Journal of Toxicology and Environmental Health, Part A

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: https://www.tandfonline.com/loi/uteh20

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To cite this article: Crispin H. Pierce , Yili Chen , William R. Hurtle & Michael S. Morgan (2004) Exponential Modeling, Washout Curve Reconstruction, and Estimation of Half-Life of Toluene and its Metabolites, Journal of Toxicology and Environmental Health, Part A, 67:14, 1131-1158, DOI: 10.1080/15287390490452344

To link to this article: https://doi.org/10.1080/15287390490452344

Journal of Toxicology and Environmental Health, Part A, 67:1131–1158, 2004

ISSN: 1528–7394 print / 1087–2620 online
DOI: 10.1080/15287390490452344

EXPONENTIAL MODELING, WASHOUT CURVE RECONSTRUCTION, AND ESTIMATION OF HALF-LIFE OF TOLUENE AND ITS METABOLITES

Crispin H. Pierce, Yili Chen, William R. Hurtle, Michael S. Morgan

Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, USA

Health risks from ostensible occupational and environmental toxicant exposure are difficult to quantify. Maximal use of limited biological measurements of xenobiotic or metabolite concentration in the body is therefore essential. Elimination rates of exhaled [$^2H_8$]toluene and urinary metabolites were analyzed from 33 exposures of males to 50 ppm [$^2H_8$]toluene for 2 h at rest. It was hypothesized that the shapes from our decay curves would be applicable to any occupational or environmental toluene exposure. Except for a rapid decline in toluene blood and breath levels in the 0–0.1 h period, this "curve reconstruction" method successfully fit data from published studies. Urinary hippuric acid concentrations were not well fit due to substantial background levels, whereas o-cresol levels were accurately described. Our approach was able to reconstruct data from studies where exposure duration ranged from 10 min to 7 h, and where activity level ranged from rest to 150 W (strenuous exercise). Using this approach, limited biological data following toluene exposure could be back-extrapolated to immediate postexposure concentrations, which in turn could be compared to biological indicators of exposure to determine risk.

Whether in the context of a "cancer cluster" investigation, the forensic analysis of crime, or the setting of occupational exposure standards, data on which to determine internal dose of a toxicant are generally limited. Fortunately, the availability of these data is expanding through advances in analytical detection methods, which provide more sensitive means to quantify intentional and unintentional exposure to xenobiotics. Measurement of parent substances or metabolites in biological samples provides information about absorbed dose, distribution, metabolism, and excretion, collectively called toxicokinetics. An understanding of toxicokinetics maximizes the exposure–internal dose and dose-response information obtained from the often sparse biological samples following a known or possible exposure.

Biological indicators of exposure, such as exhaled toluene in breath and excreted *o*-cresol in urine, provide better estimates of absorbed dose than do

Received 6 June 2003; accepted 6 February 2004.

We thank Russell Dills and David Kalman for laboratory support during the original exposures, and Danny Shen and Robert Hink for valuable discussion. This work was supported by the Superfund Basic Research program, NIEHS ES 04696, and by NIOSH SERCA grant 5 K01 OH00164.

Address correspondence to Crispin H. Pierce, Department of Allied Health Professions, UW-Eau Claire, 105 Garfield Avenue, PO Box 4004, Eau Claire, WI 54702-4004, USA. E-mail: piercech@uwec.edu

ambient exposure concentrations. The timing of sample collection, however, may be critical to estimating dose. Moreover, there is often substantial disagreement regarding the rate of chemical "washout" (decay) from the body, as evidenced by disparate half-lives for the same chemical (Pierce et al., 1998).

The toxicokinetics of toluene and its metabolites were investigated to understand how limited biological sampling could be used to reconstruct profiles of declining analytes in biological media. Certain toxicokinetic parameters, such as elimination half-lives, are generally constant across non-saturating doses in an individual; these parameters are useful in choosing sampling times, and in estimating toxicant body burden and its rate of decline. Elimination rates of exhaled [$^2H_8$]toluene and urinary metabolites from 33 exposures of males to 50 ppm of [$^2H_8$]toluene for 2 h at rest were analyzed. Mathematical techniques were developed to apply the decay curves and half-lives of exhaled toluene and excreted metabolites from this rich data set to other limited sampling data, in order to reconstruct corresponding washout curves. It was hypothesized that the shapes from our washout curves would be applicable to any occupational or environmental toluene exposure. In this way, limited biological data following toluene exposure could be back-extrapolated to immediate post-exposure concentrations, which in turn could be compared to biological indicators of exposure to determine risk.

METHODS

This work entails the analysis of our previously published toluene exhalation rates and metabolite excretion rates (Pierce et al., 1998), development of kinetic approaches to describe the data, and testing of these approaches on data sets published by others.

Exposures, Sample Collection, and Analyses

As previously described (Pierce et al., 1996), 25 nonsmoking male Caucasian subjects aged 20–62 yr without occupational solvent exposure were individually exposed to 50 ppm [$^2H_8$]toluene and 50 ppm [$^1H_8$]toluene simultaneously for 2 h at rest through a gated mouthpiece (50 ppm toluene = 2.04 mmol/m^3 = 188 mg/m^3). Five of these subjects participated in 2 or 3 replicate exposures, for a total of 33 experiments. This study was approved by the University of Washington Human Subjects Division, and all subjects provided informed written consent.

Breath samples were collected prior to exposure, at multiple times during the d1 postexposure, and twice per day for the subsequent 3 d. The contents of breath sample bags (10 and 20 L) were drawn through two-section (200 mg/100 mg) charcoal sorbent tubes using a calibrated personal sampling pump. The charcoal sections were then analyzed (Eller, 1984), through solvent extraction and gas chromatography/mass spectrometry (GC/MS) detection. Rates of exhalation were calculated as alveolar breath [$^2H_8$]toluene concentration multiplied by the subject-specific pulmonary ventilation rates.

Urine samples were collected prior to exposure, immediately following exposure, for the 24 h following exposure, and twice per day for the subsequent 3 d, at times comfortable for the subjects. To assess metabolite production rates, the elapsed time between urinations and the urine volume for each sample were recorded. Samples were analyzed for native and deuterated hippuric acid and *o*-, *m*-, and *p*-cresols (Figure 1) as previously described (Dills & Kalman, 1997; Pierce et al., 1999), through gas chromatography with electron capture (cresols) and mass spectrometer (hippuric acid) detection. Most urinary sample collection continued for 80 h, while several exposures included collection for 170 h. Some metabolite levels, particularly at later collection times, were not quantifiable and thus not included in our analysis. Because we observed substantial urinary background contributions of all metabolites (Pierce et al., 1998), only the deuterated metabolites were examined for this report. Coexposure to 50 ppm native toluene is unlikely to have influenced the toxicokinetics of the 50 ppm deuterated toluene exposure, given expected metabolic saturation at levels of 200 ppm and higher (Tardif et al., 1996). Metabolite excretion rates were plotted versus time at the midpoints of the collection intervals. Isotope effects on toluene metabolism were previously found, but these effects produced differences that were smaller than interindividual differences (Pierce et al., 1999).

Exponential Modeling

SAAM II software in the numerical mode (SAAM Institute, Seattle, WA) was used to describe toluene exhalation and metabolite excretion data. The

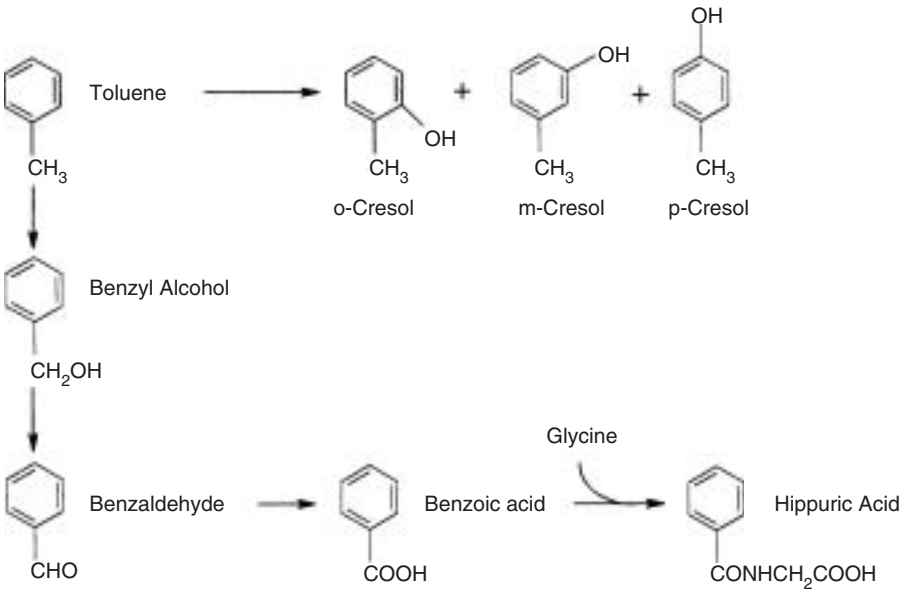

FIGURE 1. Pathways of toluene metabolism.

"model, relative" variance optimization setting was used to fit the model to each data set (toluene; hippuric acid; *o*-, *m*-, and *p*-cresol) en masse. The variance model was given by (SAAM Institute, 1998)

$$V_{i,j}(s(\hat{p},t_{i,j}),y_{i,j},\hat{v}_j)=\hat{v}_j\bullet(A+B\bullet[s(\hat{p},t_{i,j})]^C) \quad (1)$$

The model was fit to the data by minimizing the objective function

$$R(p)=\frac{1}{M}\sum_{j=1}^{J}\sum_{i=1}^{N_j}\left(\log[V_{i,j}(s(\hat{p},t_{i,j}),y_{i,j},\hat{v}_j)]+\frac{[y_{i,j}-s(\hat{p},t_{i,j})]^2}{V_{i,j}(s(\hat{p},t_{i,j}),y_{i,j},\hat{v}_j)}\right) \quad (2)$$

and the variance parameter was estimated to be

$$\hat{v}_j=\frac{1}{N_j}\sum_{i=1}^{N_j}\frac{[y_{i,j}-s(\hat{p},t_{i,j})]^2}{V_{i,j}(s(\hat{p},t_{i,j}),y_{i,j},1)} \quad (3)$$

where p is the vector of adjustable parameters, $R(p)$ the objective function, $y_{i,j}$ the ith datum in the jth data set, $s(p, t_{i,j})$ the model value corresponding to $y_{i,j}$ at time $t_{i,j}$, $V_{i,j}(s(p, t_{i,j}), y_{i,j} n_j)$ the variance model for $y_{i,j}$, J the number of data sets, N_j the number of data points in the jth data set, M the total number of data points ($M=N_1 + N_2 +\ldots+ N_J + N_b$), A, B, and C the weighting parameters associated with $y_{i,j}$, and the caret over a symbol denotes an estimated value.

