Toxicokinetics of Ethyl Tertiary-butyl Ether (ETBE) and Methyl Tertiary-butyl Ether (MTBE) in Men and Women

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GRANT NUMBER 5 KO1 OH00164

Final Performance Report

Abstract
Methyl tertiary butyl ether (MTBE) and ETBE are used as gasoline components to reduce tailpipe emissions in 39 areas of the United States which exceed the National Ambient Air Quality Standards for carbon monoxide and in the 9 metropolitan regions with the most elevated summertime ozone levels. MTBE and ETBE exposures occur during gasoline production and refueling, and as recently documented in California and Washington, potentially through contact with groundwater contaminated by leaking underground storage tanks. However, little is known about how the body handles MTBE, ETBE and their metabolites; nor about reliable biological indicators of occupational and environmental exposure. We conducted controlled exposures of men and women to 2.5 ppm $^2H_{12}$–MTBE + 2.5 ppm ETBE for two hours with alternating periods of work and rest, and sampled blood, breath and urine during and for three days following exposure. Concurrently, a physiologically-based kinetic (PBK) model was developed to include data from other research efforts, to incorporate parameter variability through Bayesian fitting and Monte Carlo simulation techniques, and to determine a biological index of exposure. Post-exposure blood and breath levels exhibited two half-lives for both $^2H_{12}$–MTBE and ETBE of about 1 and 8.4 hours, and 1.5 and 10.6 hours, respectively. Tertiary-butyl-alcohol (TBA) breath concentration from each ether appeared to be a valuable index of exposure with a single half-life of 20–40 hours.

Introduction
Ethyl tertiary-butyl ether (ETBE) has been used minimally as an "oxygenated" fuel additive, principally because of production costs, whereas the methyl ether (MTBE) has become the most-widely used gasoline additive, used in about 20% of gasoline nationwide, at a concentration of 2–15%. MTBE is now used in 35 metropolitan areas and in the entire states of California, Massachusetts, Connecticut, Rhode Island, and Delaware. To better understand occupational and environmental health risks associated with current oxygenated fuel exposures, this project has expanded to examine the toxicokinetics of ETBE, MTBE, and their common metabolite, tertiary-butyl alcohol (TBA).

While MTBE production as a fuel oxygenate has grown by 20% per year over the last decade (21×10^9 kg in 1994), and is used widely in the United States and Europe, recent concerns about groundwater contamination have led to its planned phase-out of use nationwide. This shift may lead to increased use of other, less water-soluble oxygenates, such as ETBE. A thorough understanding of the absorption, distribution, metabolism and excretion of these substances; as well as useful biological indicators of exposure, are therefore needed.

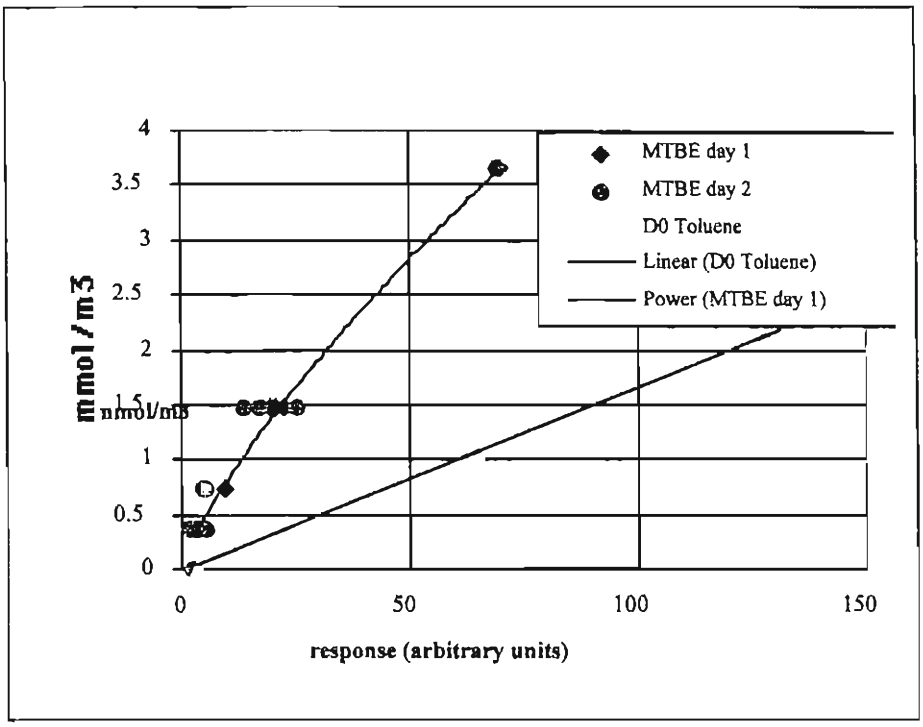
Because recent evidence suggests that following human exposure to MTBE or ETBE, TBA has a prolonged half-life (Buckley *et al.*, 1997), this primary metabolite may be a valuable indicator of longer-term exposure. To simultaneously assess the kinetics and value as indicators of exposure of all three compounds, we have chosen to co-administer low concentrations of $^2H_{12}$-MTBE and ETBE. Use of the deuterium label allowed us to distinguish the $^2H_9$-TBA arising from $^2H_{12}$-MTBE metabolism from the unlabelled TBA generated from ETBE metabolism.

Activity in the third year included the development of analytical methods for formaldehyde, acetaldehyde, and acetone, as metabolites possibly useful as biological indicators of exposure or as species central to toxicity. This brought the total number of deuterated and native analytes to 16 for each biological sample.

Specific Aim 1: Development of ETBE/MTBE/TBA GC-MS analytical methods

Exposure equipment calibration with MTBE, and comparison to response to toluene

To assess the ability of our current instruments to monitor exposure and absorption of MTBE, a series of calibrations was conducted using both MTBE and unlabelled toluene for comparison. Controlled mixtures of solvent in air were generated by metering liquid solvent with a precision



syringe pump into a heated block where complete evaporation took place. The solvent vapor was then diluted with known flow rates of air previously conditioned by passing through a bed of activated charcoal followed by a 10 μm filter. The flow rates of both liquid solvent and dilution air were verified by comparison to primary volumetric standards. The resulting concentrations were accurate to within 4%.

Figure 1. PID response to MTBE and to Toluene in air.

After thorough mixing in a glass plug-flow chamber, air was sampled by a photoionization detector (PID, Hnu Systems Inc., Newton, MA) at a rate not exceeding 0.1% of the total mixture flow rate. The detector used an 11.7 eV UV ionization source operated at approximately 60% of maximum output, and the resulting ions were collected and quantified by an electrometer. The response of the PID was compared to the known concentration of MTBE; a similar calibration was done with toluene for demonstration of the relative response of the instrument. The results for solvent concentrations up to 3.7 mmol/m^3 (89 ppm) are shown in Fig. 1.

Synthesis of $^2H_{12}$-methyl-t-butyl ether ($^2H_{12}$-MTBE).

This compound was synthesized from $^2H_3$-methanol and $^2H_9$-t-butanol in the presence of H_2SO_4 by the method described by Norris and Rigby (1932). However, the macro scale purification described in the paper did not work well on our semi-micro scale synthesis. After exhaustively pursuing another method using a spinning band column, without quantitative production success, we returned to and refined the Norris and Rigby method, ultimately producing enough labeled MTBE for the controlled exposures.

$^2H_{12}$-MTBE was synthesized by the procedure of Norris and Rigby (1932) for MTBE. $^2H_4$-Methanol (21.5 ml, 0.52 moles), 9 ml conc. H_2SO_4, and 90 ml water were heated to 60 °C with stirring. A portion (5 ml) of $^2H_{10}$-*t*-Butanol was added, then the remainder (total 25 ml, 0.27 mole) was added dropwise over 3 hr. The product was collected as a water azeotrope using a cold-finger condenser (dry ice-acetone). Water (50 ml) was added to the distillate and the azeotrope redistilled again using a cold-finger condenser; the fraction between 39–48 °C was collected. The distillate was dried overnight with K_2CO_3, decanted, and then let sit with sodium sand for 2 days. The sodium was then filtered off and the product distilled (45–48 °C). The mass spectrum was consistent with a $^2H_{12}$ substitution of MTBE; [M-CD_3]+ (100%; 82 m/z), [M-OCD_3]+ (18%, 66 m/z), and C_3D_5 (67%, m/z 46). Purity was assessed by gas chromatography with flame ionization detection; the product contained 0.16% $^2H_3$-methanol and 0.04% $^2H_9$-t-butanol. Water content was <0.1% by gas chromatography/ mass spectroscopy.

Methods of analysis. Numerous analytical problems related to the measurement of parts per billion levels of MTBE, ETBE, TBA, ethanol and methanol in breath samples have been solved. The selection of sorbent material and hand-packing of thermal desorption tubes to collect both the hydrophobic ethers and hydrophilic alcohols has been accomplished. Additional steps have been taken to develop analytical accuracy,

including the use of pre-column filters to collect water but not alcohol, and the overnight baking of tedlar used to make breath sampling bags to remove residual phenol levels, which co-chromatograped with MTBE.

Analytical range. The gas chromatography-mass spectrometry analysis was able to quantify MTBE and ETBE over a range of approximately 0.7–700 μmol/l (0.07–70 ng/μl) solution. This corresponds to an alveolar breath concentration range of 0.11–110 nmol/l, and given a blood/breath partition coefficient of about 15 (Nihlen *et al.*, 1995), a blood concentration range of about 1.58–1580 nmol/l.

Charcoal tube analysis. We previously measured the concentrations of aromatic hydrocarbons in exhaled breath samples by trapping onto industrial hygiene sampling tubes containing activated charcoal, desorption by CS_2 and analysis by gas chromatography-mass spectrometry. As a first step to see if this technique would work for MTBE, ETBE and their alcohol metabolites, methanol, ethanol and t-butanol, we looked at desorption efficiencies of these compounds from charcoal. Controlled atmospheres of the compounds were generated in ranges anticipated during subject exposures and washout. A known volume of atmosphere was passed through the charcoal tubes, and the tubes were then desorbed with 1% dimethylformamide in CS_2. The analyses revealed that methanol was poorly absorbed (25% recovery) and broke through the first absorbent bed at low sample loadings (70 ng). Ethanol recovery decreased with concentration in the atmosphere; recovery diminished from 70% to 20% as the atmospheric concentration decreased from 100 to 0.05 ppm. The recovery of t-butanol was 40% at concentrations lower than 50 ppm. MTBE and ETBE recovery was essentially complete (100%) and constant over the range of 1-100 ppm. Thus, the charcoal tube technique was adequate for the parent compounds (MTBE and ETBE) but not for the alcohol metabolites. Thermal desorption analysis was used in breath analysis of the parent compounds and their metabolites.

Preparation of tedlar bags for breath sampling and analysis

Tedlar film (2 mil, TR20SG4; Du Pont, Wilmington, DE) was placed into a vacuum oven (100 °C) overnight to remove phenol and dimethyl acetamide contamination. These contaminents arose from the manufacturing process and co-chromatographed with $^2H_{12}$-MTBE unless removed. Tedlar bags (20 L) were then made with an impulse thermal sealer. The inlet was a stainless steel fitting with buna rubber washer placed between the Tedlar film and the flange of the fitting. Subjects filled the bags by breathing through a 1/4" diameter latex tube attached to the fitting.

Breath sampling

Breath samples were collected in Tedlar bags every 15 min after exposure for the first 2 hr, then once per hr for the next four hours, and then four times a day for the next 3 days. A background sample prior to exposure was also taken. Breath volume was determined by air displacement into a spirometer. The inflated bags were spiked with an aqueous internal standard (ISTD) solution of 2-propanol and isopropyl ether (50 μl, 2 ng/μl) then let sit overnight at room temperature to allow for the dissipation of water vapor, presumably through the Tedlar film. The bag contents were then passed through a Carboxen 1000 thermal desorption tube (Supelco, Bellefonte , PA). CO_2 concentration in the bag sample was measured with a fast infra-red CO_2 monitor (Morgan *et al.*, 1993).

Breath analysis

The thermal desorption tubes were analyzed with a automated thermal desorber (ATD-400, Perkin-Elmer, Norwalk, CN) coupled with a gas chromatograph/mass spectrometer (GC/MS) (HP 5890/HP 5971, Hewlett-Packard, Avondale, PA). A low flow air monitoring trap (L427-5107; Perkin-Elmer) was installed on the ATD and operated in the backflush mode. A deactivated transfer line (Hydroguard, 0.25 mm i.d.; Restek, Bellefonte, PA) was used to reduce tailing of the alcohols. The purge time was 4 min. Desorption time was 10 min at 330 °C. The trap temperature during desorption was -30 °C and was desorbed at 360 °C for 7.5 min. Valve temperature was 175 °C. Input split flow was off and output split flow was 10 ml/min. Desorb flow was 60 ml/min. A 5%diphenyl-95% dimethyl polysiloxane analytical column (60 m, 0.25 mm i.d., 1.0 μm film thickness, RTX-5; Restek) was attached directly to the transfer line with a butt connector. The temperature

program started with at an initial temperature of 35 °C for 7.50 min. This was followed by a 10 °C/min ramp to 210 °C. The final temperature was held for 2 min. The mass spectrometer dwell time was 100 ms for each ion. Table 1 shows the retention times and quantitation ions, and Fig. 1 illustrates a chromatogram of all analytes and internal standards.

Table 1. Retention times and quantitation ions for the blood and breath analyses.

Analyte	Retention Time Breath Assay (min)	Retention Time Blood Assay (min)	Quantitation Ions (*m/z*)
$^2H_4$-Methanol	6.10	4.45	33
Methanol	6.13	4.48	31
Ethanol	7.47	5.31	31
2-Propanol (ISTD)	8.68	6.01	45
$^2H_{10}$-*t*-Butanol	9.42	6.41	65
t-Butanol	9.58	6.50	59
$^2H_{12}$-MTBE	10.94	7.11	82
MTBE	11.17	7.24	73
Isopropyl ether (ISTD)	12.28	7.88	45
ETBE	12.95	8.27	59

Blood Sampling

Blood samples were collected from the anti-cubatil vein into Vacutainers (5 ml grey-top, NaF, citrate) during exposure at approximately 15, 25, 40, 60, 75, 85, 105, 115 min ; post-exposure samples were taken with breath samples every 15 min after exposure for the first 2 hr, then once per hr for the next four hours, and then twice a day for the next 3 days. A background sample prior to the start of exposure was also collected on the first day. Blood was stored at 4 °C until analysis.

Blood analysis

One ml aliquiots were dispensed into 40 ml glass vials with Teflon-lined silicon rubber septa. Triplicate analyses were performed for each sample. Background blood was used to prepare calibration standards. Blood aliquiots were spiked with an aqueous internal standard (ISTD) solution of 2-propanol (25 µl, 78 ng/µl) and isopropyl ether (25 µl, 2.9 ng/µl). Samples were analyzed with an automated dynamic headspace sampler (Precept II-LSC 2000; Tekmar, Cincinnati, OH) coupled to a GC/MS (HP 5890/HP 5971, Hewlett-Packard). The Precept was operated in soils mode with a purge time of 14 min and a vial temperature of 40 °C. We used a Vocarb 3000 trap (Supelco) at 35 °C. Primary desorption was at 250 °C for 6 min. The moisture control module was set at 10 °C. The cyrofocus module was held at -130 °C during primary desorption and at 180 °C during the secondary desorption. A time-programed splitter was used to bypass the analytical column during the primary desorption which was at an approximate flow of 40 ml/min. A Hydroguard precolumn (0.53 mm) was used and a 5% diphenyl-95% dimethyl polysiloxane analytical column (60 m, 0.25 mm i.d., 1.0 µm film thickness, RTX-5; Restek) was attached directly to the transfer line with a 3-hole butt connector. The temperature program started with at an initial temperature of 35 °C for 3.0 min. This was followed by a 15 °C/min ramp to 200 °C. The final temperature was held for 3.5 min. The mass spectrometer dwell time was 100 ms for each ion. The retention times and quantitation ions for the blood assay are presented in Table 1.

Specific Aim 1a: Determination of adipose tissue/air partition coefficients

Tissue/Air Partition Coefficients

Tissue/air partition coefficients were determined using a vial headspace equilibration technique (Pierce *et al.*, 1996a) for MTBE and ETBE in human adipose, rat adipose, rat brain, and rat liver tissues. Unlike our findings with aromatic solvents, rat adipose tissue had a higher affinity for MTBE and ETBE than human tissue (Table 2), and thus the use of human tissue data were used in the physiologic models.

Table 2. Human and rat tissue/air partition coefficients for MTBE and ETBE.

