESULTS**

**Time Course of PGME and PGMEA in Blood following IV Administration to Male Fischer 344 Rats**

Analysis of control blood from each animal prior to administration of the test materials indicated PGME or PGMEA was not present in the blood. The time courses of PGME levels in rat blood following iv administration of 10 mg PGME/kg bw or 14.7 mg PGMEA/kg bw (equimolar doses) are shown in Figure 1, top and middle panels (square symbols). At the low dose levels administered, the concentration of PGME was nonquan-
tifiable in blood at sampling times greater than or equal to 120 min post-dosing. Administration of equimolar low doses of PGME or PGMEA resulted in similar time courses of PGME elimination from blood that occurred in a biexponential manner. At the first blood collection time point, 5 min post-dosing, the average concentrations of PGME in the blood were 14 and 12.3 μg/g in rats administered low doses of PGME and PGMEA, respectively. The concentration of PGME subsequently declined rapidly for approximately 20 min, followed by a slower, more prolonged decline to levels at the limit of detection. Specific pharmacokinetic parameters were estimated for the blood time course of PGME and were analyzed by noncompartmental analysis methods. Clearance of PGME from blood was calculated to be 3.8 and 6.7 ml/min at the low dose of PGME and PGMEA (Table 1), respectively. The blood area under the curves (AUC) were similar, 544 and 489 μg-min/ml for the low dose of PGME and PGMEA, respectively. Half-lives for the initial phase of PGME in blood were 8.1 and 10.4 min for the low doses of PGME and PGMEA, respectively. Concentrations at time equal to zero were estimated at 68.4 and 44.7 μg/ml for the low dose of PGME and PGMEA, respectively.

Time courses of PGME in rat blood following higher equimolar dosages of 100 mg PGME/kg bw or 147 mg PGMEA/kg bw are shown in Figure 1, top and middle panels (diamond symbols). The concentration of PGME was nonquantifiable in blood at sampling times of 360 min and longer following administration of PGME. Following administration of PGMEA, all animals had nonquantifiable levels of PGME in blood at 480 and 720 min post-administration. Following intravenous administration of equimolar, high doses of PGME or PGMEA the time courses of PGME in blood were similar, and elimination in blood occurred in a biexponential manner. At the first time point that blood was collected, five min post-dosing, the average concentrations of PGME in the blood were 131.3 and 135.4 μg/g blood following intravenous administration of the high doses of PGME and PGMEA, respectively. Clearance of PGME from blood was calculated to be 1.4 and 1.7 ml/min for the high doses of PGME and PGMEA. The blood AUC values were similar, 13839 and 16355 μg-min/ml following the high dose of PGME and PGMEA, respectively. Half-lives of PGME in blood were 20.1 and 38.6 min for the high doses of PGME and PGMEA, respectively (Table 1). Concentrations at time equal to zero were estimated at 199 and 252 μg/ml for the high dose of PGME and PGMEA, respectively.

Time courses of PGMEA in rat blood following intravenous administration of 14.7 mg PGMEA/kg bw or 147 mg PGMEA/kg bw are shown in Figure 1, bottom panel. The concentration of PGMEA was nonquantifiable in blood of all animals at sampling times equal to or greater than 240 min post-dosing for the high dose of PGMEA. The concentration of PGMEA was nonquantifiable in blood from three out of four animals at 45 through 240 min post-dosing for the low dose of PGMEA. At
360 min through 720 min, PGMEA was nonquantifiable in all animals. At the first time point that blood was collected, five min, the average concentrations of PGMEA in the blood were 28 and 144.3 µg/g blood following intravenous administration of the low or high doses of PGMEA, respectively. Following intravenous administration of low and high doses of PGMEA, the time courses of PGMEA in blood were similar, and elimination in blood occurred in a bi-exponential manner. A three-fold increase in the PGMEA AUC value with an increase in dose contrasted with the 10-fold increase in dose. Clearance of PGMEA from blood was calculated to be 4 and 11 ml/min for the low and high doses of PGMEA, respectively. Half-lives were 1.6 and 2.3 min following intravenous administration of the low and high doses, respectively (Table 1).

### PGMEA Hydrolysis in Rat and Human Samples of Blood and Liver Homogenates

Representative positive CI–GC/MS ion chromatograms of an extract of a 45 min sample of human blood incubated with 5 µg/ml of PGMEA with internal standards D9-PGME and D9-PGMEA (~25 µg/ml) are shown in Figure 2. The sample extract was found to contain 1.6 µg PGME/ml and 1.9 µg PGMEA/ml.

The time courses of PGMEA elimination in rat or human blood spiked with 5 or 50 µg PGMEA/ml incubation mixtures are shown in Figure 3, upper and lower panels. Note that the kinetics were linear. The concentration of PGMEA was nonquantifiable in rat blood at sampling times equal to or greater than 120 min post-dosing for the low concentration, and blood levels were nonquantifiable by 240 min for the incubations at 50 µg PGMEA/ml. The concentration of PGMEA was ~10-fold higher at each of the time points for the incubation containing 50 µg/ml of PGMEA versus 5 µg/ml.