Because most xenobiotics distribute to varying degrees into tissues outside of blood, the rate of decline in blood concentrations of a particular analyte after exposure can be described by a multiexponential equation. The number of terms in this equation generally reflects the number of major tissue groups (including blood) into which the toxicant or its metabolite distributes. The rate at which a toxicant or its metabolite is excreted from the body, such as rate of appearance in urine or exhaled air, reflects blood concentration and so can similarly be described by a multiexponential equation.

The number of terms in the exponential equations was based on a visual examination of the fit of one- to four-term exponential decay models to the toluene and metabolite data, and on models chosen by previous authors (Droz & Guillemin, 1986; Sato et al., 1974; Hjelm et al., 1994; Ernstgård et al., 1999). Two-term equations were chosen for the metabolites and a three-term equation was chosen for toluene:

$$\text{Concentration or rate of elimination}=A_1e^{-a_1t}+A_2e^{-a_2t} \quad (4)$$

$$\text{Concentration or rate of elimination}=A_1e^{-a_1t}+A_2e^{-a_2t}+A_3e^{-a_3t} \quad (5)$$

The coefficients A_1, A_2, and A_3 are dose dependent, and the rate constants a_1, a_2, and a_3 are related to the half-life of each decay phase by $a=\ln 2/t_{1/2}$. Model fits were considered successful when the curve of simulated values visually appeared to match measured values without bias, and when the model

converged on a solution. Convergence was reached when the change in the objective function value or the change in the parameter values from the previous iteration was less than the specified convergence criteria.

Washout Curve Reconstruction

Because the A_1, A_2, and A_3 coefficients vary directly with dose, varied toluene exposures were expected to yield similar values of $A_1/A_2 = x$ and $A_2/A_3 = y$. Through substitution, the equation for blood or breath toluene concentration became

$$C = A_2 x e^{-a_1 t} + A_2 e^{-a_2 t} + \frac{A_2}{y} e^{-a_3 t} \tag{6}$$

Upon rearrangement,

$$A_2 = \frac{C}{x e^{-a_1 t} + e^{-a_2 t} + \frac{1}{y} e^{-a_3 t}} \tag{7}$$

Therefore, knowledge of a_1, a_2, a_3, x, and y, which were expected to be constant with dose, would allow the estimation of A_2, and thus the entire equation for washout, given a single toluene concentration–time point.

It should be noted that A_1, A_2, A_3, and thus x and y, are mathematically dependent on duration of exposure:

$$An = \frac{K_0}{CL} Fn(1 - e^{-anT})$$

where:

K_0 rate of infusion (or inhalation exposure)
CL clearance (CL = Dose/AUC)
Fn Phase fraction (Fn = (An/an)/AUC)
T Time of infusion (or inhalation exposure)

We examined the fit of our approach to previous authors' data to see if duration of exposure was a factor in visual goodness of fit.

For substances exhibiting two-phase decay (hippuric acid and the cresols), the expression simplified to

$$C = A_2 x e^{-a_1 t} + A_2 e^{-a_2 t} \tag{8}$$

$$A_2 = \frac{C}{x e^{-a_1 t} + e^{-a_2 t}} \tag{9}$$

The published literature was then searched using Medline for reports of controlled toluene exposure with serial collection of toluene in blood and breath, and metabolites in urine. Plots from publications were then computer scanned and data points were determined using DataThief (Huyser & van der

Laan, National Institute for Nuclear Physics, Amsterdam, the Netherlands). To avoid any potential bias from choosing specific data points, each data point (C,t) from each study was then inserted into Eq. 7 (toluene) or Eq. 9 (metabolites) to determine A_2, and to subsequently define the entire washout curve equation [Eq. (6) or (8)]. The curves thus generated by all data points within a study were then examined, and the two worst fits (highest and lowest) as well as the curve obtained by averaging all fits were plotted.

Half-Life Estimation

The half-life at any time describes the rate of decline of the blood concentration as follows:

$$t_{1/2} = \frac{-\ln 2 \times \text{Concentration}}{\text{Rate of concentration change}} = \frac{-\ln 2C}{dC/dt} \tag{10}$$

Substitution of the expression for concentration [Eq. (5)] and rearrangement yielded the following expression, which was used to estimate the changing toluene half-life over time:

$$t_{1/2} = \frac{-\ln 2C}{dC/dt} = \frac{-\ln 2\left(A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t}\right)}{d\left(A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t}\right)/dt} = \frac{-\ln 2\left(x e^{-a_1 t} + e^{-a_2 t} + \frac{1}{y} e^{-a_3 t}\right)}{a_1 x e^{-a_1 t} + a_2 x e^{-a_2 t} + a_3 \frac{1}{y} e^{-a_3 t}} \tag{11}$$

For substances exhibiting two-phase decay (hippuric acid and the cresols), the half-life equation became

$$t_{1/2} = \frac{\ln 2(x e^{-a_1 t} + e_2^{-a_2 t})}{a_1 x e^{-a_1 t} + a_2 e^{-a_2 t}} \tag{12}$$

RESULTS

Exponential Model Fit to Our Data

All model fits converged and provided a good visual fit to the data. However, censorship of four data points as outliers was necessary to obtain a positive a_2 value for *o*-cresol. Three phases of decline were observed for exhaled toluene (Figure 2), and this decline was fit with three terms [Eq. (5)]:

$$\text{Rate of exhalation} = 41.7e^{-1.71t} + 7.31e^{-0.177t} + 0.865e^{-0.0178t}$$

Urinary metabolite excretion rates were described with two terms [Eq. (4)]: Hippuric acid (Figure 3):

$$\text{Rate of excretion} = 119e^{-0.366t} + 6.2e^{-0.0241t}$$

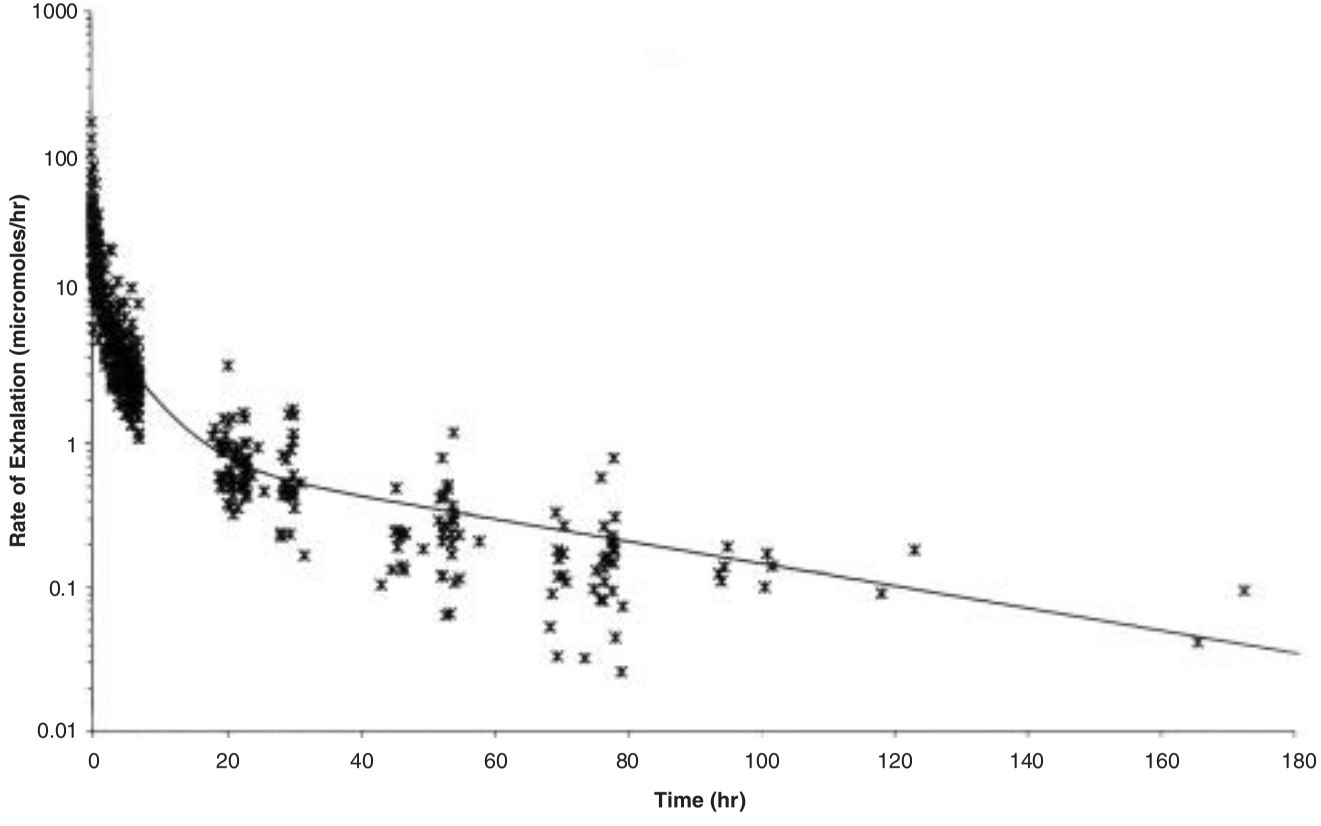

FIGURE 2. Measured (∗) rates of $^{2}H_{8}$-toluene exhalation from 33 exposures to 50 ppm for 2 h, and modeled (—) rates from the equation, Rate = 41.7 [exp(−1.71t)] + 7.31[exp(−0.177t)] + 0.865[exp(−0.0178t)].