	Human adipose MTBE / ETBE	Rat adipose MTBE / ETBE	Rat brain MTBE / ETBE	Rat liver MTBE / ETBE
Mean	110 / 166	136 / 197	13.6 / 10.9	24.3 / 24.2
s.d.	8.70 / 13.6	7.58 / 10.3	5.36 / 5.54	5.92 / 5.56
n	30 / 30	10 / 10	13 / 13	14 / 14
c.v. (%)	7.94 / 8.22	5.55 / 5.23	39.4 / 50.8	24.4 / 23.0
Low	86.0 / 128	125 / 182	5.59 / 2.69	18.6 / 18.5
High	125 / 190	147 / 214	21.9 / 19.2	43.0 / 41.9

Specific Aim 1B: Determination of blood/air partition coefficients

This aim was not pursued, given the expanded list of analytes and need to purify sample handling vials and breath bags.

Specific Aim 1C: Perform controlled human subject exposures

While the original goal of this work was to expose 20 subjects to ETBE, and examine levels of ETBE and TBA in biological samples, we expanded the number of analytes to 16 and completed eight exposures.

Specific Aim 2a: Construction of physiologic model and validation of model using previous controlled studies

Physiologic Models

The structure for data interpretation and calculation of MTBE and ETBE kinetics was comprised of five tissue groups (Fig. 2). Due to the small size and lipophilic characteristics of these ethers, absorption through the lungs was considered to be presentation rate-limited. Metabolism was expected to occur in the liver, and some storage was expected in the adipose tissue. Initial values used in the models came from our partition coefficient measurements (Table 1) and literature values for tissue volumes, blood flows, and metabolic constants (Table 2). Distinct models for MTBE, ETBE and TBA were created and run in parallel for each exposure using the SAAM II software program (SAAM Institute, Seattle, WA).

To estimate expected blood and breath concentrations during and following the controlled exposures, the MTBE and ETBE models were run using nominal parameter values (Table 3), and with the expectation of a ten-fold inter-individual range (Pierce *et al.*, 1996). These simulations also provided perspective on optimal sampling times, and on the expected differences in MTBE and ETBE levels over time. Our analytical ability to measure breath concentrations down to at least 0.11 nmol/l, corresponding to a blood concentration of 1.58 nmol/l, indicated that samples can be quantified for at least 24 hours post-exposure.

Table 3. Parameters Used In The Physiologic Models

Physiologic parameter	Tissue group			
	Slowly-perfused	Rapidly-perfused	Liver	Adipose
Volume (V, l)	0.95 BW - $V_{adipose}$[a]	0.05 BW - V_{liver}[a]	0.023 BW[a]	0.25 BW
Blood Flow (Q, l/hr)	0.24 Q_{co}- $Q_{adipose}$[b]	0.76 Q_{co}- Q_{liver}[b]	0.27 Q_{co}[a]	0.09 Q_{co}
Tissue/air partition coefficient[c], MTBE / ETBE	6.5 / 6.5[d]	35.8 / 35.8[d]	24.3 / 24.2	110 / 166
K_m (μmol/l)	—	—	264.3[d]	—
V_{max} (pmol/min-mg protein)[e]	–	—	125[f]	—

Note. BW = body weight (kg) and Q_{co} = cardiac output (l/hr) = 12.92 $BW^{0.74}$, based on similar scaling of cardiac output and alveolar ventilation rate (Tardif *et al.*, 1993) and measurements of ventilation rate in our subjects.

[a]From (Rowland and Tozer, 1989).

[b]From (Tardif *et al.*, 1993).

[c]Tissue/blood partition coefficients were determined by dividing tissue/air coefficients by the blood/air coefficient of 17.7 (MTBE) or 11.7 (ETBE), (Nihlen *et al.*, 1995).

[d]From (Borghoff *et al.*, 1996).

[e]Total activity in the liver was obtained by multiplying 125 pmol/min-mg protein by 78,750 mg protein/liver.

[f]From (Hong *et al.*, 1997).

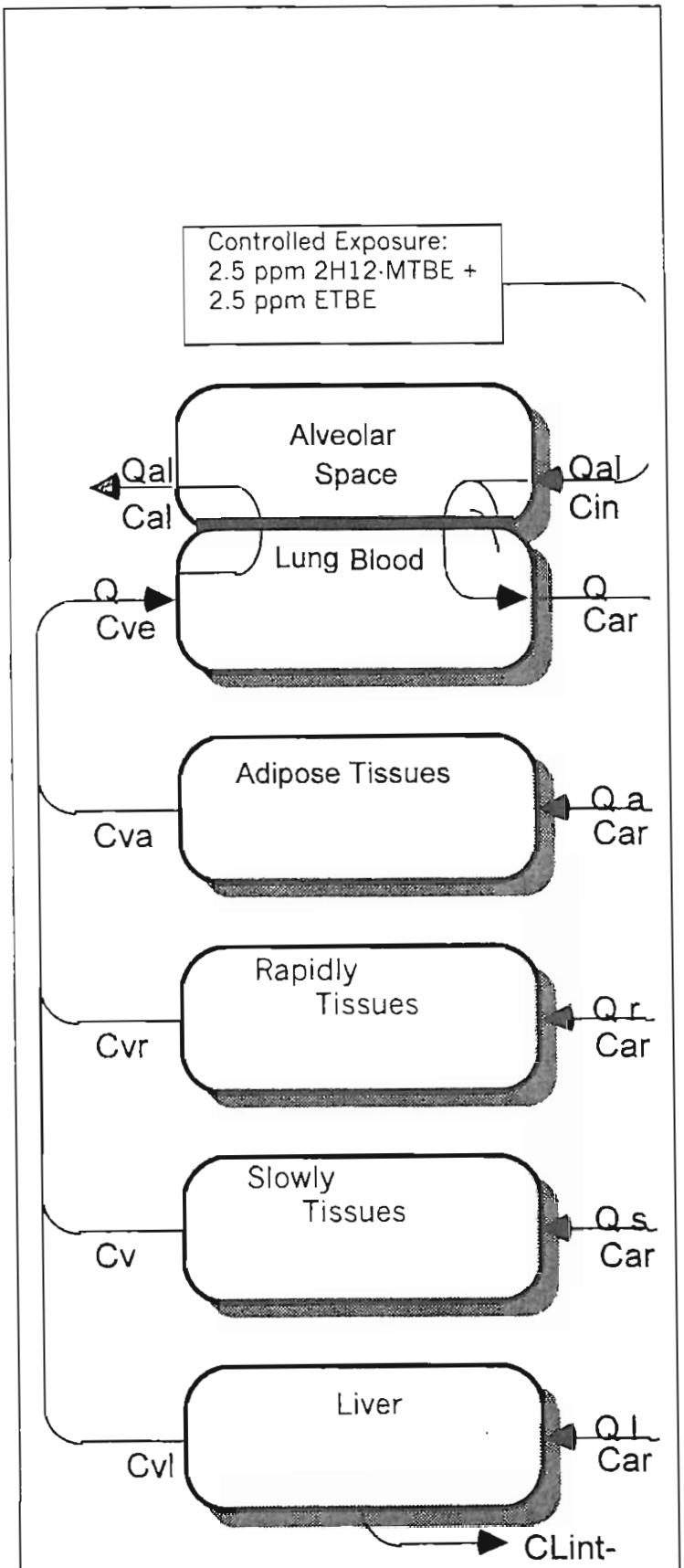


Figure 2. Physiologically-based toxicokinetic model for inhaled $^2H_{12}$-MTBE and ETBE. Abbreviations and Symbols: Q_{alv} =alveolar ventilation (l air/h); C_{inh} = concentration in inhaled air (ppm); C_{alv} = concentration in alveolar air (ppm); Q_{co} = cardiac output (l blood/h); C_{art} = concentration in arterial blood (μmol/l); C_{ven} = concentration in venous blood (μmol/l); CL_{int-h} = intrinsic metabolic clearance in the liver (l/h); Q_i = blood flow rate to tissue group i (l/h); C_{vi} = concentration in blood leaving tissue group i (mg/l). Subscripts (i) for tissue groups or compartments: a, adipose tissues; r, rapidly perfused tissues; s, slowly perfused tissues; l, liver.

Specific Aim 2b: Performance of model sensitivity analysis

Sensitivity Analyses

Initial MTBE and ETBE model sensitivity analyses were conducted to identify model parameters that were most important in determining toxicant blood concentrations. The sensitivity analysis was performed by evaluating _(blood concentration) / _(model parameter), after log transformation to reduce apparent differences due to parameter magnitude. In this way, the effects of 1% changes in each of the 17 model parameters on predicted MTBE or ETBE blood concentrations over a 24 hour exposure and washout period were determined. For both MTBE and ETBE, the 8 most influential

parameters were the breathing rate, cardiac output, exposure concentration, adiposity, adipose tissue/blood partition coefficient, blood/air partition coefficient, fraction of slowly-perfused tissues, and adipose blood flow (Fig. 3a & b). We have measured or plan to measure the first six of these parameters, will perform an extensive literature search to determine the mean and range of the fraction of slowly-perfused tissues, and will vary the adipose blood flow within physiologic bounds to fit the data. While the sensitivity plots for MTBE and ETBE were very similar, a more pronounced effect of the slowly-perfused fraction (mostly muscle) and earlier effect of adiposity and adipose blood flow in the MTBE model analysis were observed. These findings are consistent with the less lipophilic character of MTBE compared to ETBE.

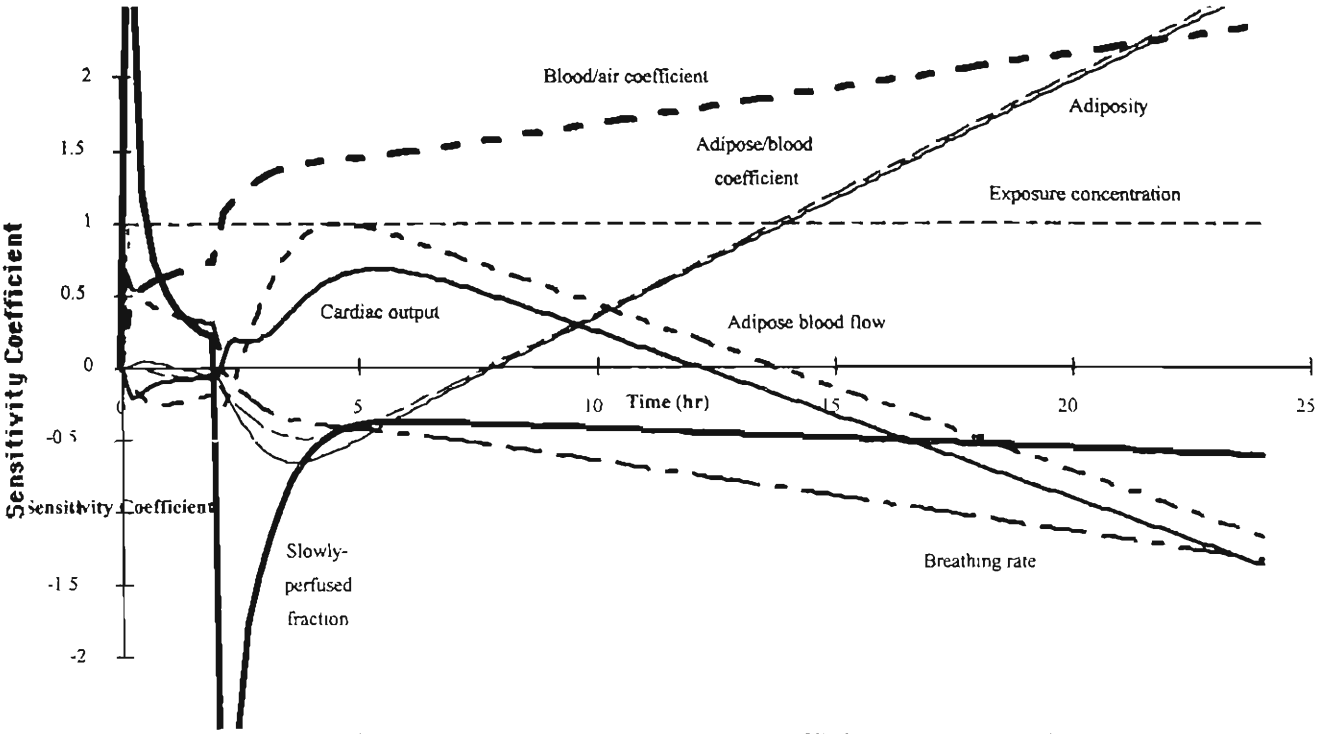


Figure 3a. MTBE model sensitivity analysis. The sensitivity coefficient represents the change in predicted blood concentration over time following a 1% increase in each model variable.

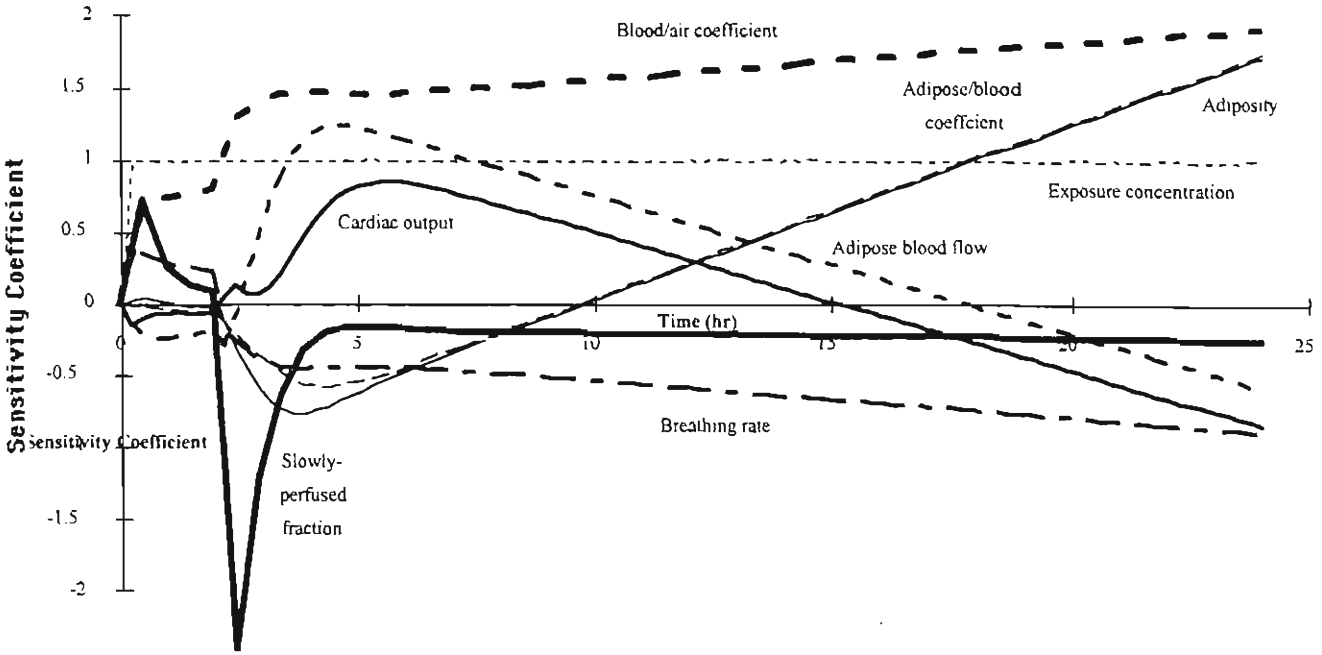


Figure 3b. ETBE model sensitivity analysis. The sensitivity coefficient represents the change in predicted blood concentration over time following a 1% increase in each model variable.

Uncertainty analysis

To evaluate the effects of parameter uncertainty on model output, each of the parameters identified as important in the sensitivity analysis was further examined with regards to population variability. Using the measured means and standard deviations for these parameters, a variability ranking was calculated for each parameter as $(s.d./mean)_i$ / $(s.d./mean)_{average}$. Because both population variability and sensitivity (effect on model output) are important, the eight model parameters with the highest scores on each of these scales were chosen for Monte Carlo simulations (Fig. 4).