Hydrolysis half-lives were calculated through 60 min, as hydrolysis was essentially complete by this time. The disappearance of PGMEA from blood was more rapid in rat blood than in human blood. The hydrolysis half-lives were 36 and 34 min for the 5 and 50 µg/ml concentrations of PGMEA in human blood, whereas, in rat blood the hydrolysis half-lives were 16 and 15 min, respectively (Table 2).

In addition to measuring the disappearance of PGMEA in the incubation mixtures, the appearance of PGME in the incubation mixtures was also measured. These data are shown in Figure 3, upper and lower panels. Approximately 10-fold more PGME is formed at the higher incubation mixture of 50 µg/ml of PGMEA.

The time courses of PGMEA in rat and human liver homogenates spiked with 5 or 50 µg PGMEA/ml incubation mixtures are shown in Figure 4, upper and lower panels. Nonquantifiable levels of PGMEA were observed at 120 min, and all samples were nonquantifiable in rat liver homogenate at 240 and 360 min for the low concentration. Nonquantifiable levels of PGMEA were observed at 360 min for the incubations containing 50 µg/ml of PGMEA. The concentration of PGMEA was nonquantifiable in all human liver homogenate samples at 360 min for the low concentration, and PGMEA levels were quantifiable through 360 min for the incubations at 50 µg/ml of PGMEA versus 5 µg/ml.

Hydrolysis half-lives were calculated through 60 min, as hydrolysis was essentially completed by this time. The disappearance of PGMEA from rat liver homogenate mixtures was similar in rat and human incubations. The hydrolysis half-life

### Table 1

<table>
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<tr>
<th>Kinetic Parameters of PGME and/or PGMEA in Blood from Fischer 344 Male Rats Administered PGME or PGMEA Intravenously</th>
<th>Blood PGME</th>
<th>Blood PGMEA</th>
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<tr>
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<td>14.7 mg PGME/kg</td>
<td>100 mg PGME/kg</td>
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<tr>
<td>10 mg PGMEA/kg</td>
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was 28 and 30 min for the 5 and 50 $\mu$g/ml concentrations of PGMEA in rat liver homogenate, whereas in human liver homogenate the hydrolysis half-life was 34 min for both the 5 and 50 $\mu$g/ml concentrations (Table 2).

In addition to measuring the disappearance of PGMEA in the incubation mixture, the appearance of PGME in the incubation mixture was also measured. These data are shown in Figure 4, upper and lower panels. Approximately 10-fold more PGME was formed at the higher incubation mixture of 50 $\mu$g/ml of PGMEA.

**DISCUSSION**

Data from this study corroborate the findings of Miller *et al.* (1983, 1984) of the rapid hydrolysis of PGMEA in rats. Once PGMEA is hydrolyzed, the kinetic parameters determined for PGME derived from the ester are identical to the kinetics for PGME. The kinetics of PGMEA in blood from rats following iv administration of 14.7 or 147 mg PGMEA/kg bw resulted in half-lives of 1.6 and 2.3 min. These findings are also consistent with those of Stott *et al.* (unpublished data) in which a blood plasma half-life of 2.2 min was determined in rats following an intermediate iv dose of 50 mg PGMEA/kg bw. The slopes of the PGME blood curve in rats following iv administration of PGME or PGMEA were indistinguishable. Calculated AUC, half-life, and $C_{\text{initial}}$ values for PGME were similar following administration of equimolar dosages (low) of 10 mg PGME/kg bw or 14.7 mg PGMEA/kg bw. Half-lives for PGME in blood following administration of a low dose of PGME or PGMEA were estimated to be approximately 8.1 and 10.4 min, respectively. These data are in agreement with a study conducted by Stott *et al.* (unpublished data) where the blood plasma half-life of PGME was determined to be approximately 5.7 min following iv administration of 10 mg PGME/kg bw. AUC, half-life, and $C_{\text{initial}}$ values were similar following administration of 100 mg PGME/kg bw or 147 mg PGMEA/kg. Half-lives for PGME in blood following administration of the high dose of PGME or PGMEA were 20.1 and 38.6 min, respectively.

There was no evidence of a dose dependency in the pharmacokinetic fate of PGMEA following iv administration to rats. A 10-fold increase in the dose of PGMEA resulted in similar blood half-lives for PGMEA of 1.6 and 2.3 min for the
low and high dose, respectively, indicating that metabolism of PGMEA was not saturated. The AUC for PGMEA following the high dose was expected to be 10-fold higher than for the low dose, when in fact the AUC for PGMEA was only 3-fold higher. Examination of individual time points (mean values) on the concentration-time curve for PGMEA at both dose levels showed that there was approximately a 10-fold difference between the low and the high dose, although this difference was not reflected in the AUC values. Since the first time of blood collection was 5 min and the determined half-life was on the order of 2 min, earlier blood collection time points would have better defined the curve and would have given a more accurate determination of the true AUC value. Following iv administration of the high dose, the metabolism of PGMEA was not saturated, since the concentration of circulating PGMEA was similar following the low and high doses of PGMEA and the half-life of PGMEA in blood is approximately 2 min for both doses.