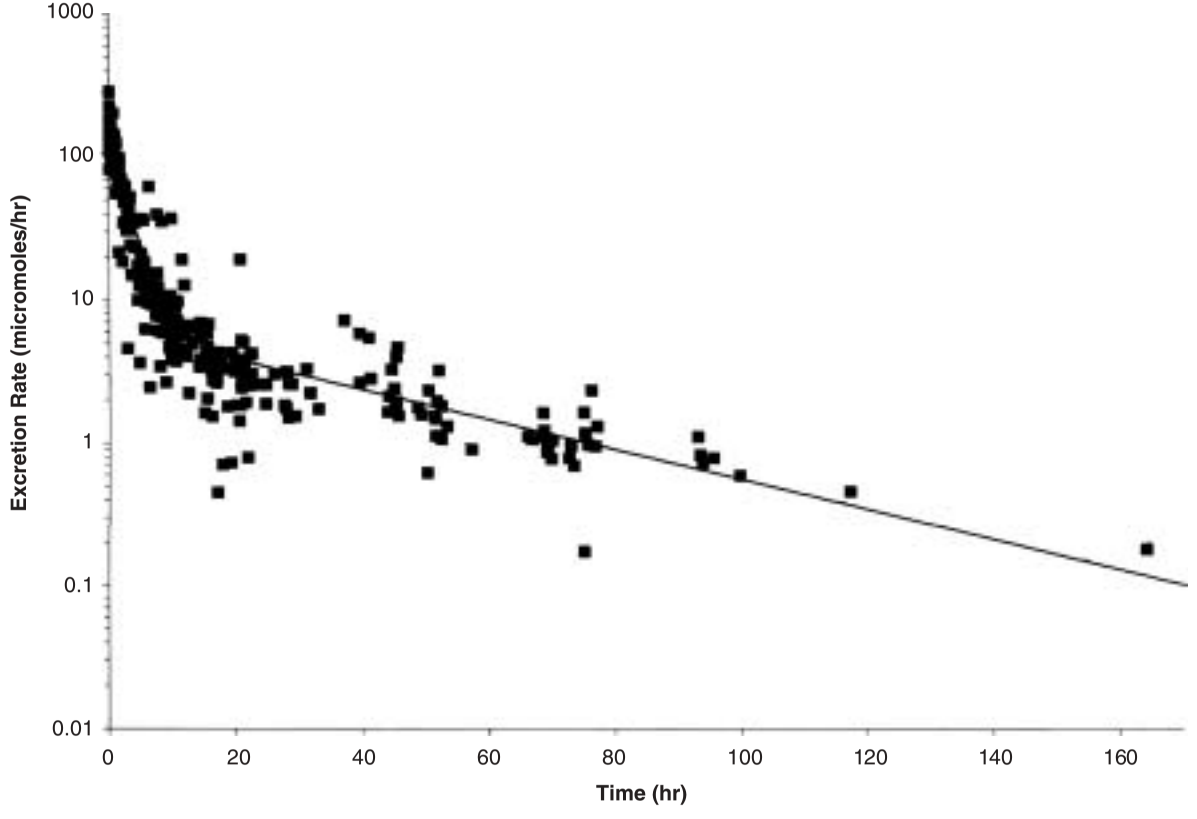

FIGURE 3. Measured (■) rates of [$^2H_5$]hippuric acid excretion from 33 exposures to 50 ppm [$^2H_8$]toluene for 2 h, and modeled (—) rates from the equation, Rate = 119[exp(–0.366t)] + 6.2[exp(–0.0241t)].

o-Cresol (Figure 4):

$$\text{Rate of excretion} = 0.296e^{-0.205t} + 0.0213e^{-0.0309t}$$

m-Cresol (Figure 5):

$$\text{Rate of excretion} = 0.332e^{-0.164t} + 0.0364e^{-0.0114t}$$

p-Cresol (Figure 6):

$$\text{Rate of excretion} = 7.36e^{-0.163t} + 1.41e^{-0.0291t}$$

The exponential parameter values (a_1, a_2, and a_3) were generally 10-fold different within each analyte (Table 1). The value of a_2 for toluene and the values of a_1 for the metabolites were within a 2.2-fold range, with overlap of the 95% confidence intervals for *o*-, *m*-, and *p*-cresol but not hippuric acid. The final exponential parameters for each analyte (a_3 for toluene and a_2 for the metabolites) were within a 2.7-fold range, with overlap of the 95% confidence intervals for hippuric acid, *o*- and *m*-cresol, but not *p*-cresol. The closeness of the second and third toluene values (a_2 and a_3) to the two metabolite values (a_1 and a_2) suggested that metabolite disposition is limited by the rate of toluene metabolism.

Washout Curve Reconstruction

Toluene Application of Eqs. (6)–(9) to each concentration–time point of published data provided a test of our approach. Kezic et al. (2000) exposed 5 male and female subjects, aged 22–50 yr old, to a toluene concentration "below the present occupational exposure limit in the Netherlands" for a 10-min period, and collected 11 alveolar breath samples over the first 1.4 h postexposure. Curve reconstruction [Eq. (7)] was unsuccessful for the samples collected from 0 to 0.2 h, where the measured levels declined much more rapidly than predicted levels (Figure 7A). In contrast, the concentration decline in the 0.2–1.4 h samples was closely approximated.

Ernstgård et al. (1999) exposed 10 male Caucasian subjects (ages 24–49, weights 68–88 kg) to 50 ppm toluene for 2 h with a 50-W workload. They collected and analyzed 13 blood samples over a 22-h postexposure period. Curve reconstruction was successful in describing the observed blood concentrations of toluene (Figure 7B).

Carlsson (1982) exposed 12 male subjects to a toluene concentration of about 80 ppm during a 2-h period under 3 protocols of rest, 50-W workload, and rest/150-W workload. He collected and analyzed 10 or 11 alveolar breath samples over the first 5 h postexposure. Curve reconstruction was successful for both protocols (Figure 7, C–E), although much like the fitting to the Kezic et al. data, the first several points (0–0.1 h for rest and 0–0.2 h for rest/150 W)

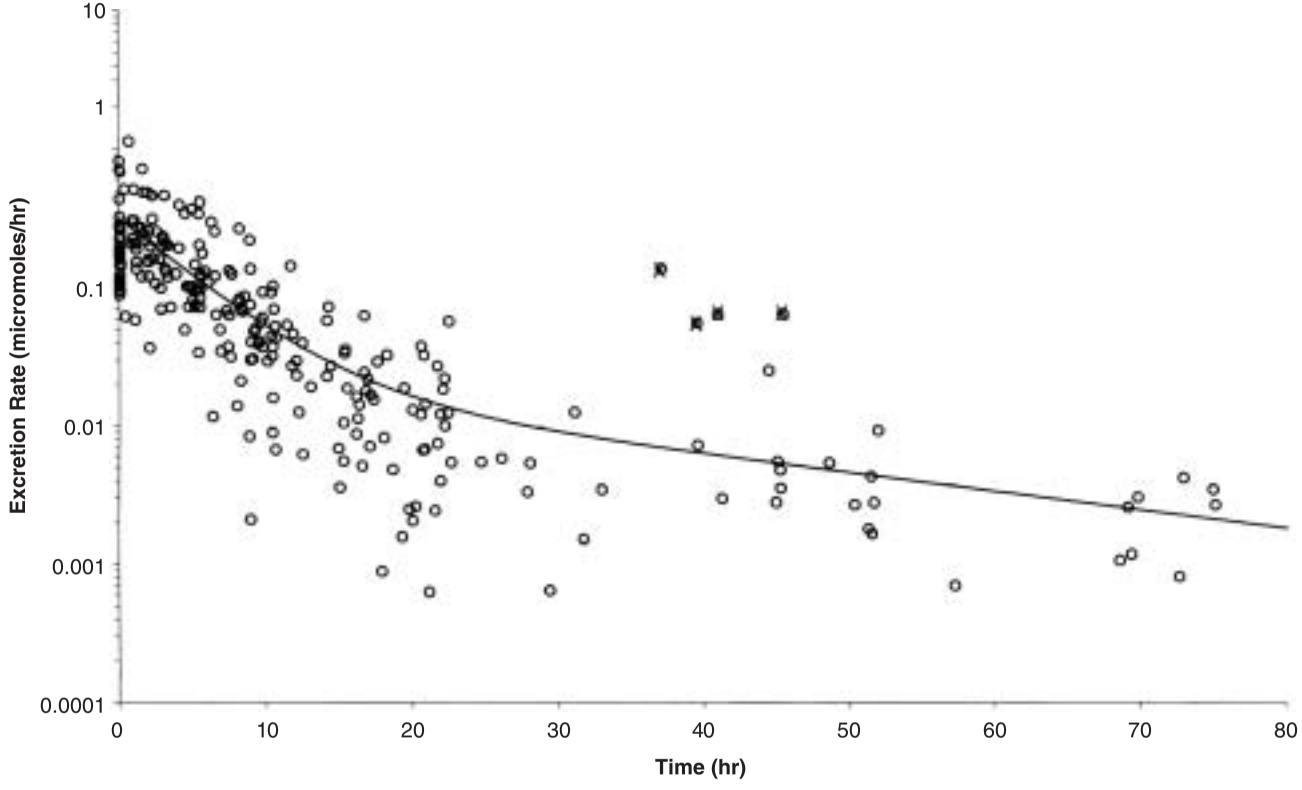

FIGURE 4. Measured (o) rates of [$^2H_7$]-*o*-cresol excretion from 33 exposures to 50 ppm [$^2H_8$]toluene for 2 h, and modeled (—) rates from the equation, Rate = 0.296[exp(–0.205*t*)] + 0.0213[exp(–0.0309*t*)]. The four data points indicated with an "X" were excluded from the fitting process as outliers.