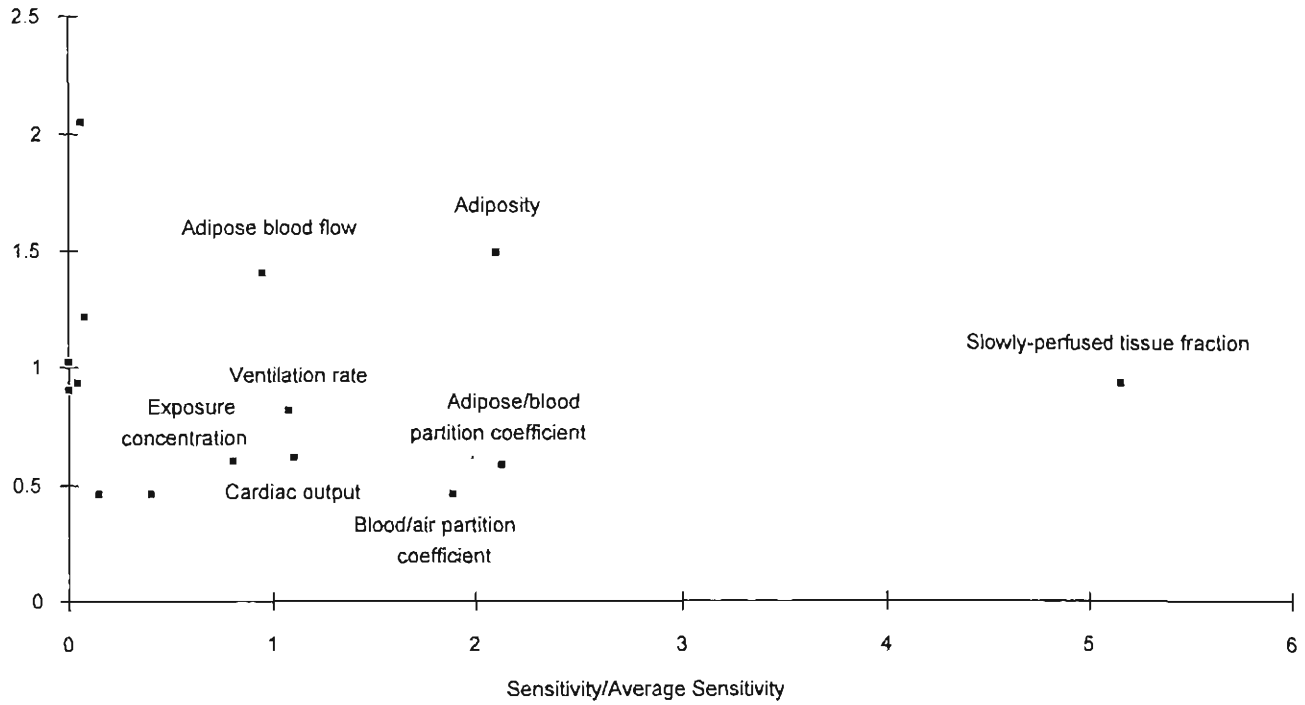


Figure 4: Results of model parameter uncertainty analysis illustrating both sensitivity (change in blood

concentration predictions to changes in parameter values), and population variability in measured parameter values.

Model Testing

In an initial effort to see whether the physiologic model was predictive of findings from other laboratories, we requested data from Dr. Annsofi Nihlen. Model parameters were allowed to vary within physiologic bounds, providing a close fit of simulated values to measured concentrations of MTBE in blood and breath (Fig. 5 a and b).

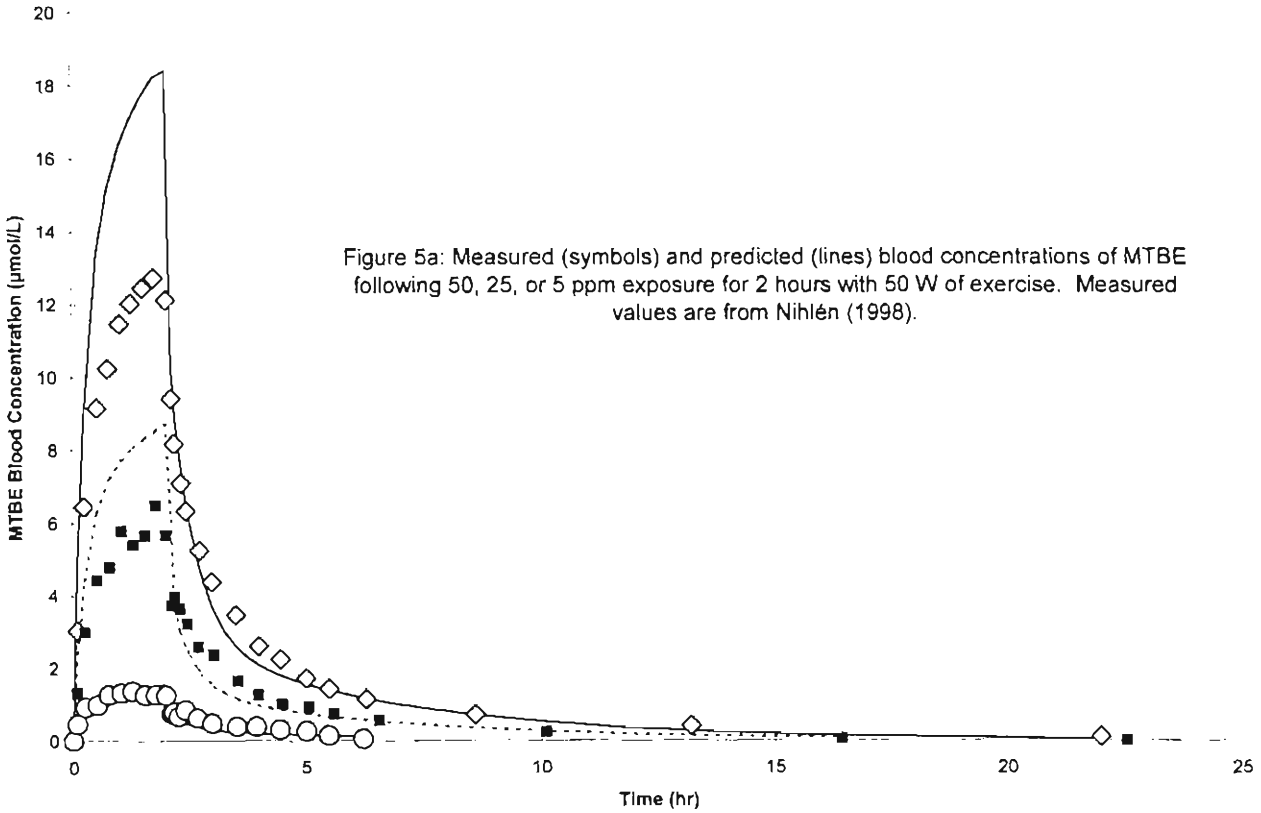


Figure 5a: Measured (symbols) and predicted (lines) blood concentrations of MTBE following 50, 25, or 5 ppm exposure for 2 hours with 50 W of exercise. Measured values are from Nihlén (1998).

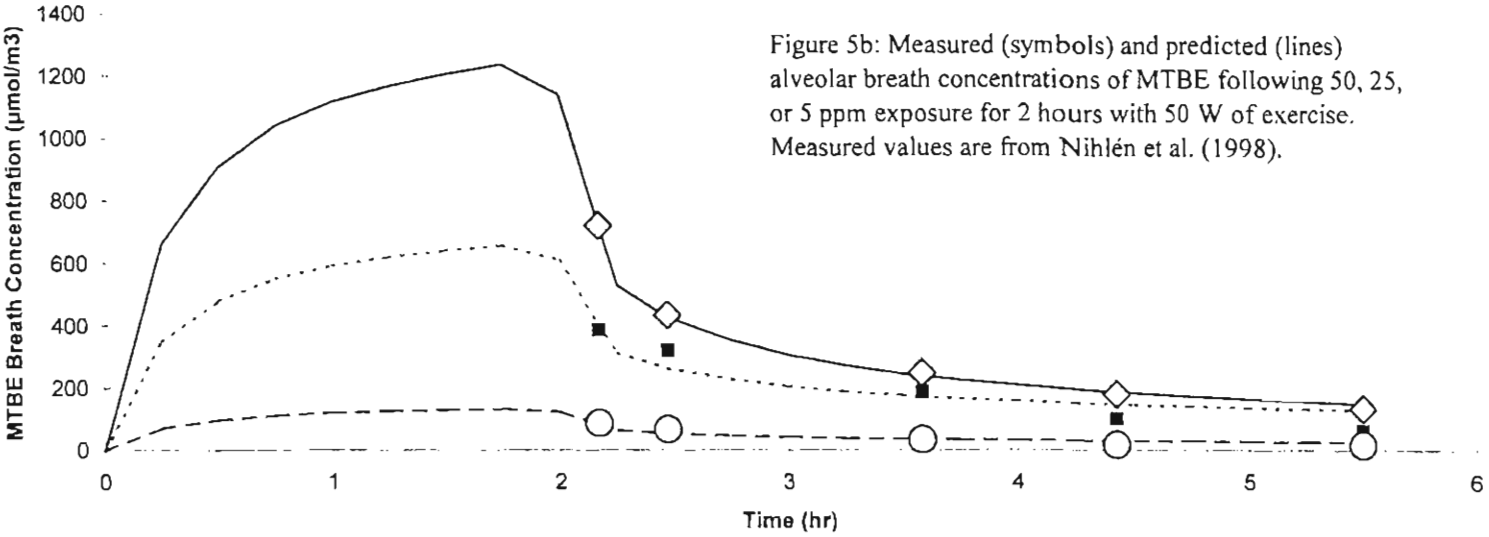


Figure 5b: Measured (symbols) and predicted (lines) alveolar breath concentrations of MTBE following 50, 25, or 5 ppm exposure for 2 hours with 50 W of exercise. Measured values are from Nihlén et al. (1998).

Selection of Final Model Parameter Values

Because the model is innately an incomplete representation of the physiologic processes that govern xenobiotic kinetics, selected parameters were allowed to vary within expected physiologic ranges to provide a close fit to the observed data. The tissue:blood and blood:air partition coefficients and the metabolic parameters K_M and V_{max} for MTBE and ETBE were varied using the averages and standard deviations of measured values (Hong 2000; Borghoff 2000, Licata 2000, Leavens 2000, and Table 2). Measured body weight and adiposity for each subject were used as model inputs. Based on the sensitivity/variability analysis (Fig. 4), weight was allowed to vary by its measurement error (4%), and adipose blood flow was allowed to vary by 29% (Pierce et al., 1998). All parameters were varied using the Bayesian fitting option (Vicini et al., 1999) of SAAM II.

Models for $^2H_9$-TBA and TBA used the tissue:blood and blood:air partition coefficients suggested by Leavens (2000), allowing a 25% variability in the values for slowly- and rapidly-perfused tissues for fitting using the Bayesian option. Appearance of TBA in blood was treated as a first-order absorption process using a mass of 156 micromoles of either MTBE or ETBE (corresponding to a 2.5 ppm exposure). Elimination was also treated as a first-order process, consistent with the initial observations from our group and others that after exposure cessation, TBA declines monoexponentially. Both the rates of absorption and elimination were allowed to vary without constraint to best describe the data.

Specific Aim 2c: Calculation of toxicokinetic parameters

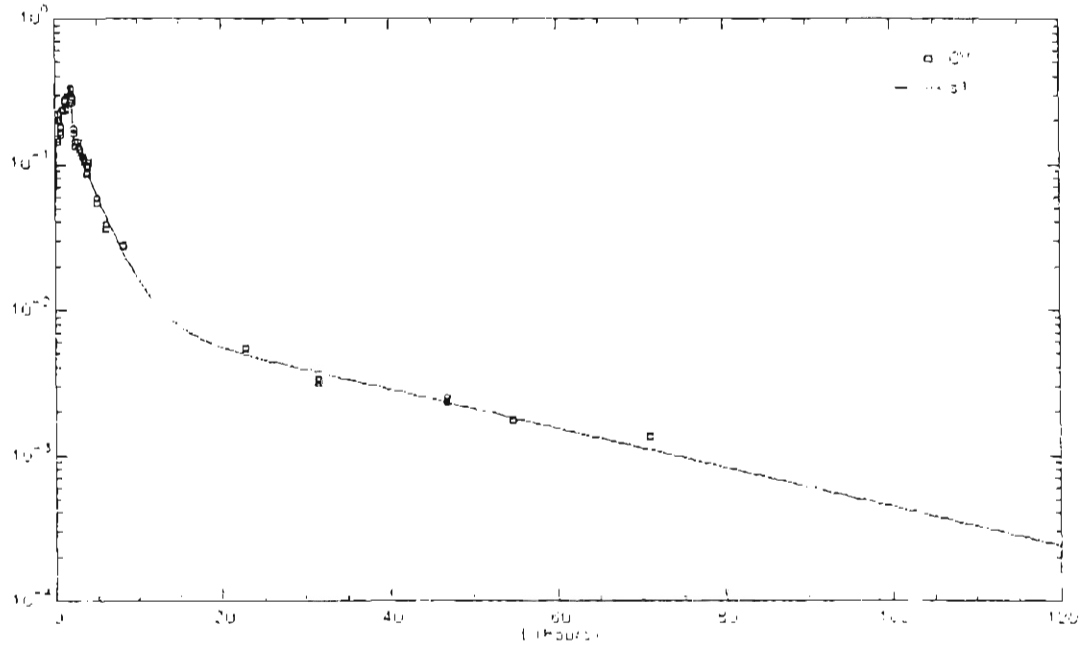
Systemic clearance (CL) was calculated as F x Dose/AUC, where bioavailability (F) was estimated as 17/18 (governed by the blood:air partition coefficient), dose was estimated as inhaled concentration x ventilation rate x two hours, and AUC was the area under the fitted blood concentration-time curve. The terminal volume of distribution (V) was calculated by the model as Amount of Analyte in the Body/Concentration in Blood. Terminal half-life was determined as ln(2) x V / CL (Rowland and Tozer, 1989).

Correspondence of Model and Data

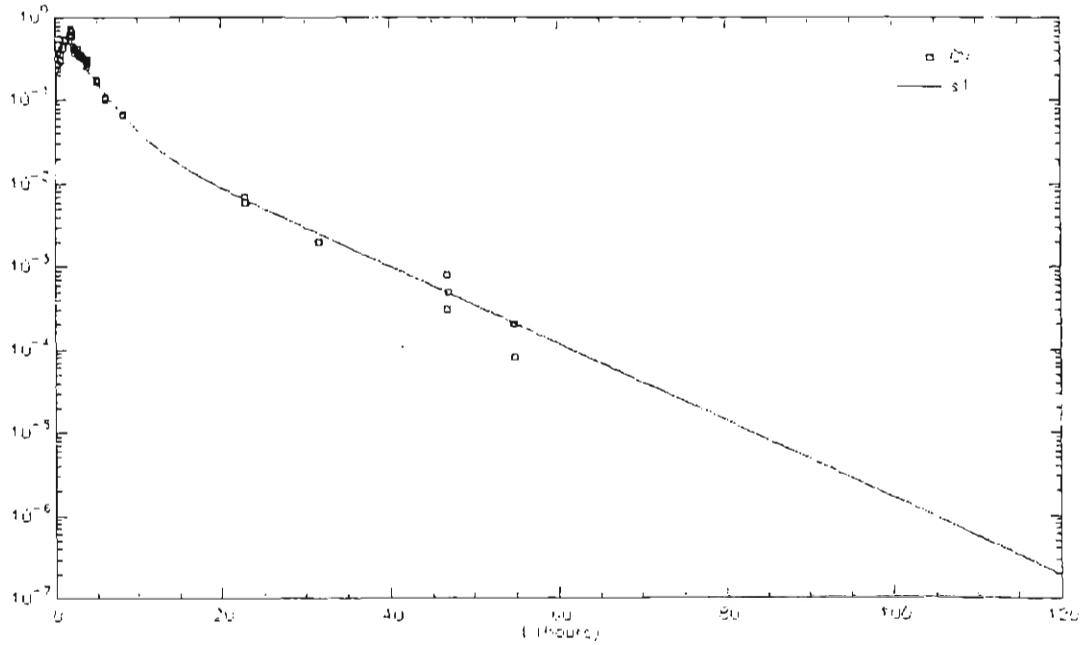
There was a close correspondence of simulated to measured blood concentrations of $^2H_8$-MTBE and ETBE, for all subjects except #5 (Fig. 6A–BB); this may have been due to the model's inability to simulate this subject's low body fat of 12.83% and high degree of physical activity, which were in contrast to the other subjects. Simulated and measured levels of $^2H_9$-TBA and TBA were close for all subjects (Fig. 6A–BB).

Figure 6: Measured (▫) and model-simulated (—) concentrations of analytes in blood following two-hour exercise/resting exposures of subjects to $^{2}H_{8}$-MTBE and ETBE.