Saturation of elimination process(es) for PGME from the blood of treated rats was observed. A 10-fold increase in the dosages of PGME or PGMEA resulted in more than a doubling in the half-lives of resultant PGME in blood: 8.1 and 10.4 min versus 20.1 and 38.6 min. Further noted was a disproportionate 25-fold increase in AUC values from 544 and 489 (low dose) μg-min/ml to 13839 and 16355 (high dose) μg-min/ml, for PGME and PGMEA, respectively.

Consistent with the relatively rapid elimination of PGMEA from blood of rats in vivo, PGMEA is also rapidly hydrolyzed in vitro by tissue and blood carboxylesterases to PGME. PGMEA was hydrolyzed approximately twice as fast in incubations with rat blood as compared to human blood: 15–16 min and 34–36 min, respectively. The in vitro hydrolysis rate of PGMEA in rat blood was somewhat faster in our study than the rate reported by Sumner et al. (1999); half-lives of 21.3 and 28.4 min were determined for the 5 and 50 μg PGMEA/ml incubations, respectively. Results from this study are consistent with the findings of Hoffman and Jackh (1985, unpublished data), where the in vitro hydrolysis plasma half-life of PGMEA in Wistar rats was determined to be 10 min. Studies with other glycol ethers have also demonstrated that in vitro hydrolysis rates are rapid. Deisinger and Guest (1989) found that diethylene glycol monobutyl ether acetate was rapidly hydrolyzed in vitro with a half-life of less than 3 min in rat blood, to produce diethylene glycol monobutyl ether. Hoffman and Jackh (1985, unpublished data) also found that in Wistar rats the in vitro plasma half-life of ethylene glycol monobutyl ether acetate was one min.

In vitro hydrolysis rates of PGMEA using rat liver homogenates were similar to rates observed in incubations with hu-

<table>
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<th>TABLE 2</th>
<th>Summary of PGMEA Hydrolysis Half-Lives and Rate Constants Determined From in Vitro Incubations of Rat and Human Blood and Liver Homogenates</th>
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<tr>
<td></td>
<td>μg PGMEA/ml</td>
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<td>Blood</td>
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<td>Human</td>
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*PK Solutions, Summit Research Services, Montrose, CO, calculated from 0–60 min incubation data.
60 mg protein/ml.
*Data from Sumner et al., 1999.
*Supplied as 20 mg protein/ml, diluted to 2 mg protein/ml.
man liver homogenates. The in vitro hydrolysis half-lives ranged from 28 to 34 min when incubated with a 2 mg protein/ml liver homogenate. The in vitro half-lives of PGMEA in blood and liver incubations are similar. In contrast, a study by Stott and McKenna (1985) examining the in vitro hydrolysis of the related glycol ether acetate, ethylene glycol monomethyl ether acetate, by mouse tissues reported greater hydrolysis by liver homogenate than that found with blood.

The basis for a shorter half-life of PGMEA in vivo than in vitro is likely due to the dynamic interplay of the physiological processes in the whole organism, in contrast to the controlled conditions of an in vitro system. Nevertheless, the in vitro hydrolysis half-lives for PGMEA in human and rat liver incubations were similar (approximately 34 and 30 min, respectively) suggesting a similar first pass effect capability of both species in vivo. The more rapid hydrolysis of PGMEA in rat blood as compared to human blood (15 and 34 min, respectively) suggests a somewhat faster potential for elimination may occur in rats; however, a more integrated evaluation employing a physiologically based-pharmacokinetic (PBPK) model for PGMEA presently under development will provide a more accurate estimation of the potential impact of this difference.

The results of this study support the existing metabolism and pharmacokinetic database on PGMEA. For example, PGMEA was not detected in the plasma or urine of rats exposed to PGMEA via inhalation (Miller et al., 1984), indicating rapid and extensive hydrolysis, via tissue and plasma carboxylesterases. The results of this study and hazard data for PGME and PGMEA reveal that these two chemicals are essentially equivalent, since PGMEA is rapidly hydrolyzed to PGME and subsequently metabolized by common metabolic pathways. The extensive toxicological database that has been developed for PGME should thus serve with confidence as surrogate data for PGMEA. As noted, the only exception to this would be the development of lesions of the nasal mucosa of rodents following exposure to PGMEA vapor. This appears to be the direct result of inhaled PGMEA and generation of acetic acid in surrounding nasal epithelium which has been demonstrated by Stott and McKenna (1985). Kinetic and biochemical parameters generated as part of this study will help facilitate development and validation of a PB-PK model for PGMEA and PGME. This model will provide a basis for a more scientifically sound and appropriate human health risk assessment for PGMEA.

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