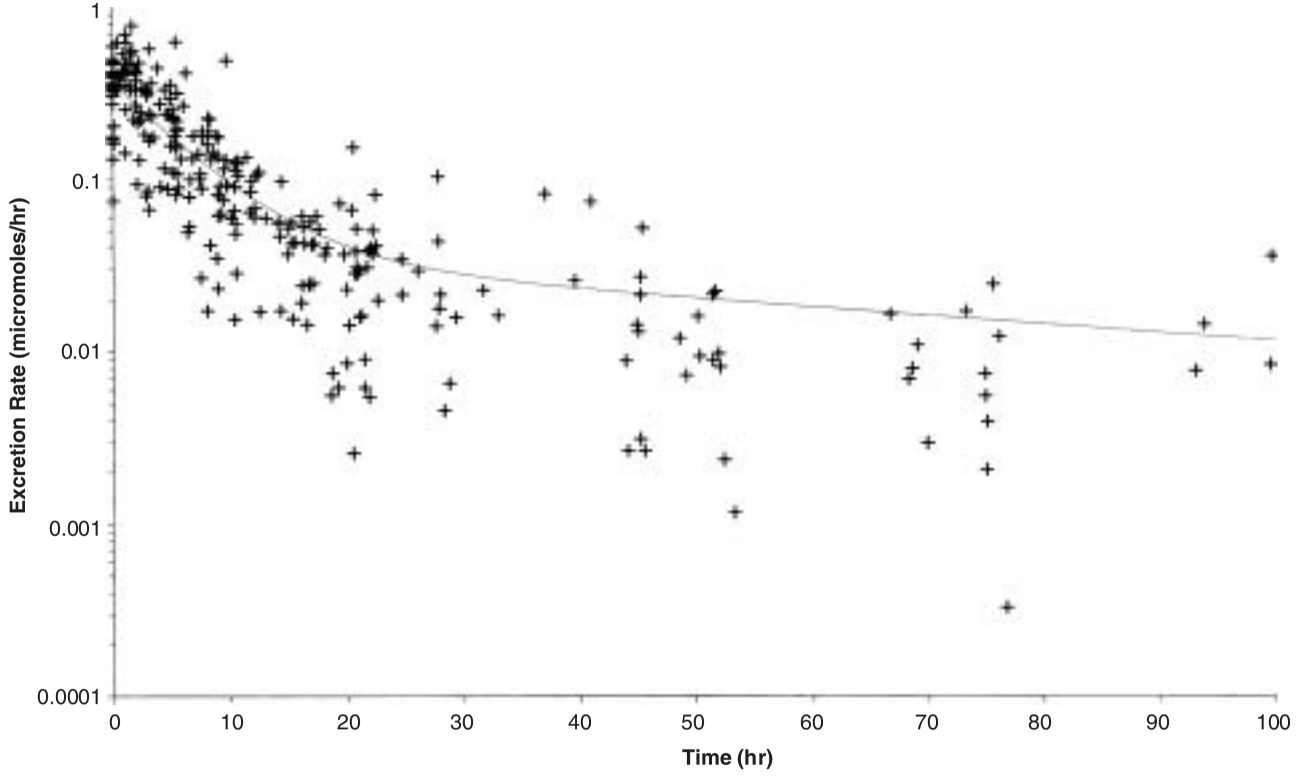

FIGURE 5. Measured (+) rates of [$^2H_7$]-*m*-cresol excretion from 33 exposures to 50 ppm [$^2H_8$]toluene for 2 h, and modeled (—) rates from the equation, Rate = 0.332[exp(−0.164*t*)] + 0.0364[exp(−0.0114*t*)].

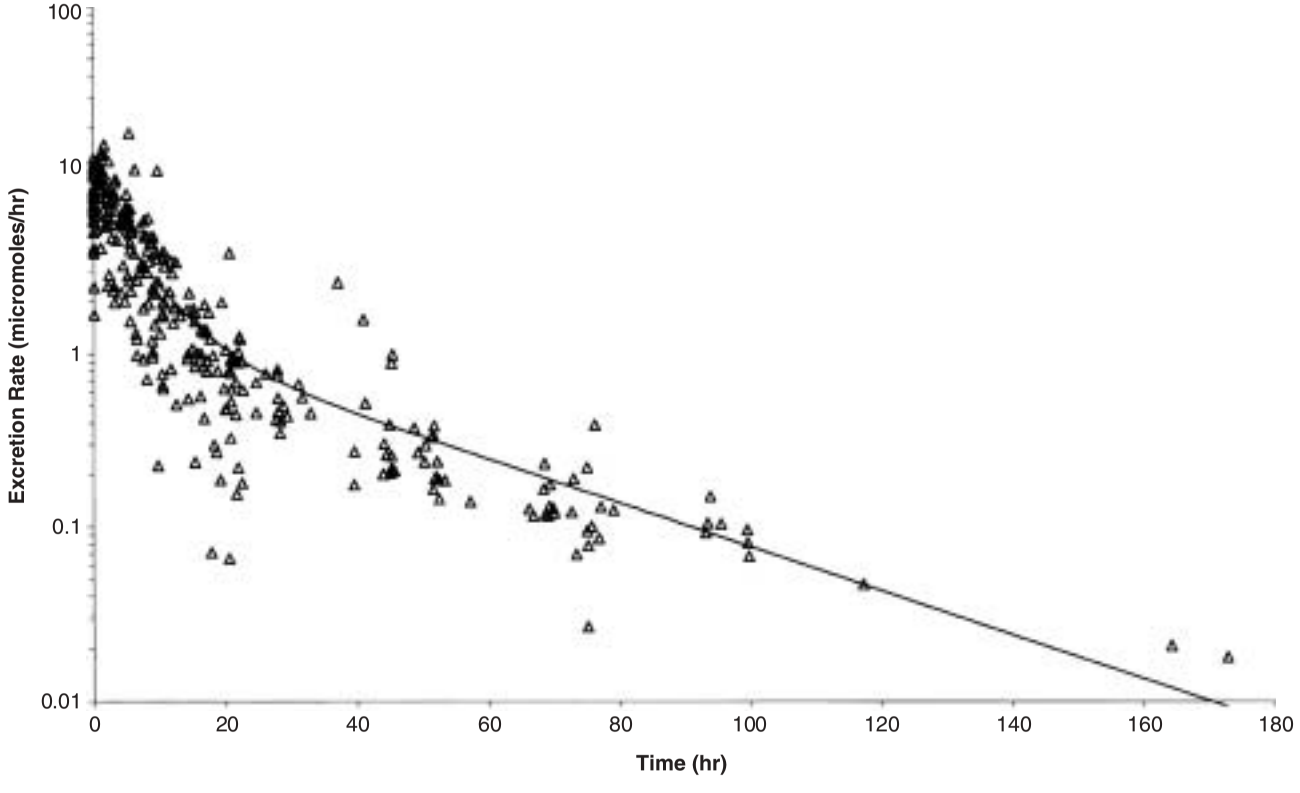

FIGURE 6. Measured (△) rates of [$^2H_7$]-*p*-cresol excretion from 33 exposures to 50 ppm [$^2H_8$]toluene for 2 h, and modeled (—) rates from the equation, Rate = 7.36[exp(−0.163*t*)] + 1.41[exp(−0.0291*t*)].

TABLE 1. Exponential Fit Parameter Values for Toluene and Metabolites

	A_1 (μmol/h)	A_2 (μmol/h)	A_3 (μmol/h)	a_1 (h^{-1})	a_2 (h^{-1})	a_3 (h^{-1})	x	y
Toluene	41.7 (33.7–49.7)	7.31 (5.60–9.61)	0.865 (0.635–1.09)	1.71 (1.33–2.09)	0.177 (0.130–0.225)	0.0178 (0.0140–0.0215)	5.71	8.45
Hippuric acid	119 (102–136)	6.20 (5.20–7.19)	—	0.366 (0.330–0.403)	0.0241 (0.0210–0.0273)	—	19.2	—
o-Cresol	0.296 (0.249–0.343)	0.0213 (0.00936–0.0332)	—	0.205 (0.172–0.239)	0.031 (0.0199–0.0419)	—	13.9	—
m-Cresol	0.332 (0.285–0.379)	0.0364 (0.0218–0.0511)	—	0.164 (0.134–0.194)	0.0114 (0.00451–0.0184)	—	9.13	—
p-Cresol	7.36 (6.28–8.43)	1.41 (1.00–1.82)	—	0.163 (0.133–0.193)	0.0291 (0.0249–0.0332)	—	5.21	—

Note. Values are expressed as mean (95% confidence interval).

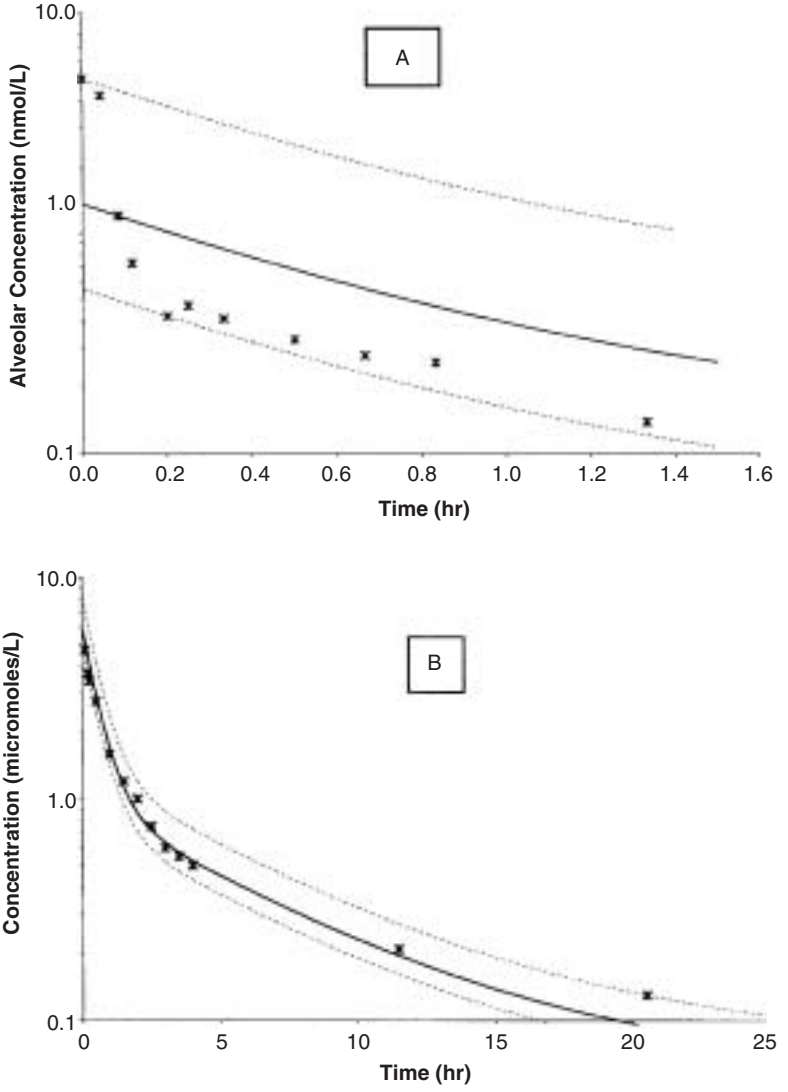

FIGURE 7. Highest and lowest (——), and average (—) fits to published toluene data. (A) Kezic et al. (2000). (B) Ernstgård et al. (1999).

declined more rapidly than predicted. The final 2 points in the 50-W and rest/150-W exposures were also lower than predicted.

Sato et al. (1974) exposed 3 men (average body weight of 60 kg) to 100 ppm toluene for 2 h at rest, and collected samples of cubital venous blood and end-tidal air for 5 h postexposure. Curve reconstruction generally described the measured values of end-tidal air and blood well, with some underestimation of the 0–0.1 h slope (Figure 7, F and G).