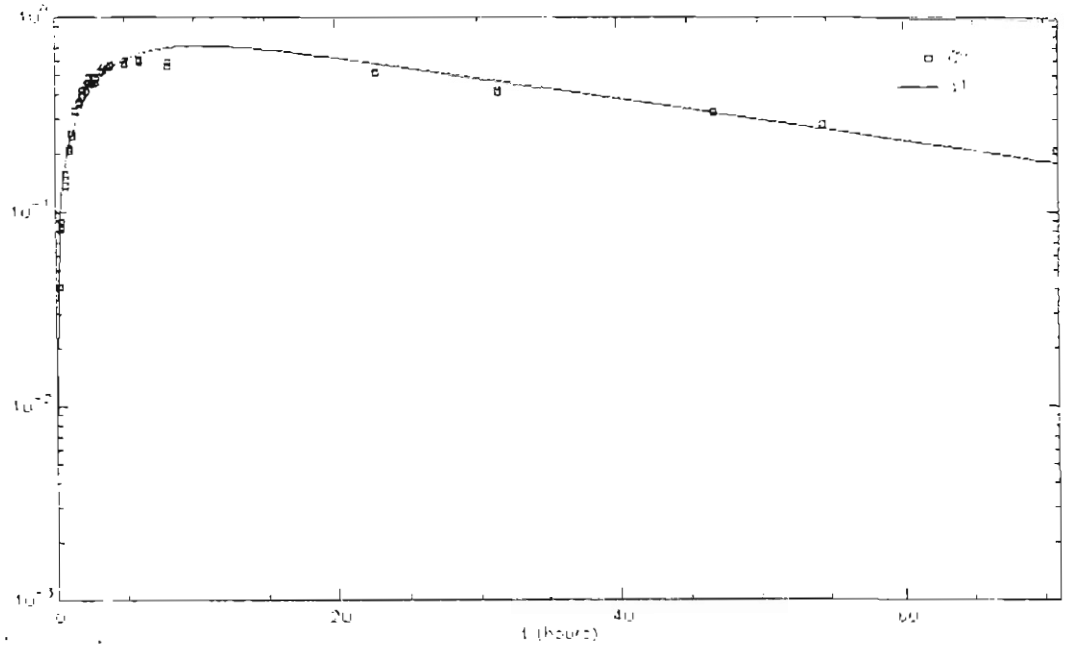
A: Subject 1A, $^{2}H_{12}$-MTBE



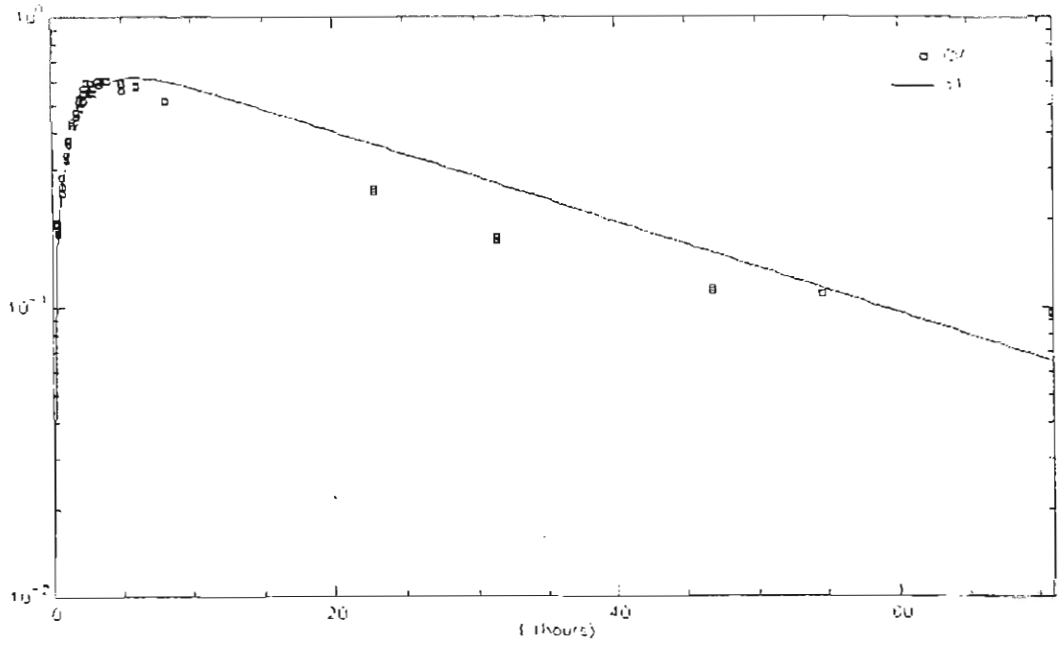
B: Subject 1a, ETBE



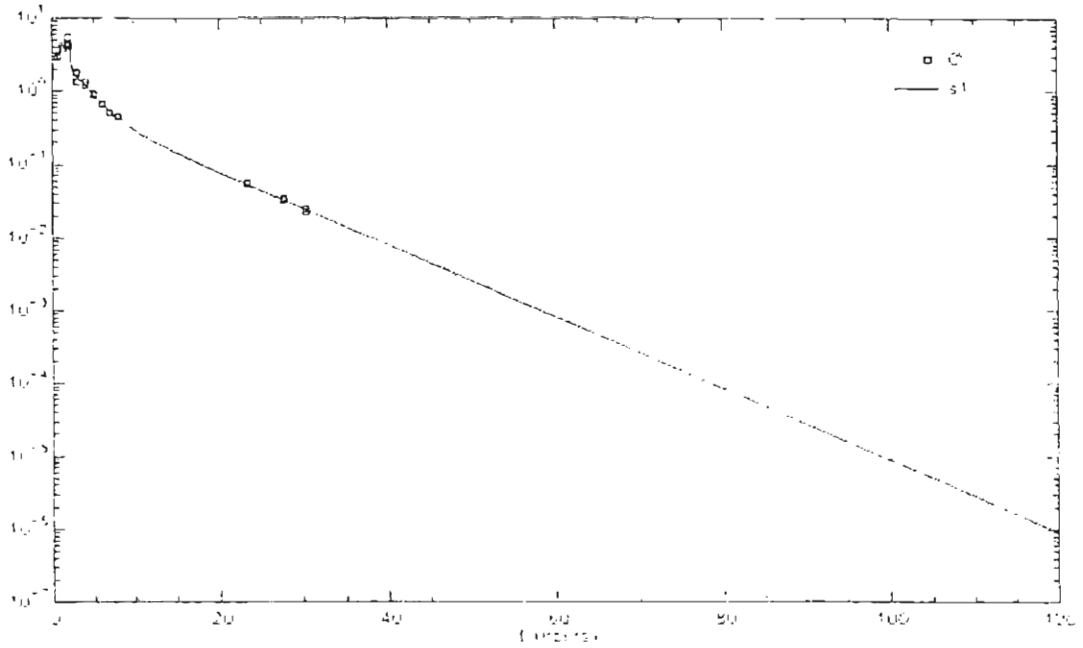
C: Subject 1a, $^{2}H_{9}$-TBA



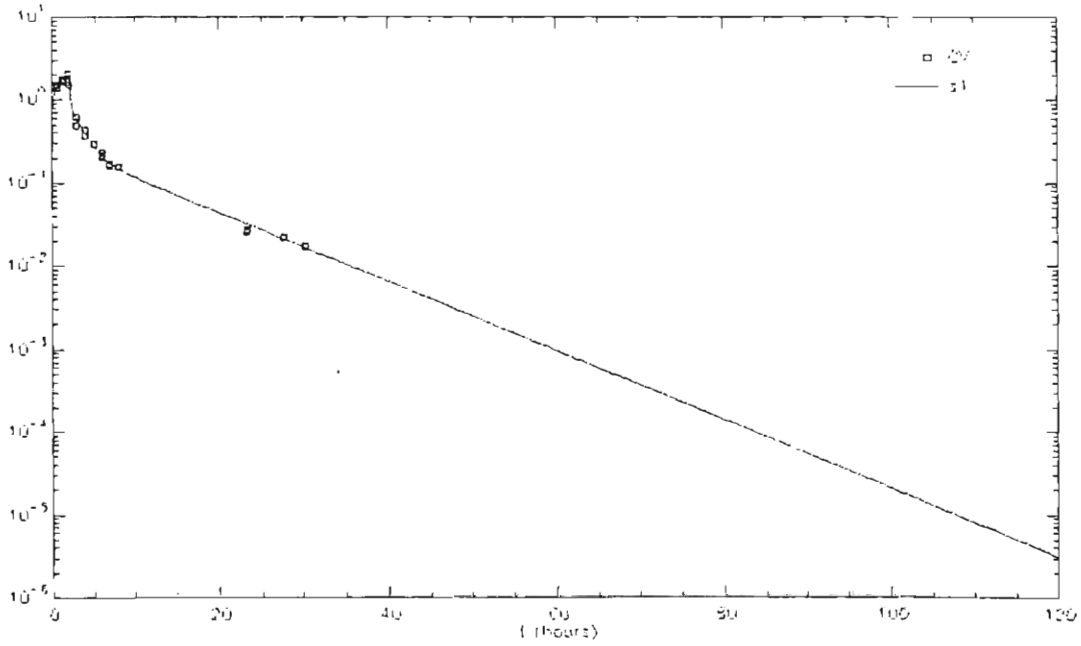
D: Subject 1a, TBA



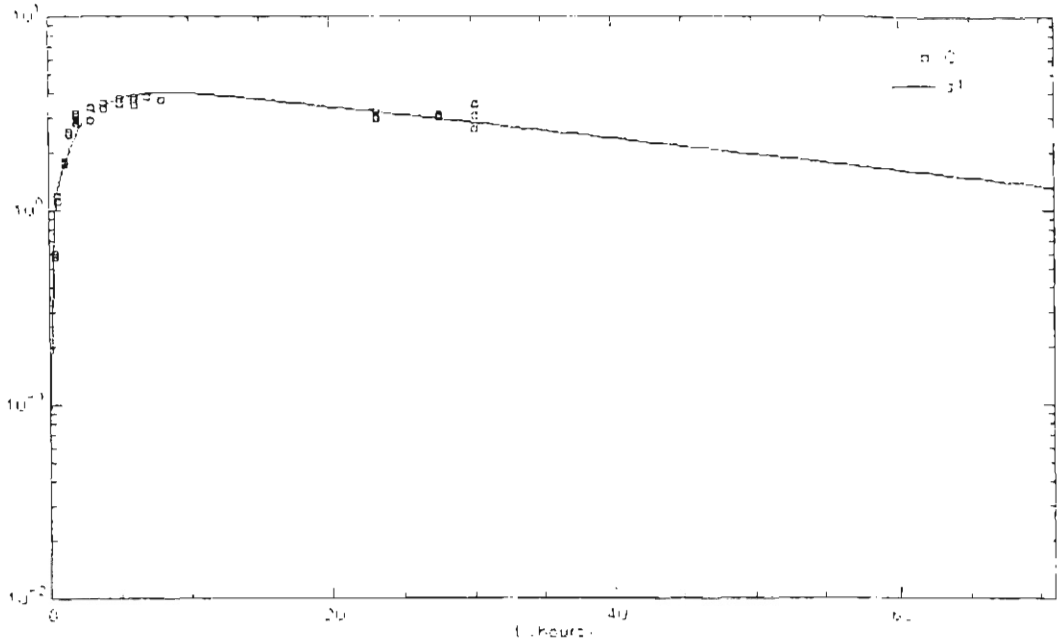
E: Subject 1B, $^{2}H_{12}$-MTBE



F: Subject 1b, ETBE



G: Subject 1b, $^{2}H_{9}$-TBA



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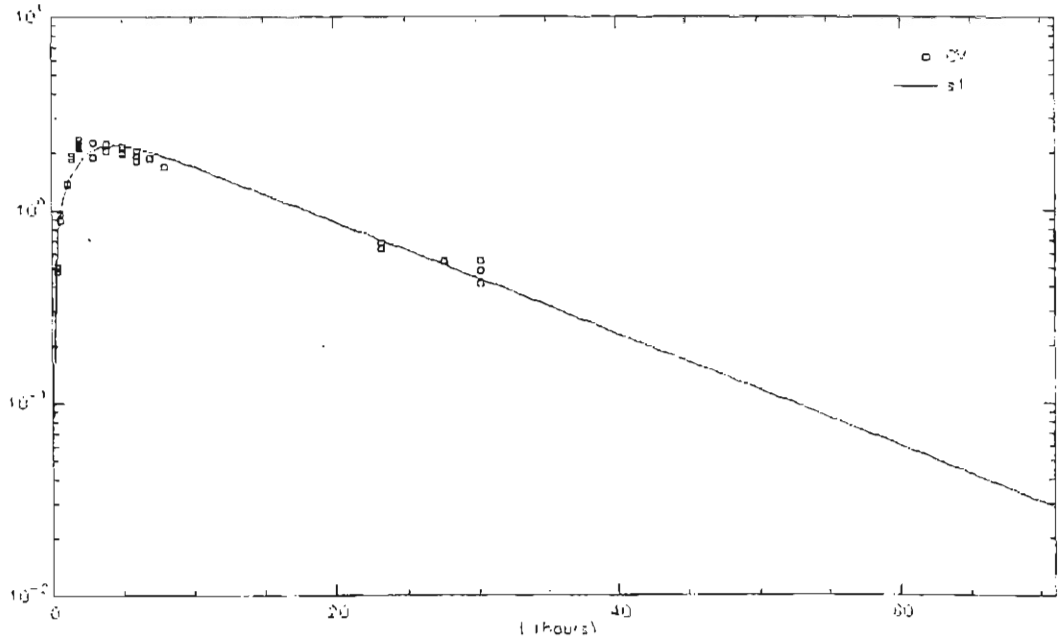

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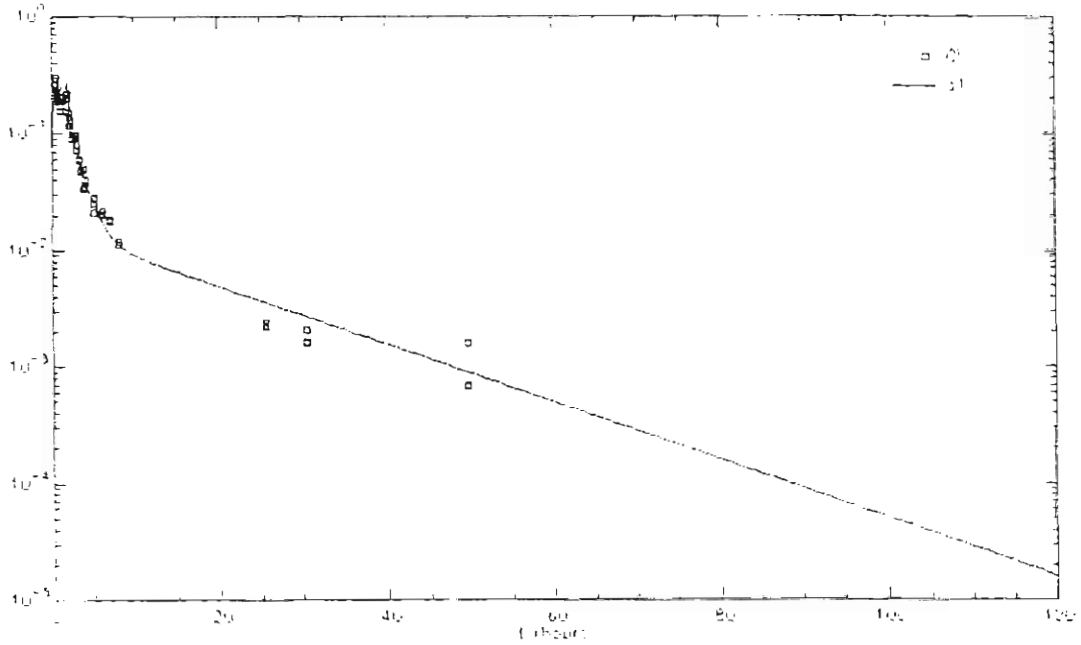

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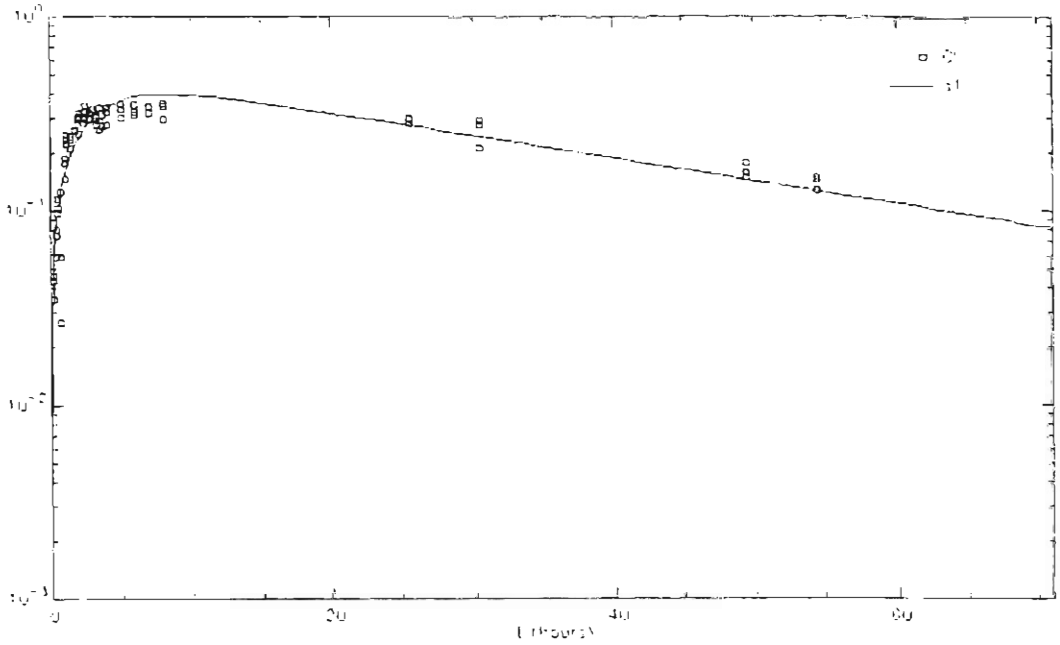

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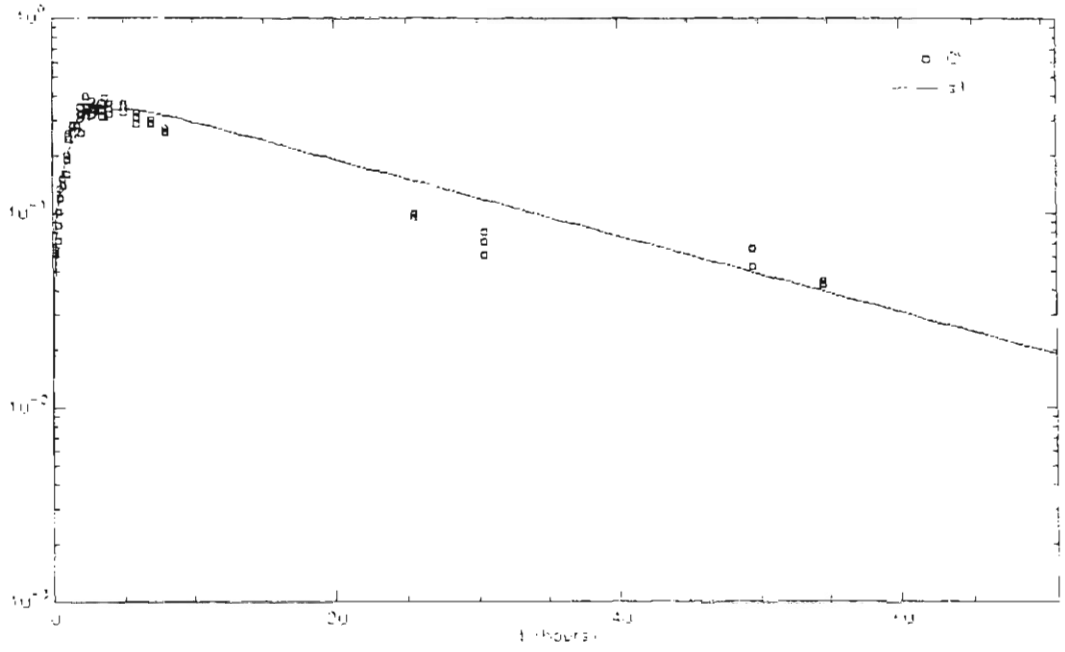
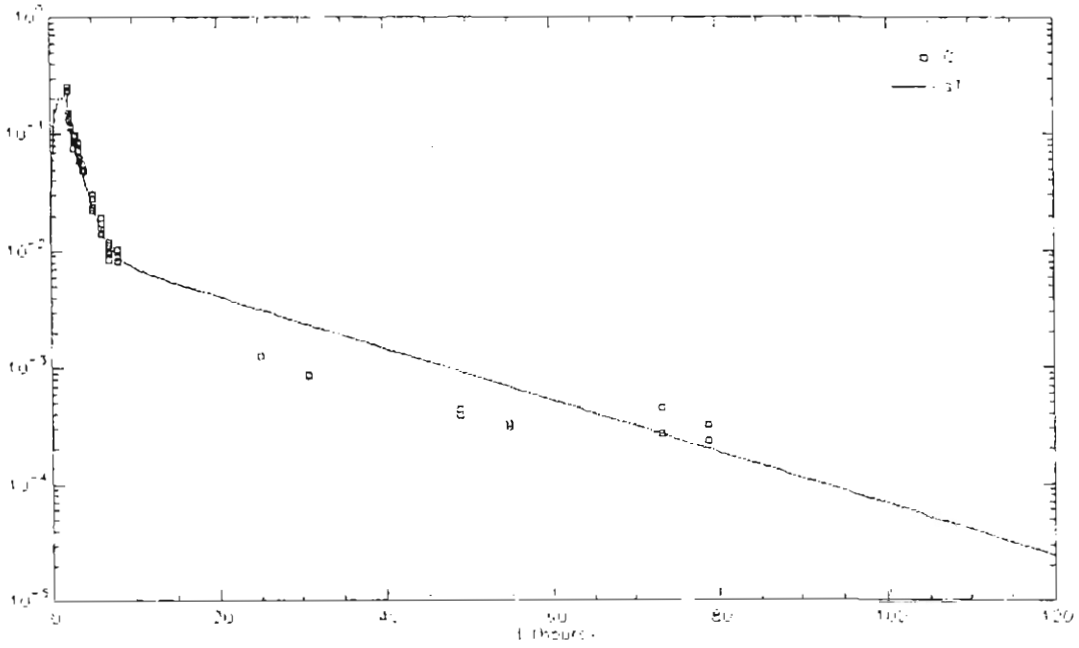
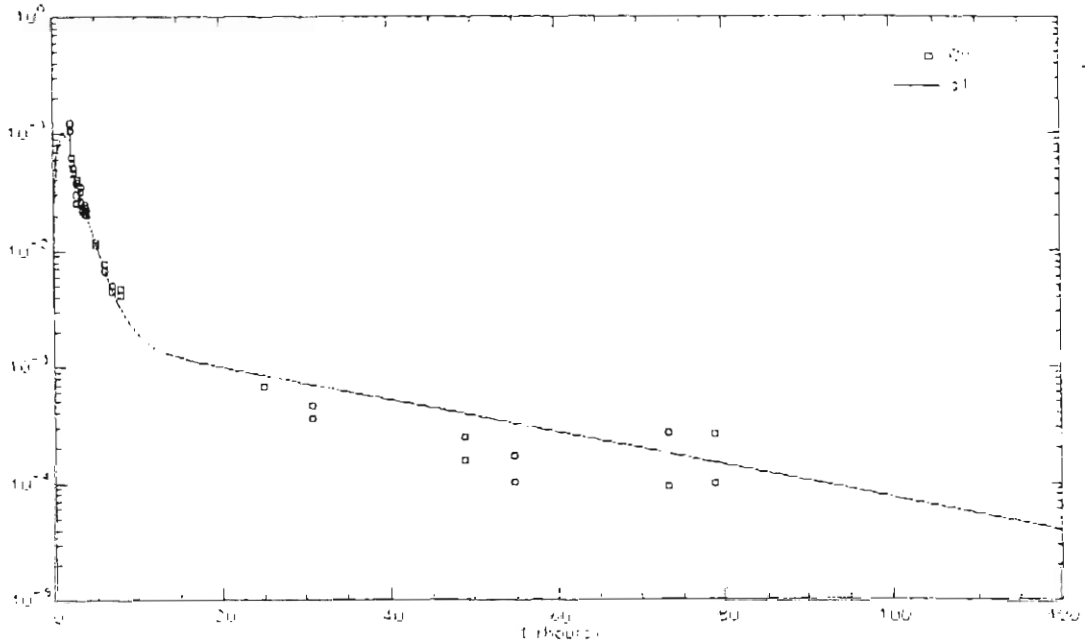


L:Subject 3, $^{2}H_{12}$-MTBE



M: Subject 3, ETBE



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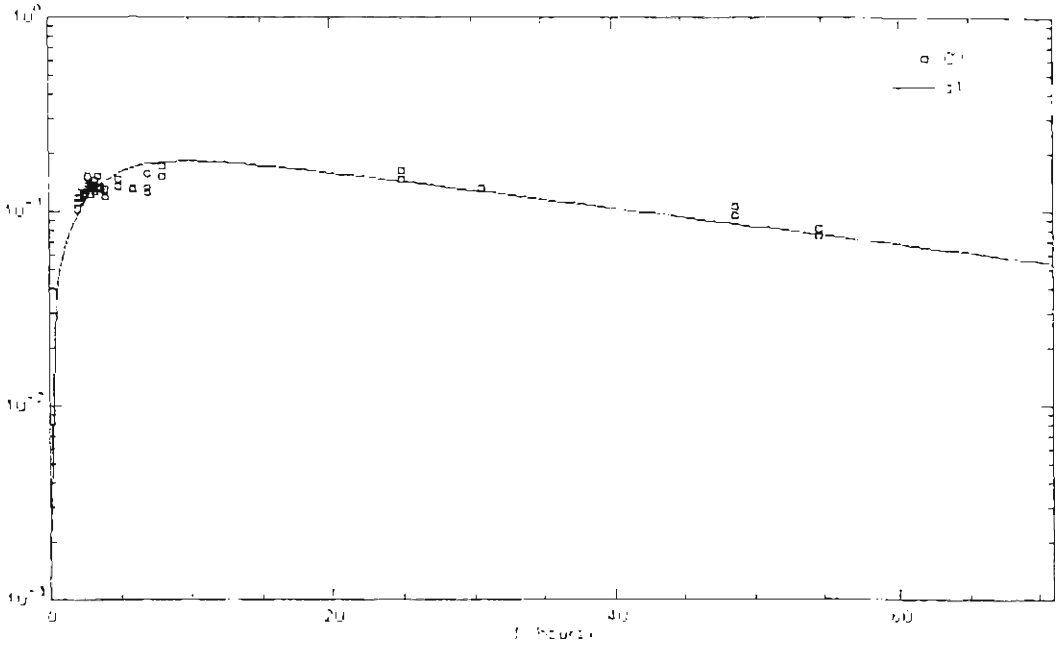
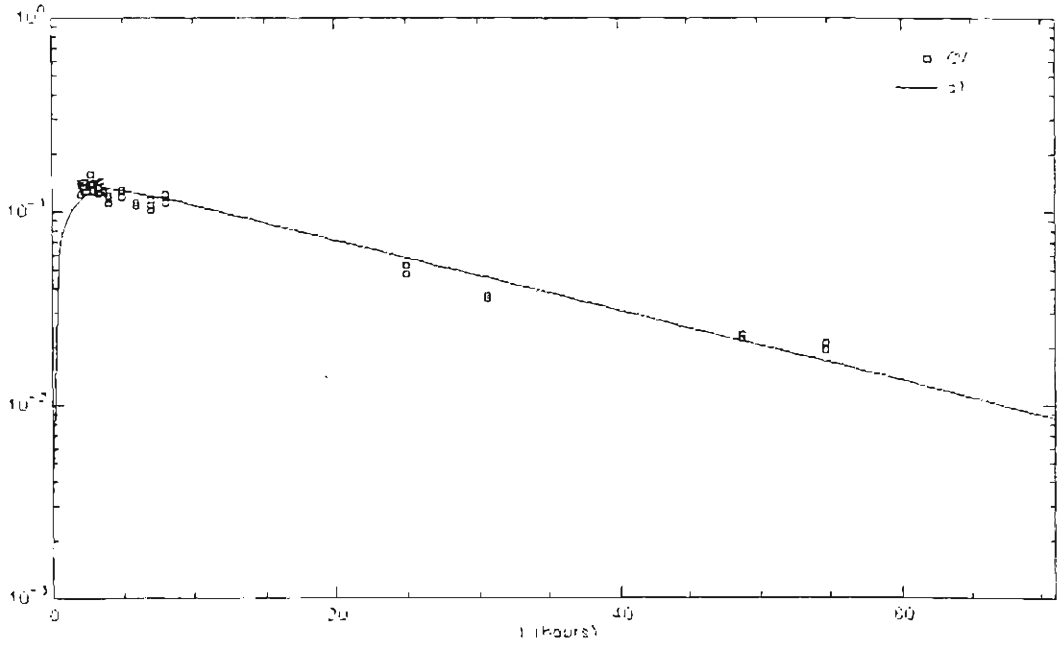


O: Subject 3, TBA



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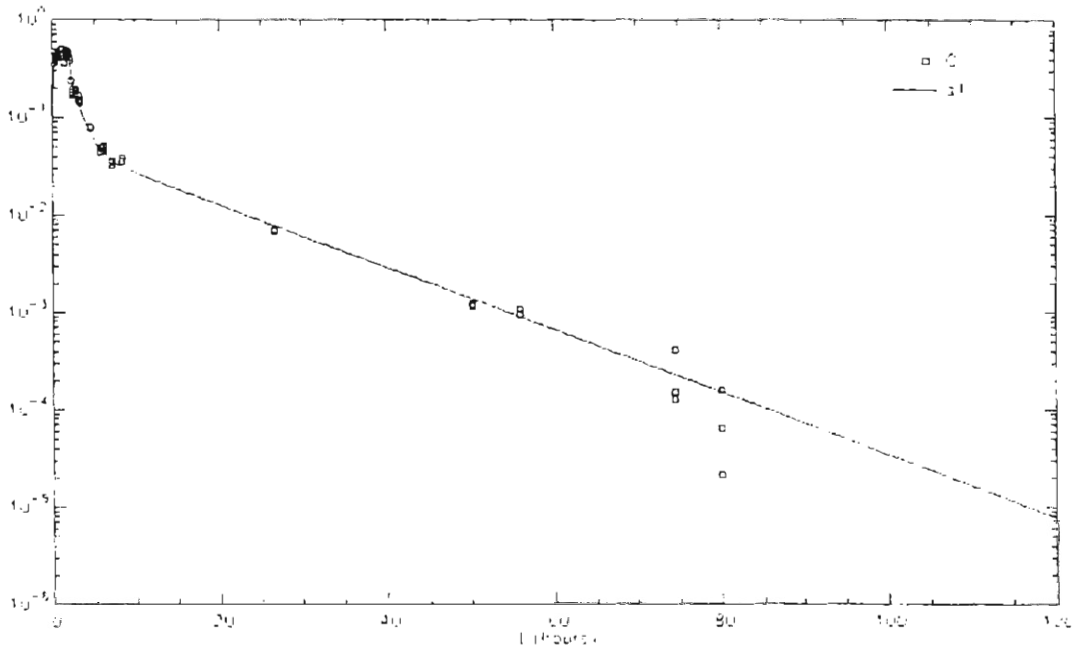

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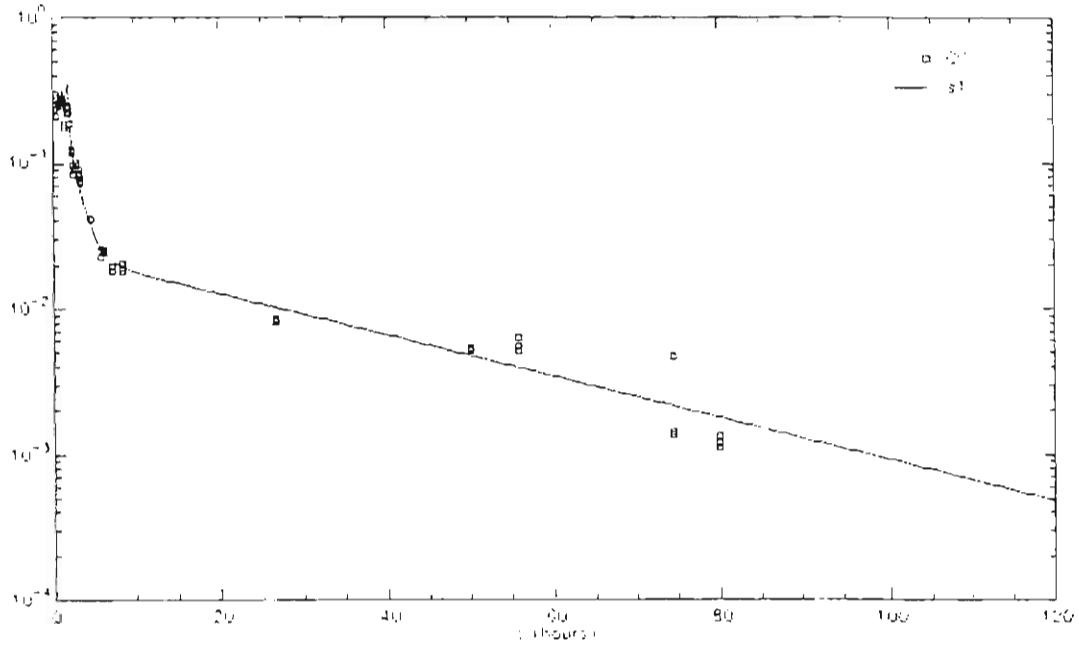