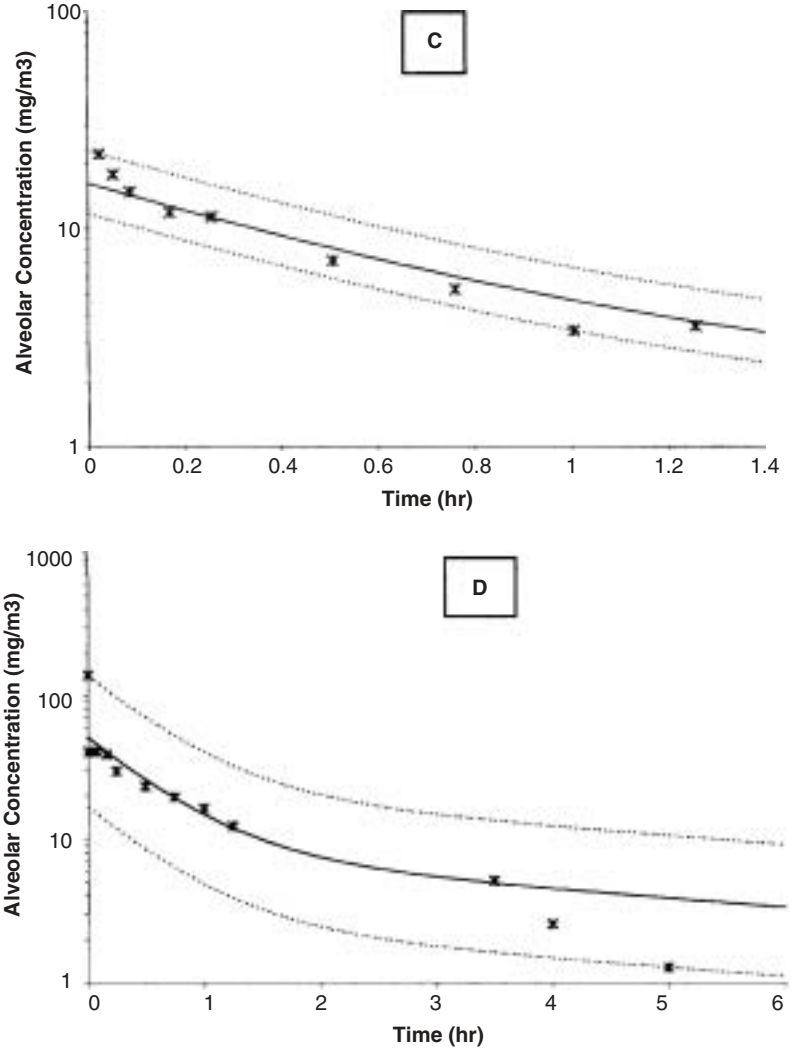

FIGURE 7. (*Continued*) Highest and lowest (——), and average (—) fits to published toluene data. (C) Carlsson (1982) under resting conditions. (D) Carlsson (1982) under 50-W exercise conditions.

Löf et al. (1990) exposed 6 women (ages 26–40, weights 53–73 kg), phenotyped as "slow" or "rapid" debrisoquin hydroxylators, to 80 ppm toluene for 4 h at rest. They found no differences in toluene blood concentration or clearance across debrisoquin phenotype. Curve reconstruction provided a close description of data collected in the 0.1–2 h period, but underestimation of the 0–0.1 h values and overestimation of the 2–3 h values (Figure 7, H and I).

Hjelm et al. (1994) exposed 8 men (average age 29, range 18–42; average weight 75 kg, range 71–90 kg) to 53 ppm [$^2H_8$]toluene for 2 h with mild exercise

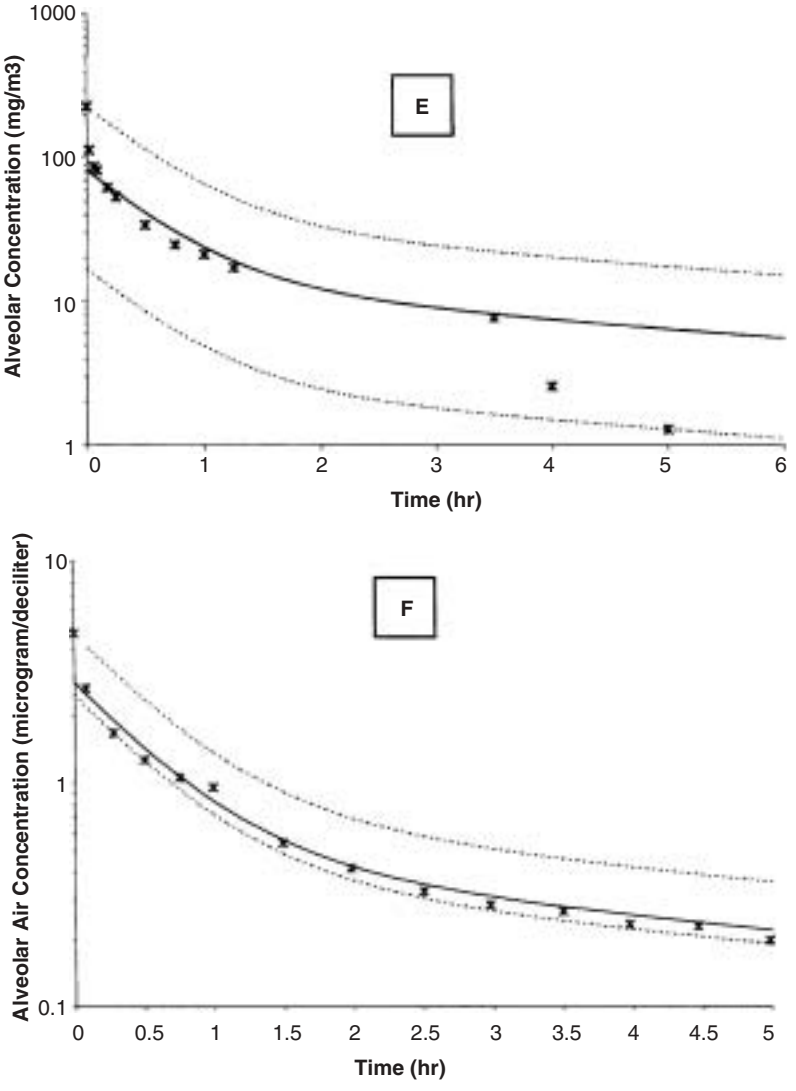

FIGURE 7. (*Continued*) Highest and lowest (——), and average (—) fits to published toluene data. (E) Carlsson (1982) under rest/150-W exercise conditions. (F) Sato et al. (1974) end-tidal (alveolar) air.

(50 W workload). Curve reconstruction provided a close fit to data collected in the 0–5 h period, but underestimation of the three values collected in the 10–25 h period (Figure 7J).

Wallén et al. (1984) exposed 11 men to 78.4 ppm toluene for 4.5 h under resting conditions, and collected finger capillary blood samples for 3 h postexposure. Curve reconstruction closely fit all data points (Figure 7K).

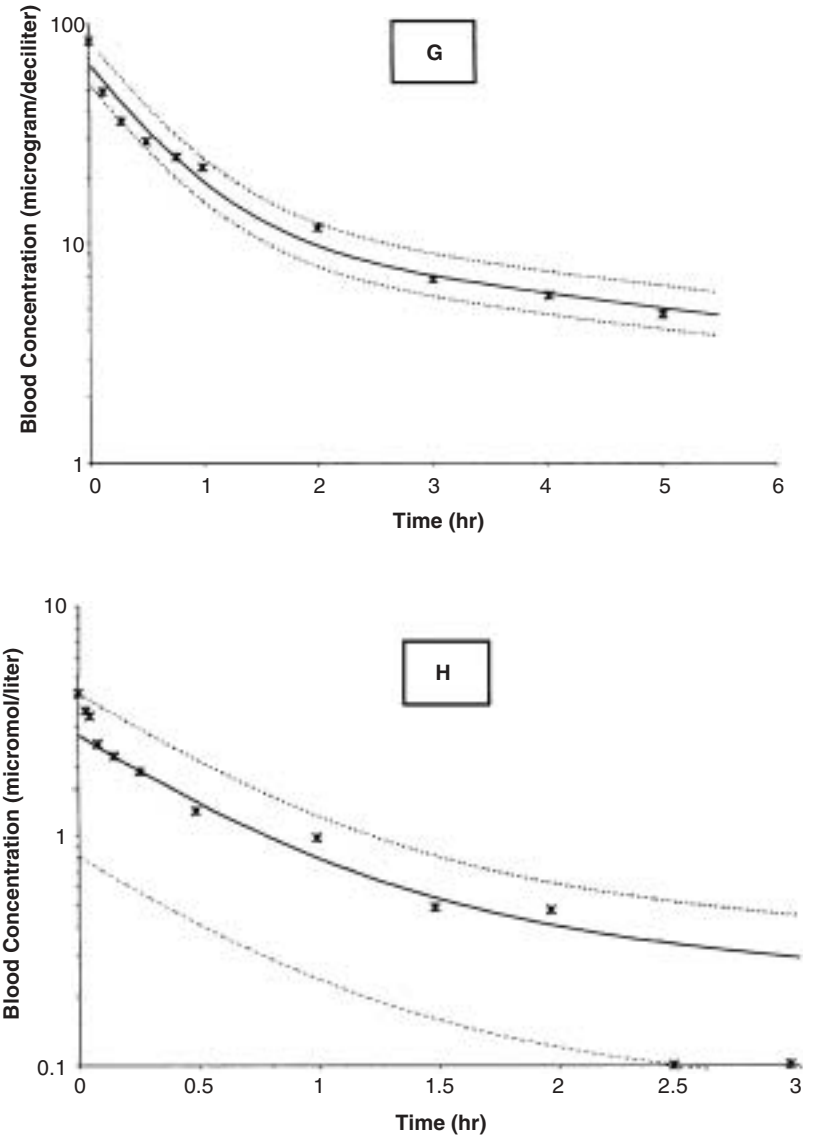

FIGURE 7. (*Continued*) Highest and lowest (——), and average (—) fits to published toluene data. (G) Sato et al. (1974) venous blood. (H) Löf et al. (1990), "slow hydroxylators."

Hippuric Acid Baelum (1991, studies B and D) exposed 4 subjects to toluene for 7 h, 2.3 h of which involved a 100-W workload, with collection of 6 urine samples for 16 h postexposure. Curve reconstruction [Eq. (9)] was able to describe the shape of the three 0–4 h samples, but not the flat slope of the

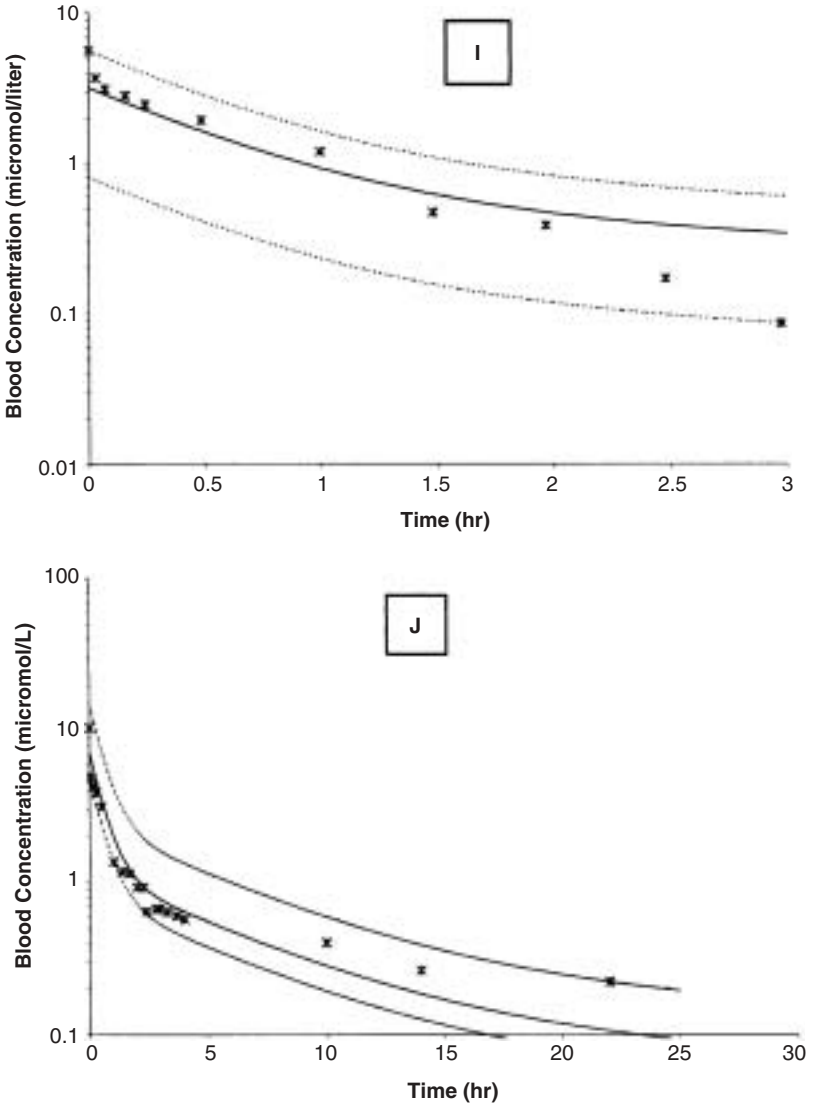

FIGURE 7. (*Continued*) Highest and lowest (——), and average (—) fits to published toluene data. (I) Löf et al. (1990), "rapid hydroxylators." (J) Hjelm et al. (1994).

final three samples (Figure 8A), nor the flat slope of all values in the second study (Figure 8B). Similarly, reconstruction was not able to mimic the relatively flat slope of the 4 hippuric acid excretion rates collected over the 1–16 h postexposure period by Ernstgård et al. (1999) (Figure 8C). In contrast, the deuterated hippuric acid excretion rates measured by Hjelm et al. (1994) were well described (Figure 8D).

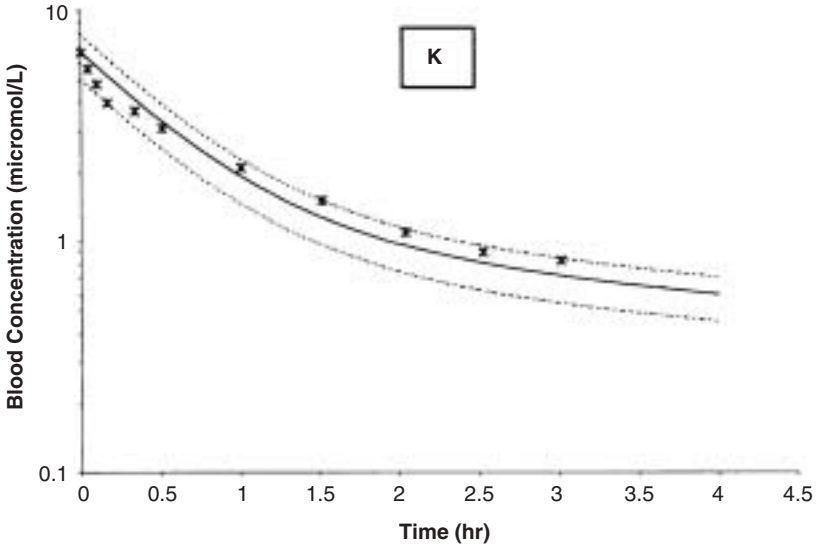

FIGURE 7. (*Continued*) Highest and lowest (——), and average (—) fits to published toluene data. (K) Wallin et al. (1984).

o-*Cresol* Curve reconstruction [Eq. (9)] was able to closely describe the *o*-cresol excretion rates measured in 6 urine samples taken in the 0–16.5 h postexposure period by Baelum (1991) (Figure 9, A and B).

Half-Life Plots

As expected, the plot of changing half-life over postexposure time [Eq. (11)] revealed three relatively constant periods for toluene: 0–2, 4–10, and 50+ h (Figure 10). The changing value of half-life for toluene is reflected in the disparate values reported by different authors (Table 2). The metabolites [Eq. (12)] exhibited two relatively constant periods each: hippuric acid, 0–6, 30+ h; *o*-cresol, 0–10, 50+ h; *m*-cresol, 0–6, 60+ h; and *p*-cresol, 0–6, 50+ h.

DISCUSSION

The principal challenges in ascribing observed human toxicity to a potential occupational or environmental exposure are the measurements of dose and knowledge of the elapsed time between exposure and effect. Toxicokinetic interpretation of biological monitoring data can provide key estimates of these values. For example, previous work has suggested that metabolite ratios in biological fluids can be used to estimate the elapsed time between exposure and measurement (Pierce et al., 2002).

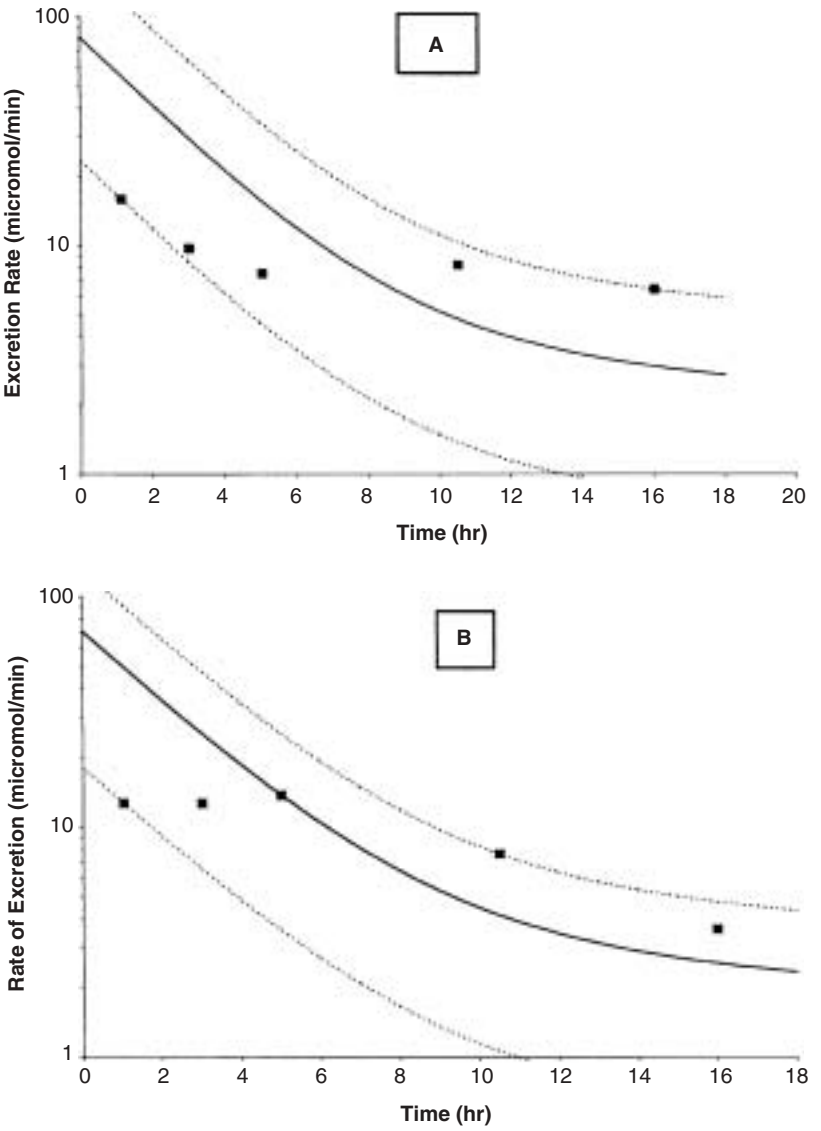

FIGURE 8. Highest and lowest (——), and average (—) fit to published hippuric acid excretion data. (A) Baelum (1991), study B. (B) Baelum (1991), study D.