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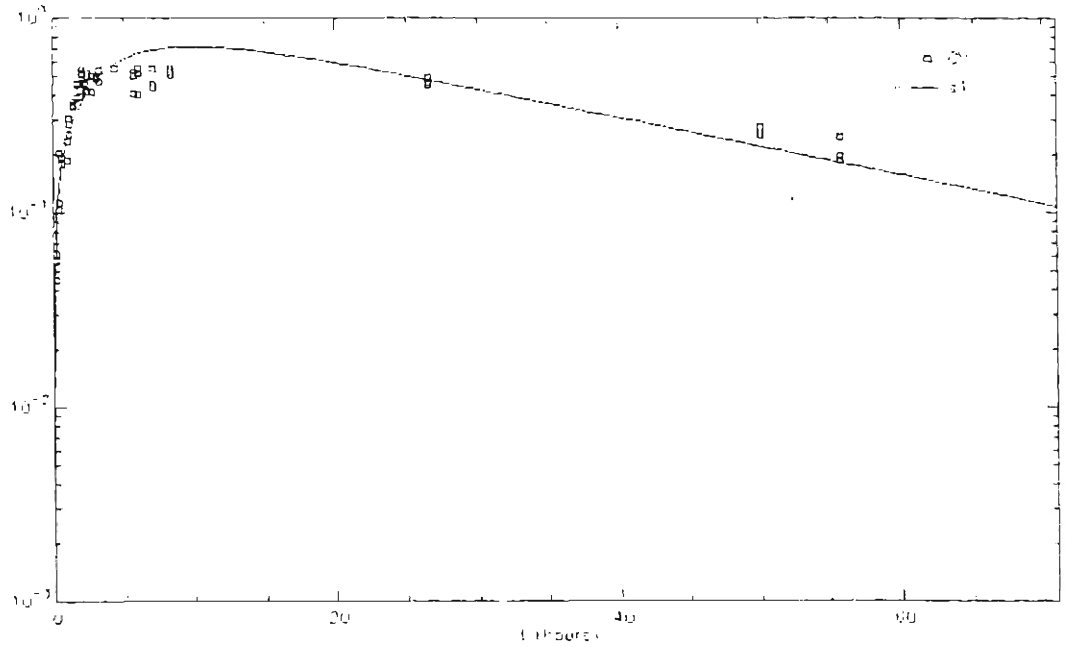
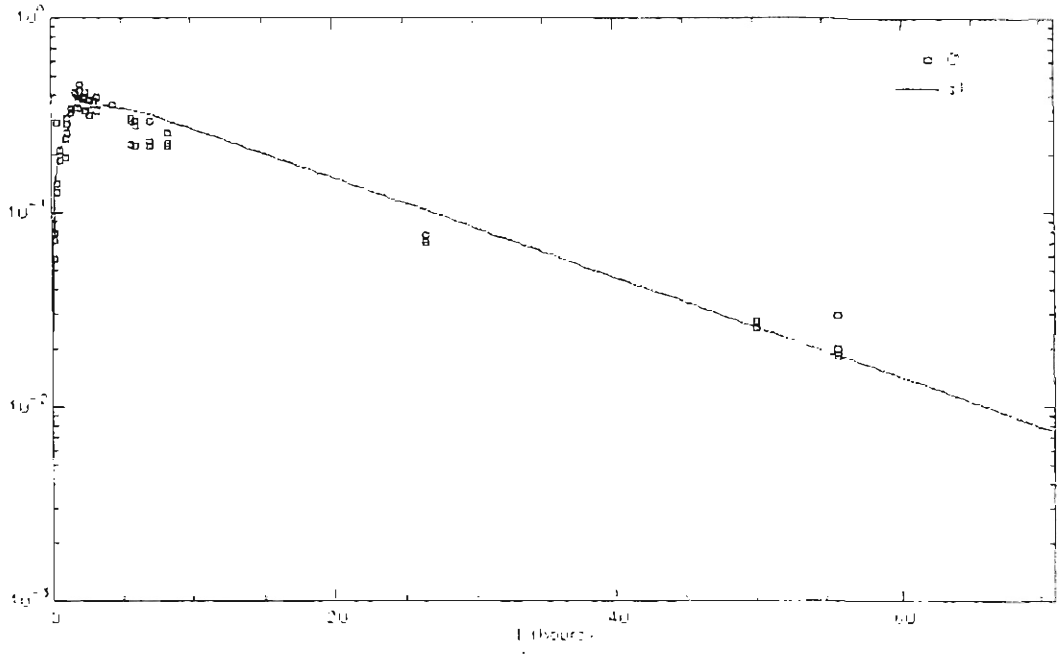


S: Subject 4, TBA



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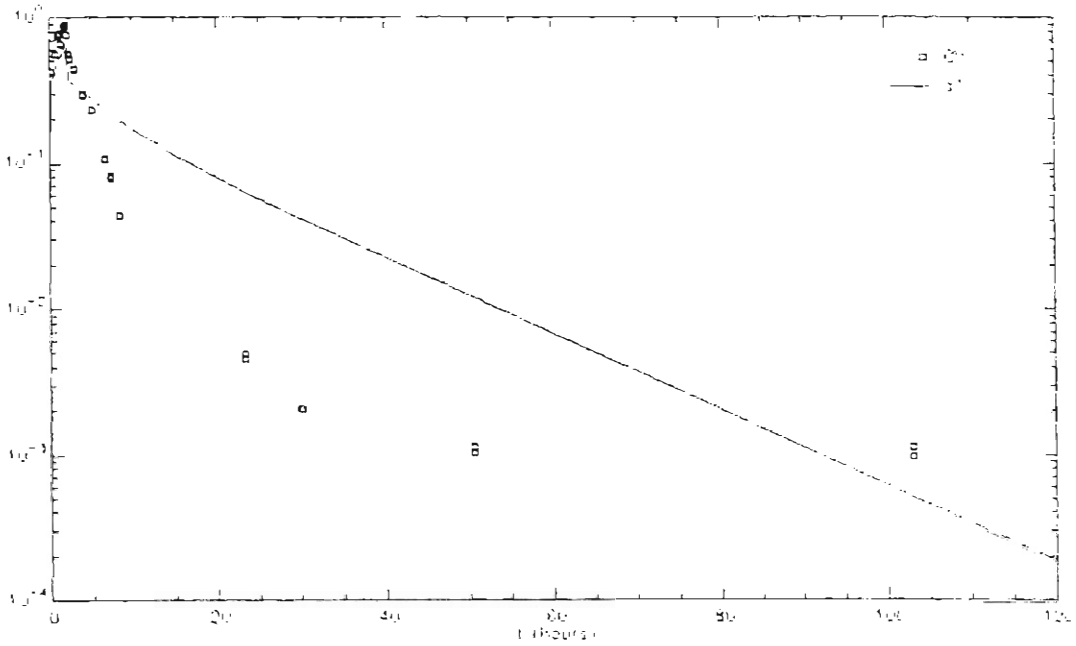
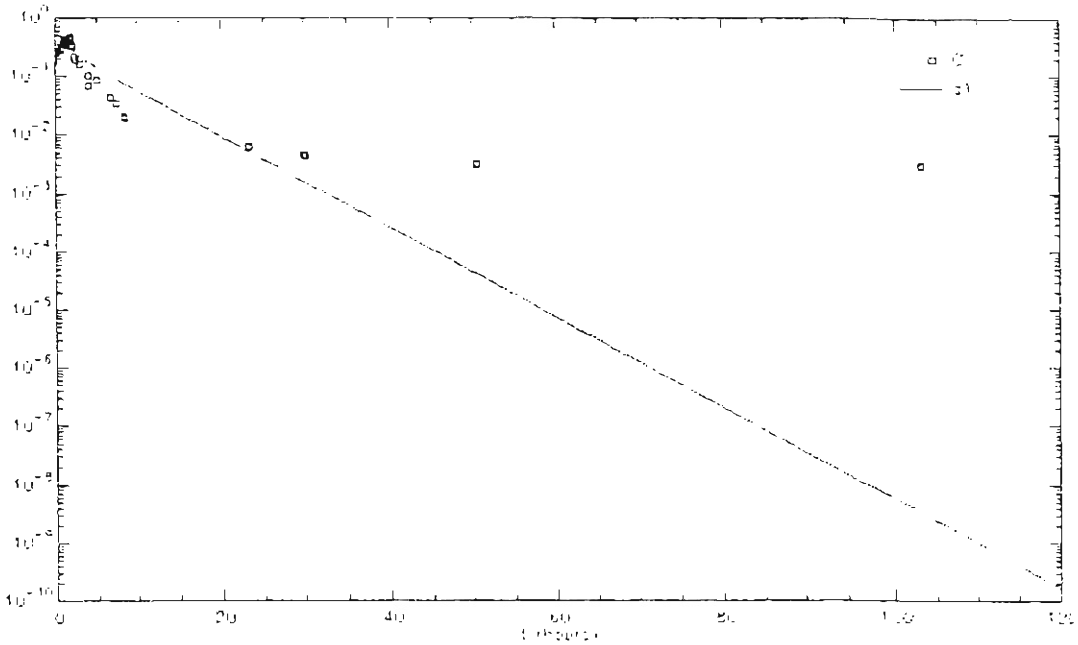


U: Subject 5, ETBE



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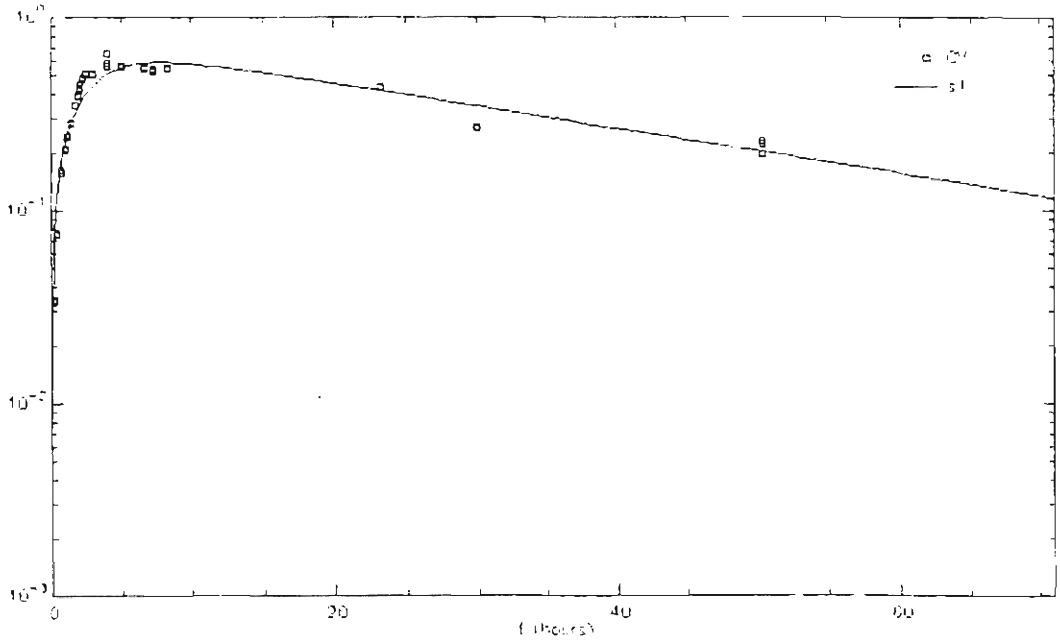
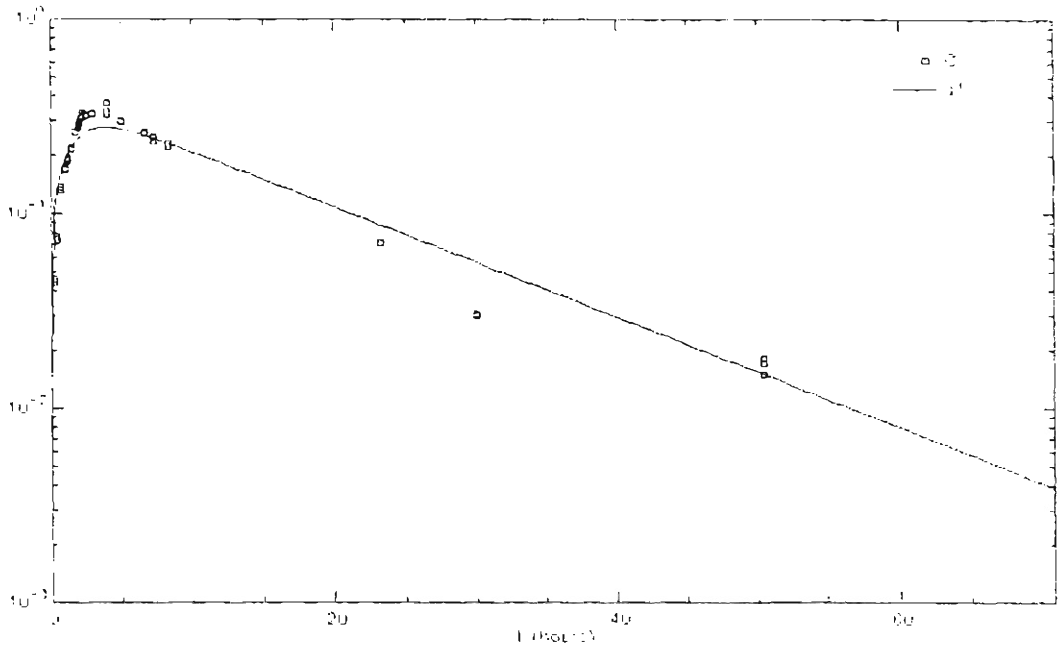
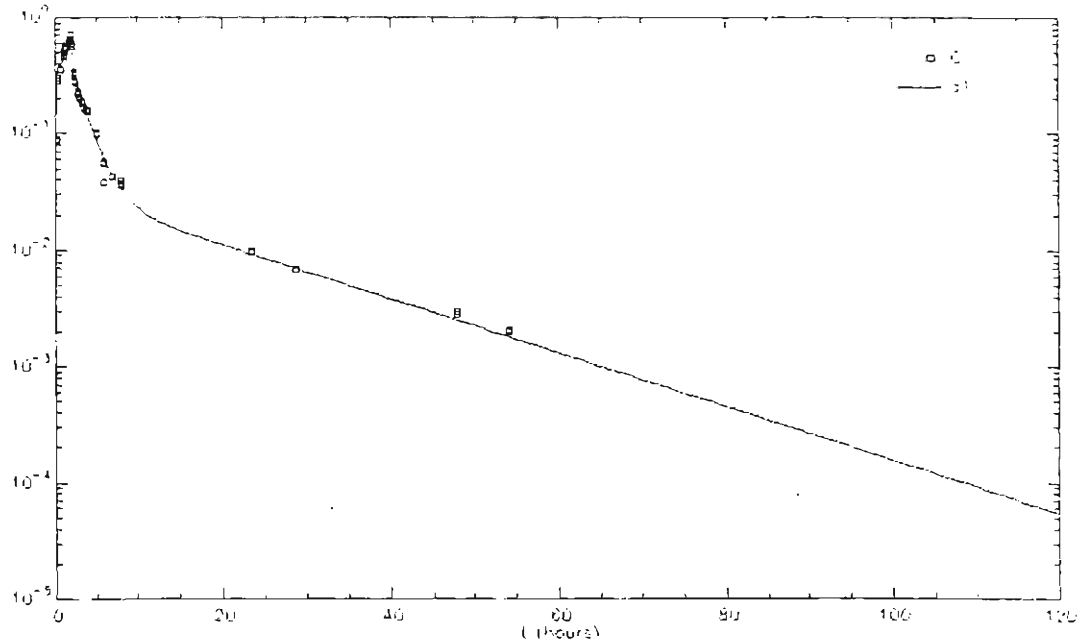


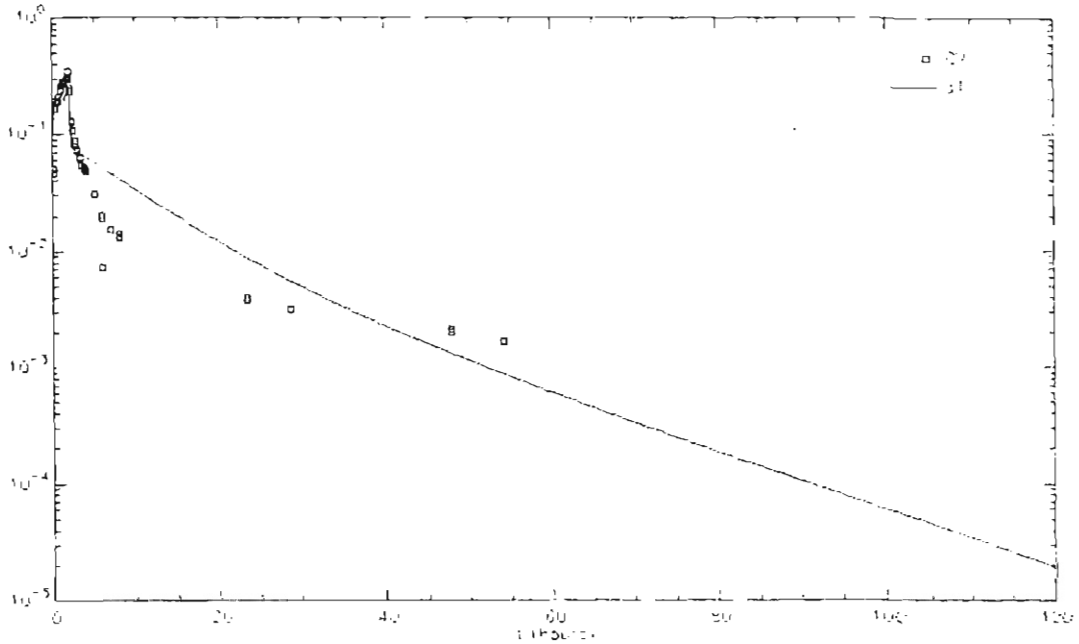
X: Subject 5, TBA



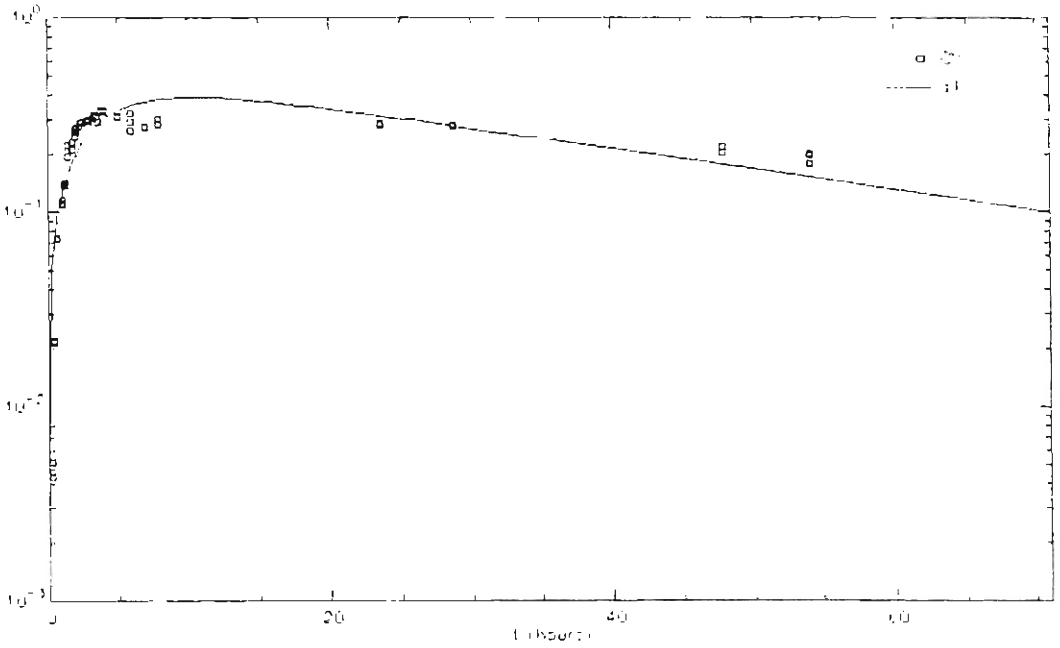
Y: Subject 6, $^{2}H_{12}$-MTBE



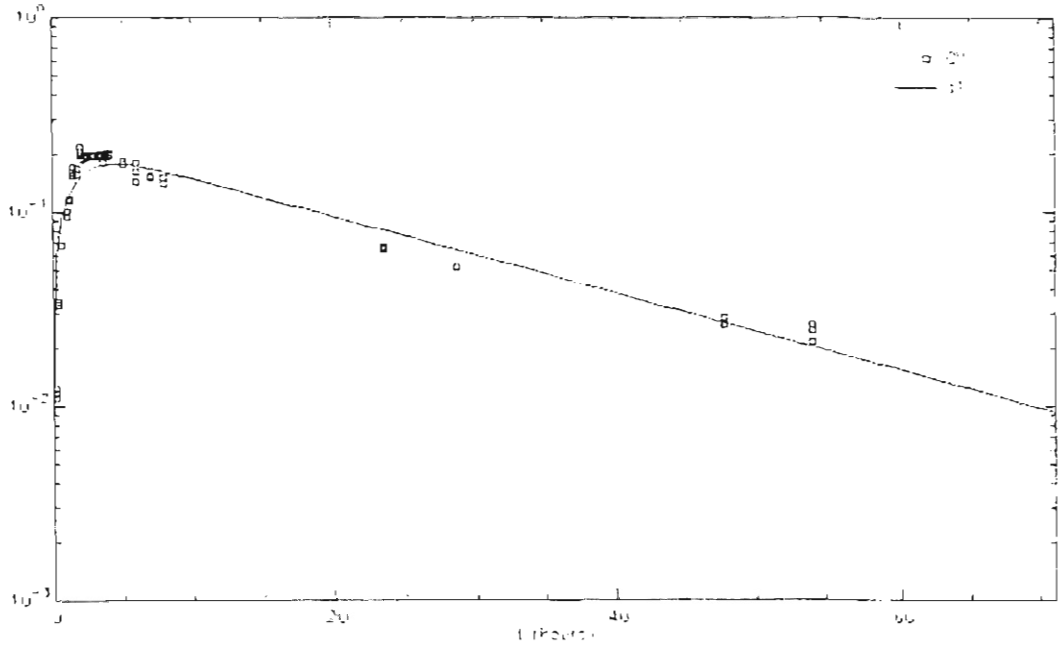
Z: Subject 6, ETBE



AA: Subject 6, $^{2}H_{9}$-TBA



BB: Subject 6, TBA



One subject (#1) was exposed twice, once to 2.5 ppm each of $^2H_{12}$-MTBE and ETBE, and once to 40 ppm $^2H_{12}$-MTBE and 10 ppm ETBE; this second exposure was designed to create higher levels of metabolites in blood for analytical detection purposes. Several exposures provided inconsistent results: blood samples from subject 2 were stored in a refrigerator with $^2H_{12}$-MTBE contamination, and so this analyte was not modeled; subject 3 was exposed to approximately 0.9 ppm of each ether; and one subject (data not shown) did not receive any ether exposure, and so provided an unintentional background control.

Analyte Candidates for Biological Monitoring

Neither formaldehyde nor acetaldehyde could be detected in blood samples during or following exposure. Background acetone and methanol levels appeared to be too high to allow the use of this metabolite to estimate MTBE exposure. Methanol and ethanol concentrations were inconsistent, perhaps due to solubility in blood and breath water vapor. TBA levels appeared to be the best indicator of MTBE and ETBE exposure, given a longer half-life and monoexponential decay.

Interindividual Differences

Although our small sample size did not allow statistical evaluation of the effects of body weight, age, height, adiposity and gender on the kinetic parameters clearance, volume of distribution, and half-life, several general observations could be made. Peak levels of $^2H_{12}$-MTBE varied by about 40% (Fig. 7a), levels of ETBE varied by about 200% (Fig. 8a), levels of $^2H_9$-TBA varied by about 175% (Fig. 9), and levels of TBA varied by about 350% (Fig. 10).

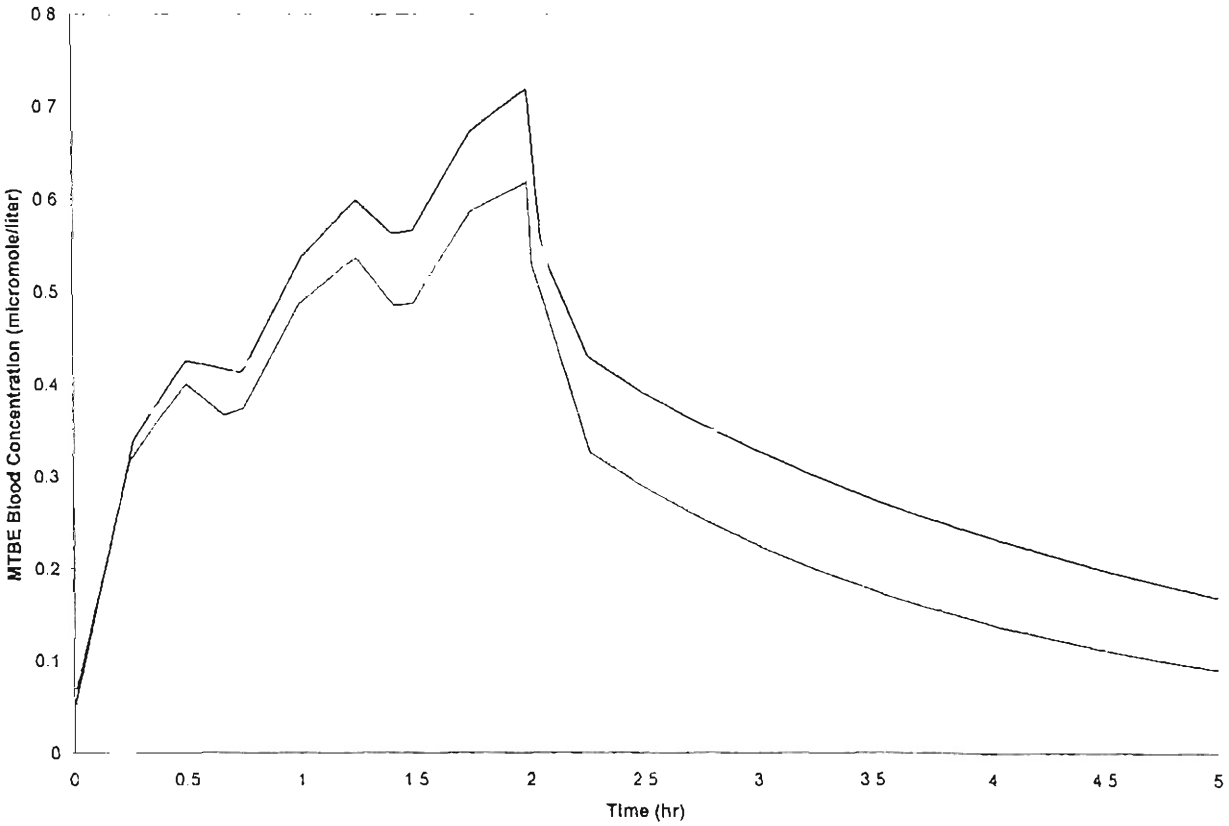
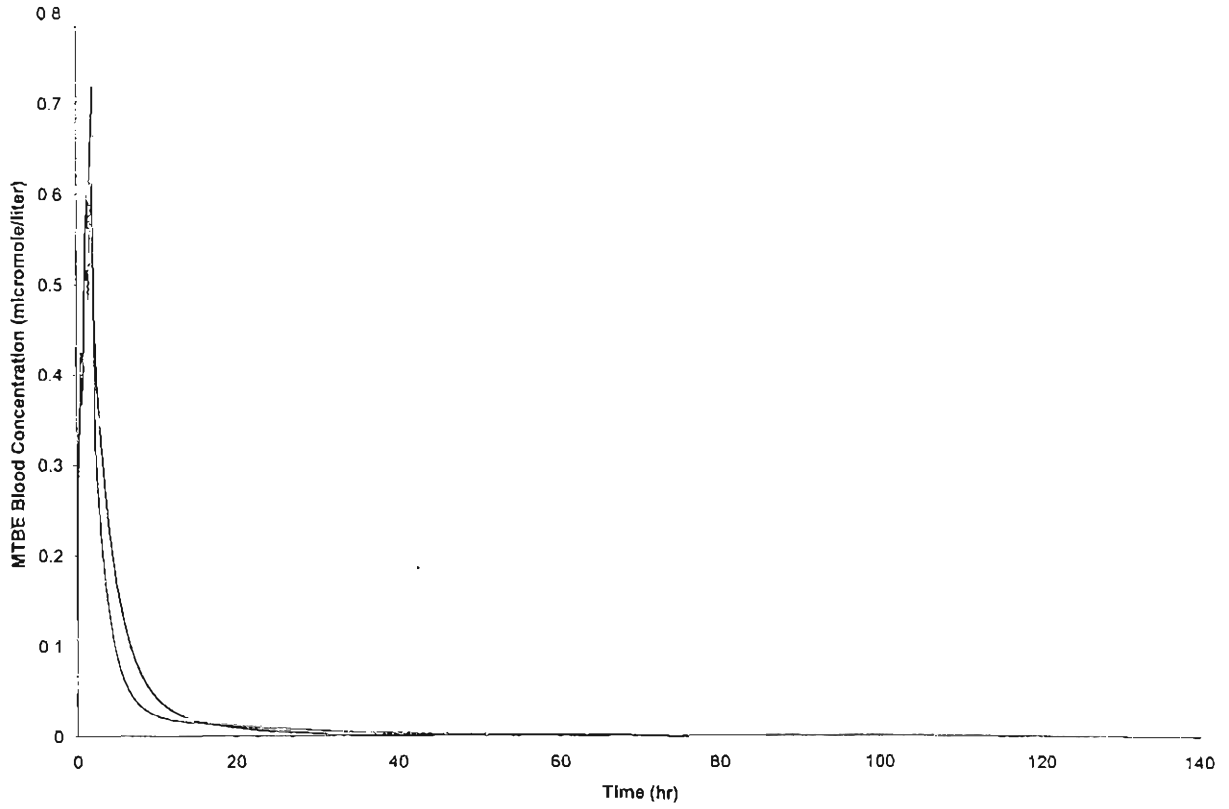


Figure 7a, b: Model-fit multiple subject blood concentrations of $^{2}H_{12}$-MTBE (upper plot, 0–5 hr, lower plot, 0–140 hr).



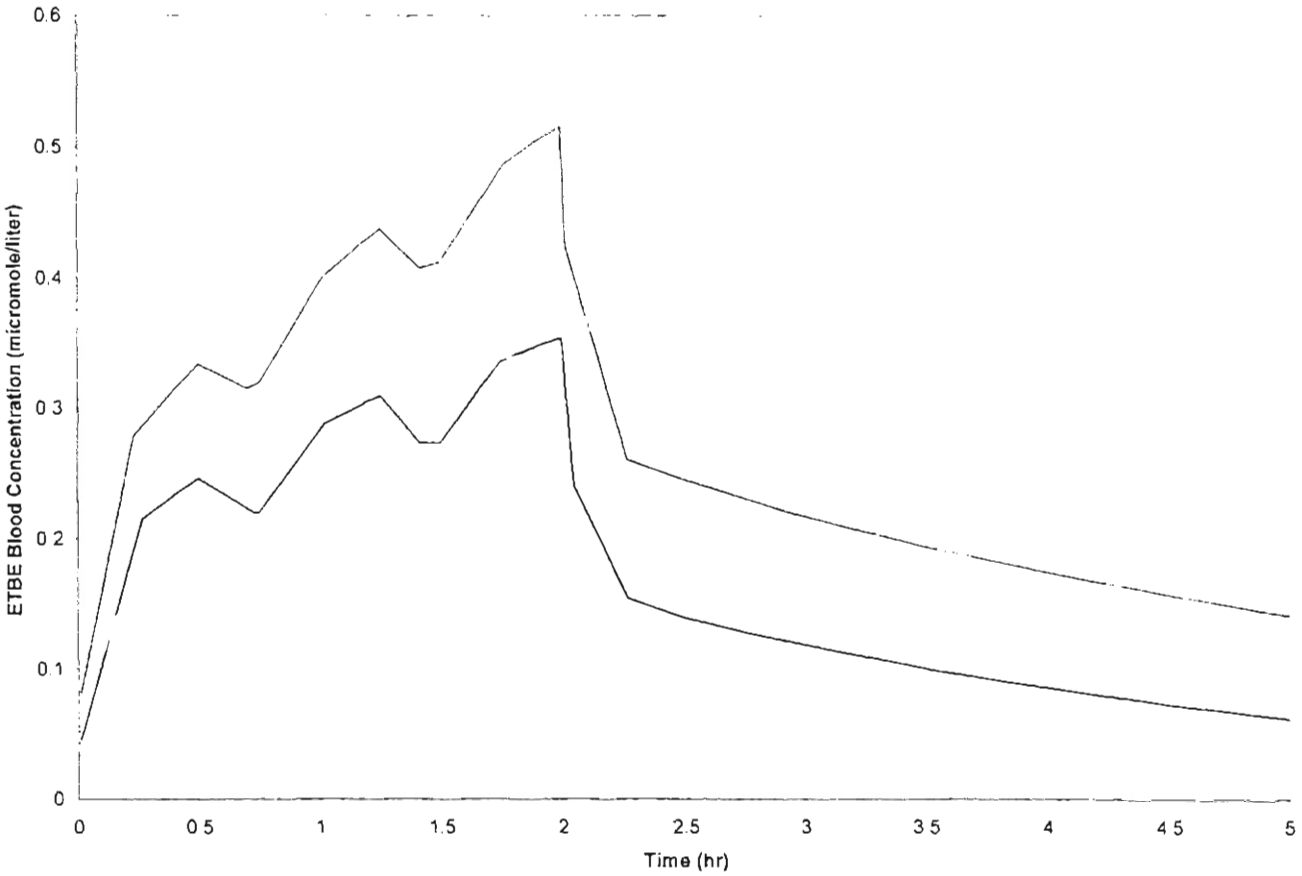
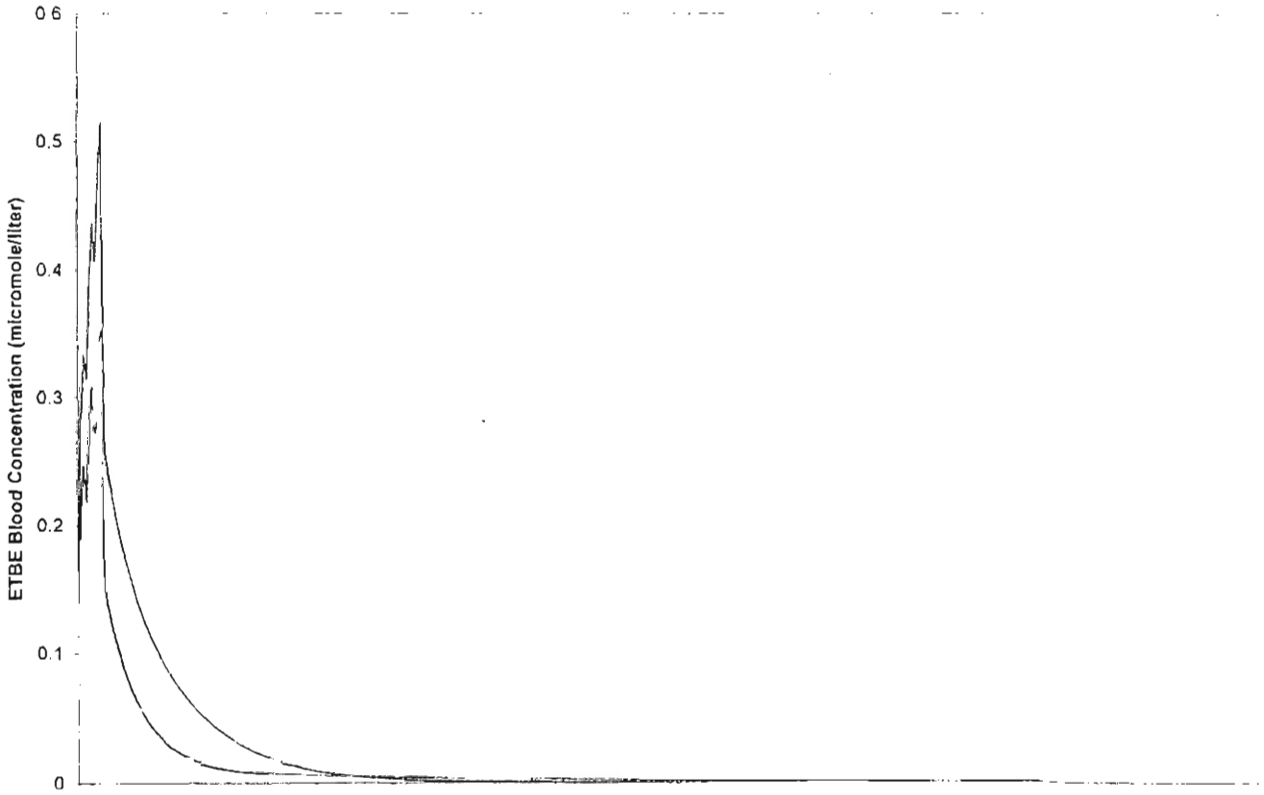