It was hypothesized that the shapes of toluene, hippuric acid, and cresol washout curves found in our study would be parallel to those of any other nonsaturating toluene exposure. This notion is supported by observations that the primary determinants of toxicant half-lives—tissue blood flows, tissue volumes, tissue–blood partition coefficients, and first-order elimination rate

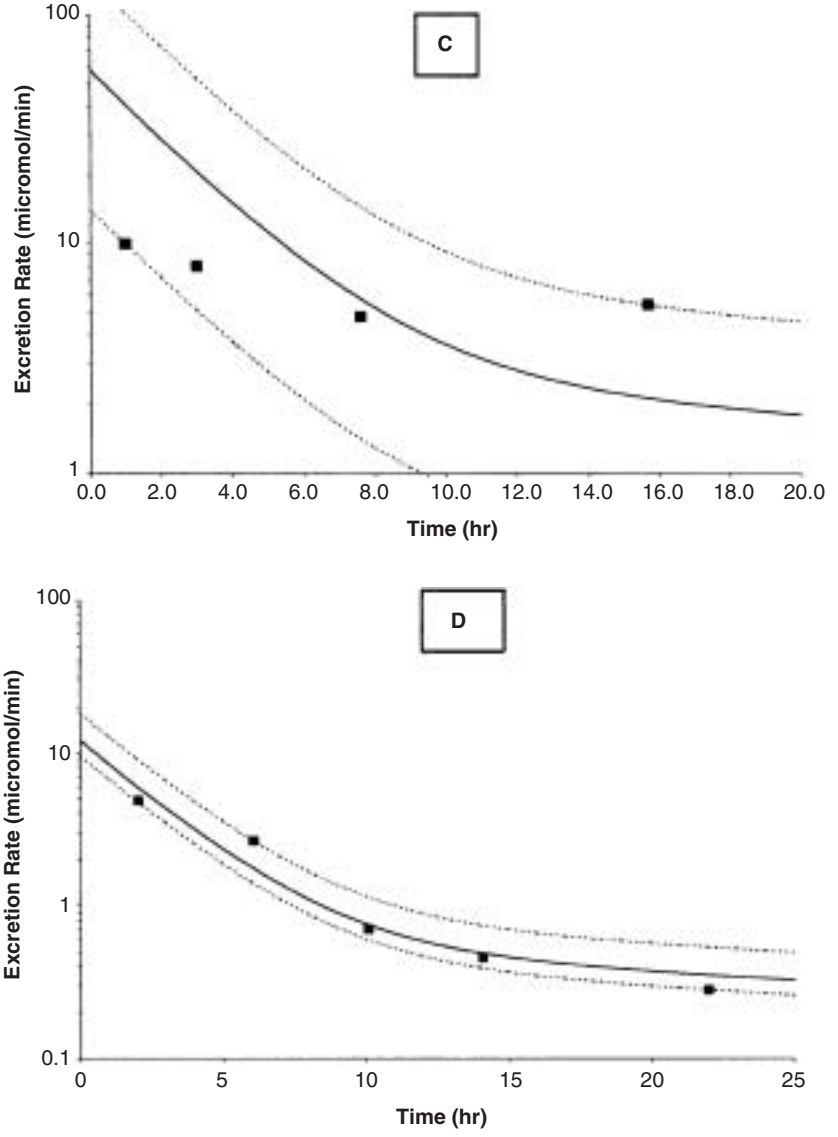

FIGURE 8. (*Continued*) Highest and lowest (——), and average (—) fit to published hippuric acid excretion data. (C) Ernstgård et al. (1999). (D) Hjelm et al. (1994).

constants—are independent of dose. However, the generalized approach this study presents does not account for interindividual characteristics, which may affect washout curve shape. For toluene, these factors include genotype, weight, and adiposity (Wallén, 1986; Sato, 1991; Inoue et al., 1986); alcohol consumption (Wallén et al., 1984); and interaction among toxicants (Inoue

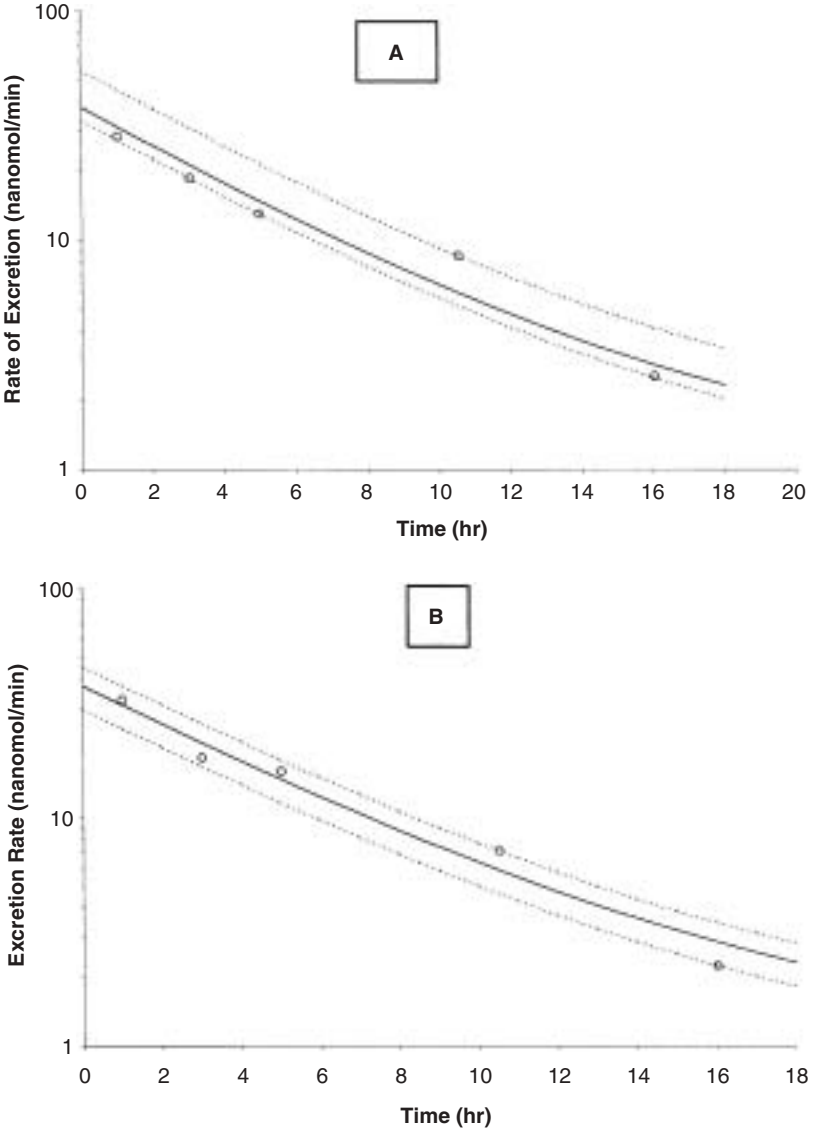

FIGURE 9. Highest and lowest (——) and average (—) fit to published *o*-cresol excretion data. (A) Baelum (1991), study B. (B) Baelum (1991), study D.

et al., 1988). Nonetheless, the 10-fold interindividual differences in our study population (nonsmoking Caucasian men aged 20–62) were generally evinced as parallel curves with proportional areas, suggesting differences in absorption and clearance rather than half-lives (Figure 11).

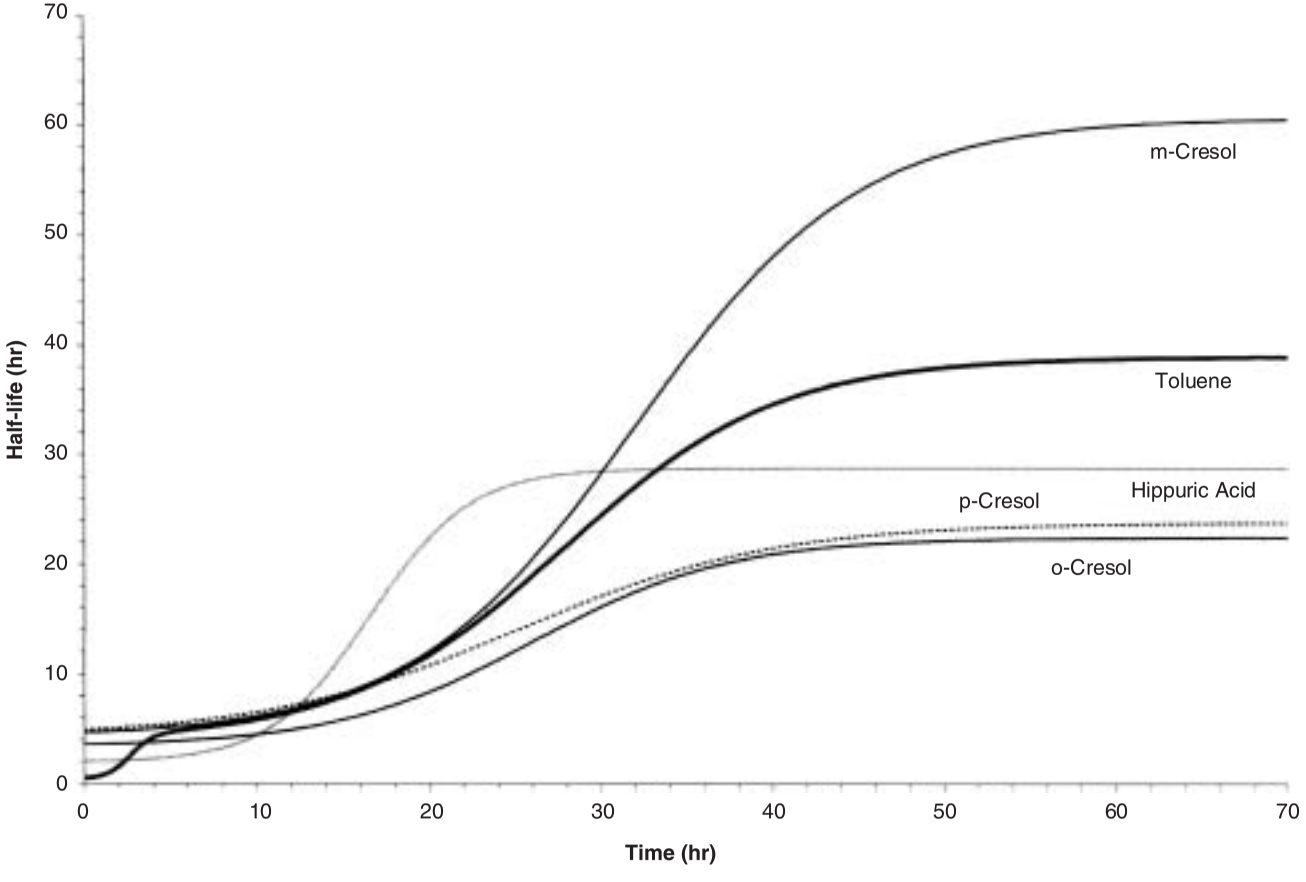

FIGURE 10. Toluene and metabolite half-lives over postexposure time.

TABLE 2. Toluene and Metabolite Half-Lives (h)

Analyte	Our study	Droz and Guillemin (1986)	Sato et al. (1974) Blood	Sato et al. (1974) Breath	Hjelm et al. (1994)	Löf et al. (1990)	Ernstgård et al.(1999)
Toluene 1[a]	—	—	0.0325	0.0264	0.0533	—	0.00183
Toluene 2	0.405	0.108	0.586	0.441	0.666	0.575/0.73[b]	0.090
Toluene 3	3.91	1.29	3.41	3.69	12.3	—	0.825
Toluene 4	38.9	15.8	—	—	—	—	15.3
Hippuric acid 1	1.89	—	—	—	5.63	—	—
Hippuric acid 2	28.8	—	—	—	—	—	—
o-Cresol 1	3.38	—	—	—	—	—	—
o-Cresol 2	22.4	—	—	—	—	—	—
m-Cresol 1	4.22	—	—	—	—	—	—
m-Cresol 2	60.8	—	—	—	—	—	—
p-Cresol 1	4.26	—	—	—	—	—	—
p-Cresol 2	23.8	—	—	—	—	—	—

[a] Number refers to first, second, third, or fourth apparent half-life.
[b] For "rapid" and "slow hydroxlyators," respectively.

Duration of exposure is expected to be a determinant of subsequent washout curve kinetics, particularly for toxicants with long terminal half-lives. Shorter durations tend to produce steeper curves, and the effect of exposure duration on curve shape diminishes with time. Droz and Fiserova-Bergerova (1992) found that the longer the elimination half-life of a biological indicator of exposure, the larger is the contribution of past exposures to measured concentrations. As an example, they found that an indicator with an elimination half-life of 10 h is influenced primarily (70%) by the past day's exposure, but also includes a contribution from the past week's exposures (20%) and the past hour's exposure (10%). In contrast, an indicator with an elimination half-life of 100 h is influenced primarily by the past week's exposure (55%), with important contributions from the last day's exposure (20%) and the past month's exposure (25%) (Droz & Fiserova-Bergerova, 1992). Effects of exposure duration on toluene and metabolite curve reconstruction were not, however, observed in our use of controlled exposure studies of 10 min–7 h. Exposure duration, when avaliable, could be used in the equation discussed above to more exactly determine curve shape:

$$An = \frac{K_0}{CL} Fn(1 - e^{-anT})$$

Our washout curve reconstruction approach provided mixed results. Decline in toluene blood and breath levels in the first 0.1 h (12 min) was underestimated (Figure 7, A, C, H, and I). This is likely due to a very rapid and short-lived decline phase immediately following the cessation of exposure. Indeed, Ernstgård et al. (1999) identified 4 half-lives, suggesting a very rapid

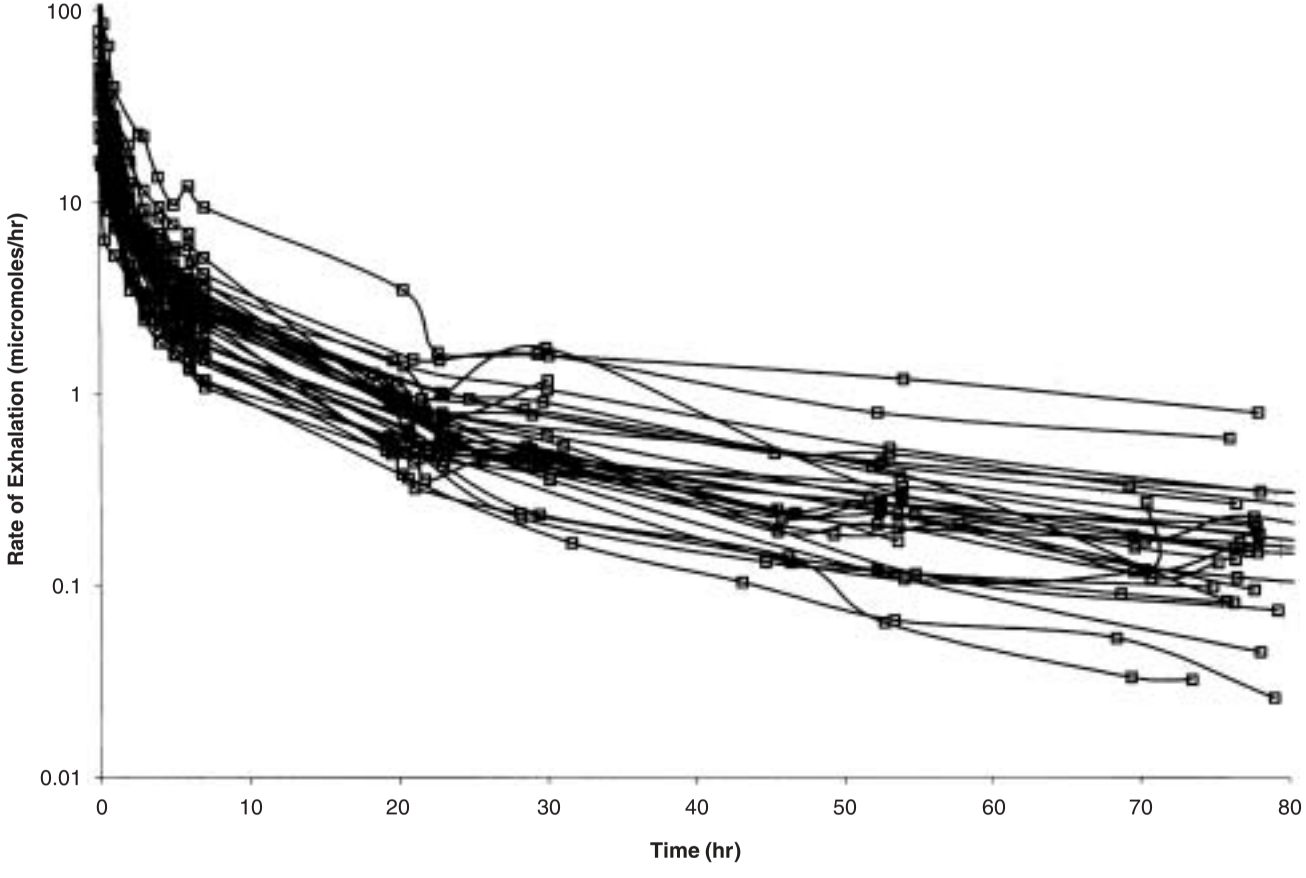

FIGURE 11. Individual rates of toluene exhalation (data truncated at 80 h postexposure for interindividual comparison).

initial half-life of 0.00183 h (0.11 min) (Table 2). It was not possible to measure this decline due to the time needed for each blood and breath sample in our experiments. Inclusion of this phase in estimating occupational and environmental postexposure blood and breath levels is impractical, however, due to the necessity of rapid serial sampling immediately following a known exposure. With the exception of three studies in which the terminal time point value appeared to be unusually low (Figure 7, D, E, and H), the curve reconstruction approach worked well for samples taken in the 0.1–23 h period (Figure 7, A–K).

Our use of [$^2H_8$]toluene allowed distinction of the [$^2H_5$]hippuric acid from the native compound in urine. A corresponding successful curve reconstruction was thus seen with the Hjelm et al. (1994) study, which also used deuterated toluene (Figure 8D). However, the expected biexponential decay in urinary hippuric acid excretion rates did not match the relatively unchanging values collected by Baelum (1991) or Ernstgård et al. (1999, Figure 8, A–C). This is likely due to the high background excretion rates of 60–1220 μmol/h observed in numerous studies (Pierce et al., 1998; Hjelm et al., 1988; Kawamoto et al., 1996; Angerer & Krämer, 1997). Thus, curve reconstruction for this metabolite would likely be useful only for toluene exposures much higher than 50 ppm.

In contrast, the *o*-cresol data of Baelum (1991) were closely approximated (Figure 9, A and B). Previously, *o*-cresol was found to be the most reliable metabolic indicator of toluene exposure, given the lower background urinary levels of this analyte (Pierce et al., 2002). As an example of the applicability of the reconstruction technique, the measured *o*-cresol excretion rates of Baelum (1991) could be used to estimate an immediate postexposure rate of 30–45 nmol/min (Figure 9B). Assuming a urinary formation rate of 1 ml/min, this would result in a concentration of 3.2–4.9 mg/L, higher than the ACGIH 8-h biological exposure index (BEI) standard of 0.5 mg/L (ACGIH, 2000).

The relatively stable half-lives of toluene in the first 2 h and of metabolites in the first 10 h postexposure (Figure 10) recommend these periods for sampling to easily back-extrapolate the immediate postexposure rate. This could be done by using the first half-life values (Table 2). The 2.7 fold differences in the terminal half-lives of toluene and metabolites (Figure 10) suggested that terminal metabolite disposition is limited by the rate of toluene metabolism. Measurement of all analytes for at least three terminal half-lives, over a period of 40–220 h, could resolve this question.

A novel approach to estimating postexposure biological levels of parent toxicant and metabolites without knowing the time since exposure involves metabolite ratios and curve reconstruction. For example, data showed that hippuric acid/cresol excretion ratios decline predictably in the first 5 h postexposure (Pierce et al., 2002). Thus, a single urinary sample could be measured for hippuric acid and *o*-cresol, and the ratio of these analytes would yield an estimate of time since exposure. This time and the *o*-cresol urinary concentration could then be used for curve reconstruction and estimation of postexposure concentration, for subsequent comparison to occupational exposure standards.

Curve reconstruction for other toxicants and drugs could be accomplished by fitting exponential models to comprehensive data sets to find rate constants a_1, a_2, a_3, and so on [Eqs. (4) and (5)], and coefficient ratios x, y, and so on [Eq. (6)], and then reconstructing the curve from a single or several data points [Eq. (7)]. This approach assumes nonsaturating doses and should be carefully used for toxicants with widely differing multiple half-lives (e.g., lead). In this case, exposure duration for the data to determine a_1, a_2, a_3, and x, y, . . . should be matched to the duration of curves to be reconstructed. Otherwise, a potential accumulation and long half-life of toxicant in depot tissue (e.g., lead in bone) would not be adequately represented. Physiologically based kinetic (PBK) modeling offers an alternative approach that would account for accumulation in tissue; however, simultaneous measurement of toxicant in at least two tissues (e.g., lead in blood and in bone) would be necessary for curve reconstruction.

Curve reconstruction successfully described published toluene blood and breath concentrations, and *o*-cresol excretion rates from different studies, where exposure duration ranged from 10 min to 7 h (Kezic et al., 2000; Baelum, 1991), and where activity level ranged from rest to 150 W (strenuous exercise). Hippuric acid excretion data were not well fit due to the substantial background levels of this analyte in urine. Knowledge of toxicant or metabolite excretion slope characteristics—exponential rate constants a_1, a_2, and a_3 (for a three-term model) and the coefficient ratios $x = A_1/A_2$ and $y = A_2/A_3$—allowed washout curve reconstruction from limited biological sampling. Once reconstructed, this curve was used to estimate immediate postexposure (or potentially pre-next shift) excretion rates or biological concentrations, providing a versatile measure of internal exposure and determination of risk.

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