Figure 8a, b: Model-fit multiple subject blood concentrations of ETBE



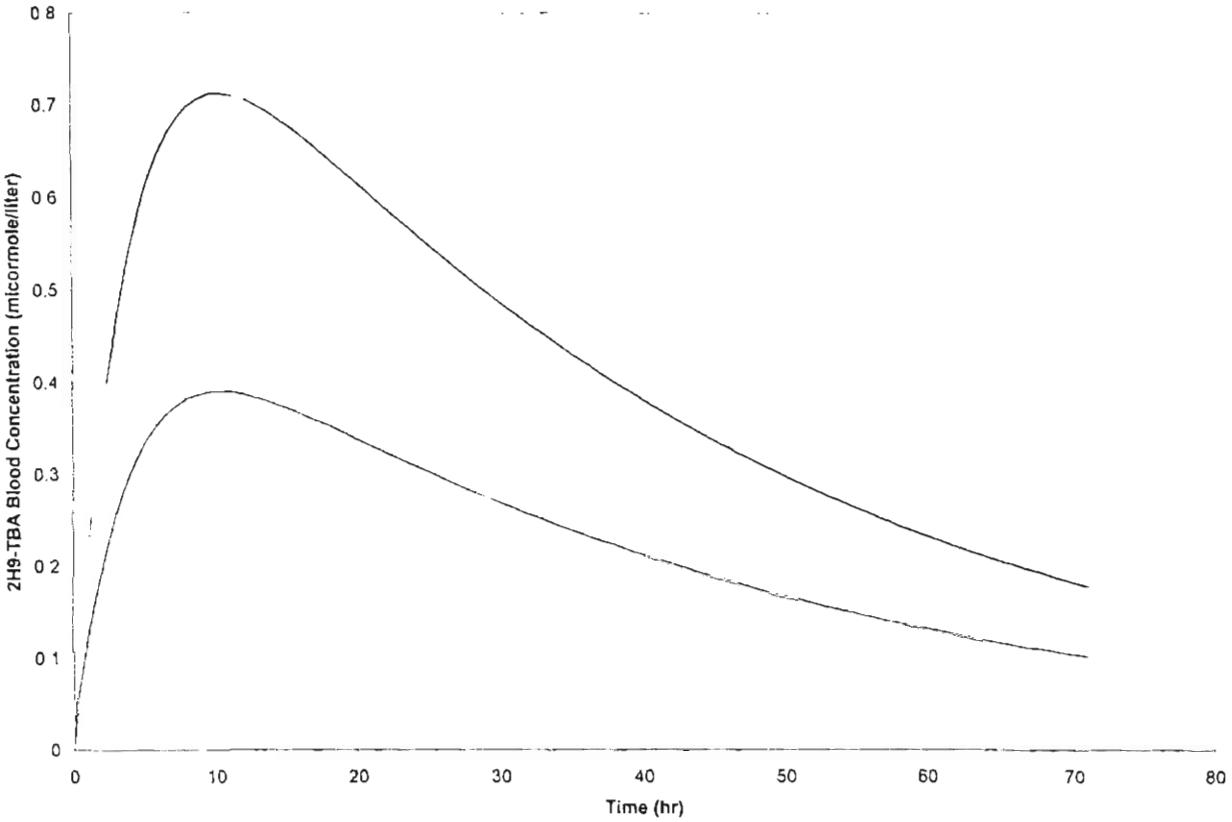


Figure 9: Model-fit multiple subject blood concentrations of $^{2}H_{9}$-TBA

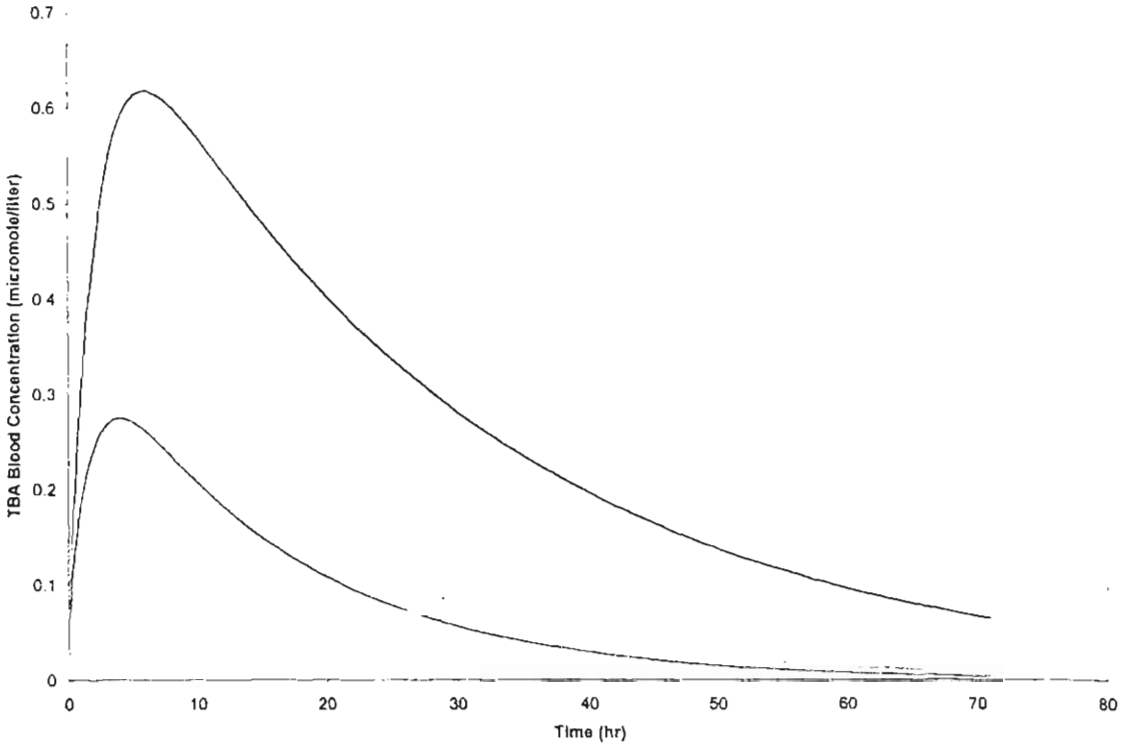


Figure 10: Model-fit multiple subject blood concentrations of TBA

Table 4: Kinetic parameter values for all subjects.

Subject	Analyte	AUC	VD	Dose	CL	t 1/2
1a	mtbe	2.61E+00	460	156	5.99E+01	5.33E+00
	etbe	1.27E+00	2860	156	1.23E+02	1.61E+01
	d9 tba	3.01E+01	22	156	5.18E+00	2.95E+00
	tba	1.95E+01	19	156	7.98E+00	1.65E+00
1b	mtbe	1.68E+01	928.738	2496	1.49E+02	4.33E+00
	etbe	6.79E+00	724.39	624	9.19E+01	5.46E+00
	d9 tba	1.82E+02	28.591	2496	1.37E+01	1.45E+00
	tba	4.32E+01	27.494	624	1.44E+01	1.32E+00
2	mtbe					
	etbe	8.37E-01	2490.103	156	1.86E+02	9.26E+00
	d9 tba	1.56E+01	30.384	156	9.97E+00	2.11E+00
	tba	9.05E+00	32.745	156	1.72E+01	1.32E+00
3	mtbe	7.59E-01	1197.938	54.423	7.17E+01	1.16E+01
	etbe	3.30E-01	3855.266	60.54	1.83E+02	1.46E+01
	d9 tba	8.03E+00	23.181	54.423	6.78E+00	2.37E+00
	tba	3.54E+00	22.99	60.54	1.71E+01	9.31E-01
4	mtbe	1.78E+00	896.386	156	8.78E+01	7.07E+00
	etbe	1.41E+00	2421.599	156	1.11E+02	1.51E+01
	d9 tba	2.67E+01	27.562	156	5.84E+00	3.27E+00
	tba	7.54E+00	27.579	156	2.07E+01	9.24E-01
5	mtbe	5.48E+00	406.841	156	2.85E+01	9.91E+00
	etbe	2.13E+00	316.407	156	7.32E+01	2.99E+00
	d9 tba	2.25E+01	20.459	156	6.92E+00	2.05E+00
	tba	5.50E+00	36.238	156	2.84E+01	8.86E-01
6	mtbe	2.07E+00	1334.535	156	7.52E+01	1.23E+01
	etbe	1.21E+00	2011.217	156	1.29E+02	1.08E+01
	d9 tba	1.67E+01	34.797	156	9.35E+00	2.58E+00
	tba	4.63E+00	43.931	156	3.37E+01	9.03E-01

Table 5: Kinetic parameter summary

	Volume of distribution (l)		Clearance (l/hour)		Half-life (hour)	
	Average	**S.D.**	**Average**	**S.D.**	**Average**	**S.D.**
mtbe	870.74	376.85	78.59	39.76	8.42	3.33
etbe	2097.00	1224.44	128.25	42.98	10.62	5.06
d9 tba	26.71	5.11	8.25	2.97	2.40	0.61
tba	30.00	8.39	19.93	8.66	1.13	0.30

Kinetic Parameter Values

Because clearance, volume of distribution, and half-life are independent of dose, route of exposure, and sampling time (in the terminal phase), these kinetic parameters are valuable in assessing interindividual differences and setting occupational exposure standards. Thus, while the exposure for subject 1b was intentionally higher, and that of subject 3 unintentionally lower, kinetic parameters for these two cases could be compared with the nominal exposures. The kinetic parameter values are presented in Table 4, and a summary is presented in Table 5. While the half-life values for TBA were about 1–2.5 hr when calculated from V and CL, examination of plots reveals a range of 20–40 hr, indicating the value of this analyte as a biological indicator of exposure.

Publications and Presentations

Three manuscripts presenting the results of this research are currently in preparation:

- Controlled $^2H_{12}$-MTBE and ETBE Exposures in Human Subjects (2001) Pierce, Dills, Kalman, and Morgan.
- Modeling Interindividual Differences in $^2H_{12}$-MTBE, ETBE, and TBA Toxicokinetics (2001) Pierce, Hurtle, Dills, Gonzalez, Kalman and Morgan.
- Biological Monitoring of Controlled $^2H_{12}$-MTBE and ETBE Exposures (2001) Pierce, Hurtle, Dills, Kalman, and Morgan.

An abstract for consideration of presentation at the July 2001 NIOSH NORA meeting has been submitted. Additionally, two undergraduates, Amanda Gonzalez and Yi Li Chen, have each spent one quarter of independent study on this work, and one graduate student, Bill Hurtle, is further analyzing the data from this work as a one-third portion of his thesis project.

References

Buckley, T.J., Prah, J.D., Ashley, D., Zweidinger, R.A., and L.A. Wallace (1997) Body burden measurements and models to assess inhalation exposure to methyl tertiary butyl ether (MTBE). *J Air Waste Manag Assoc* **47**: 739-52.

Borghoff, S.J., Murphy, J.E., and M.A. Medinsky (1996) Development of a physiologically based pharmacokinetic model methyl *tertiary*-butyl ether and *tertiary*-butanol in male Fischer-344 rats. *Fundamen Appl Toxicol* **30**: 264–275.

Borghoff, S.J. (2000) Summary and update of animal data used for PBPK modeling. EPA MTBE PBPK Modeling Workshop, May 24–25, 2000, Research Triangle Park, NC.

Hong, J.Y., Chung, C.S., Lee, M., Wang, Y.Y., Huang, W., Tan, Y., Pattern, C.J., and F.Y. Bondoc (1997) Role of cytochromes P450 in the metabolism of methyl *tert*-butyl ether in human livers. *Arch Toxicol* **71**: 266–269.

Hong, J.Y. (2000) Role of cytochrome P450 in the metabolism of methyl *tert*-butyl ether and other gasoline ethers. EPA MTBE PBPK Modeling Workshop, May 24–25, 2000, Research Triangle Park, NC.

Leavens, T.L. (2000) Physiologically based pharmacokinetic model for disposition of mehtyl *tertiary*-butyl and tertiary-butyl alcohol in humans. EPA MTBE PBPK Modeling Workshop, May 24–25, 2000, Research Triangle Park, NC.

Licata, A.C. (2000) A physiologically based pharmacokinetic model for methyl *tert*-butyl ether in humans following inhalation exposure. EPA MTBE PBPK Modeling Workshop, May 24–25, 2000, Research Triangle Park, NC.

Morgan, M.S., Dills, R.L., and Kalman, D.A. (1993). Evaluation of stable isotope-labeled probes in the study of solvent pharmacokinetics in human subjects. Int. Arch. Occup. Environ. Health 65, S139–S142.

Nihlen-A., Lof-A., and G. Johanson (1995) Liquid/air partition coefficients of methyl and ethyl T-butyl ethers, T-amyl methyl ether, and T-butyl alcohol. *J Expo Anal Environ Epidemiol.* **5**: 573-82.

Nihlen, Annsofi (1998) Ethers as gasoline additives---Toxicokinetics and acute effects in humans. Ph.D. dissertation, Institute of Environmental Medicine, Karolinska Institute, Solna, Sweden.

Norris, J. F., and Rigby, G. W. (1932). The reactivity of atoms and groups in organic compounds. XII. The preparation and properties of mixed aliphatic ethers with special reference to those containing the tert.-butyl radical. *J Am Chem Soc* **54**: 2088–2100.

Pierce, C. H., Dills, R. L., Silvey, G. W., and D. A. Kalman (1996a) Partition coefficients between human blood or aidpose tissue and air for aromatic solvents; *Scand J Work Environ Health* **22**:112–118 .

Pierce, C. H., Dills, R.L., Morgan, M.S., Nothstein, G.L., Shen, D.D., and D. A. Kalman (1996b) Interindividual differences in $^{2}H_{8}$-toluene toxicokinetics assessed by a semi-empiric physiologically-based model.*Toxicol Appl Pharmacol* **139**:49–61.

Pierce, C. H., Dills, R.L., Morgan, M.S., Vicini, P., and D. A. Kalman (1998) Biological monitoring of controlled toluene exposure. *Int Arch Occup Environ Health* **71**:433–444.

Rowland, M., and T.N. Tozer (1989). *Clinical Pharmacokinetics: Concepts and Applications*, 2nd ed. Lea and Febiger, Philadelphia, PA.

Tardif, R., Laparé, S., Krishnan, K., and J. Brodeur (1993). Physiologically based modeling of the toxicokinetic interaction between toluene and m-xylene in the rat. *Toxicol. Appl. Pharmacol.* **120:** 266–273.

Vicini, P, Pierce, C. H., Dills, R.L., Morgan, M.S., and D. A. Kalman (1999). Individual prior information in a physiological model of $^{2}H_{8}$-toluene kinetics: an empirical Bayes estimation strategy. *Risk Analysis* **19:**1127–1134.