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Biological monitoring of controlled toluene exposure

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Abstract ***Objectives:*** Widespread exposure to toluene occurs in the printing, painting, automotive, shoemaking, and speaker-manufacturing industries. The relationship between air concentrations and the absorbed dose is confounded by dermal exposure, personal protective devices, movement throughout the workplace, and interindividual differences in toluene uptake and elimination. ***Methods:*** To determine the best biological indicator of exposure we examined the blood and alveolar breath concentrations of toluene as well as the urinary excretion rates of hippuric acid and of *o*-, *m*-, and p-cresols from 33 controlled human inhalation exposures to 50 ppm for 2 h. ***Results:*** Among the metabolites, o-cresol was least influenced by background contributions, whereas the p-cresol and hippuric acid rates were obscured by endogenous and dietary sources. Toluene levels in alveolar breath proved to be the most accurate and noninvasive indicator of the absorbed dose. A physiologic model described blood and breath data using four measured anthropometric parameters and the fit values of extrahepatic metabolism and adipose-tissue blood flow. ***Conclusions:*** After breathing rate and extrahepatic metabolism had been set to conservative (protective) values (the 97.5th and 2.5th percentiles, respectively) the model predicted that pre-final-shift breath levels of ≤10 μmol/m^3 and post-final-shift levels of ≤150 μmol/m^3 corresponded to average workplace exposure levels of ≤50 ppm toluene. Alternately, we used the distributions and covariances of the measured and fit model parameters to yield conservative pre-final-shift levels of ≤7.3 μmol/m^3 and post-final-shift breath levels of ≤120 μmol/m^3 that were reflective of workplace exposure levels of ≤50 ppm toluene.

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Introduction

Toluene is the most widely used organic solvent [34], constituting up to 80% of paints, 62% of inks, 56% of thinners, and 51% of adhesives [35, 44]. Gasoline contains 5–7% toluene by weight, and about 92% of the United States production, 6.03 billion pounds in 1992 [1], is used for gasoline formulation. The highest current levels of toluene exposure are seen in the printing, painting, automotive, shoemaking, and speaker-manufacturing industries. Occupational overexposure is associated with mucous membrane irritation [29], decrements in central nervous system function [64], and endocrine disruption. Quantification of exposure to women is particularly important, given reports of increased spontaneous abortion and menstrual dysfunction from occupational exposure to toluene [46, 47, 53]. Reports of women exposed to a mixture of benzene, toluene, and xylene in a shoe factory [49], to benzene and toluene in another shoe factory [28], and to white spirit, toluene, xylene, and organosilicon varnishes [65, 66] revealed increased rates of prolonged or heavy menstrual bleeding, dysmenorrhea, and irregular cycles.

For the protection of workers from toxicity, toluene concentrations measured in workplace air have traditionally been compared with standards such as the time-weighted average threshold limit value (TWA TLV), the short-term exposure limit (STEL), and the permissible exposure limit (PEL). However, personal protective devices, dermal absorption, the degree of exertion, movement throughout the workplace, and interindividual anthropometric and biochemical characteristics modulate the relationship between the exposure concentration and the body burden. Interindividual factors that have been shown to contribute to variability in the toluene body burden are personal characteristics such as genotype [32] and body size and composition [63, 72],

life-style factors such as alcohol consumption [73] and smoking [34, 43], nutrition and health status, and interactions among toxicants [67]. We have found that body weight, the fraction of adipose tissue, the blood/air partition coefficient, and the fitted values for the extrahepatic rate of metabolism and adipose blood flow significantly affect blood concentrations of [$^2H_8$]-toluene (a surrogate for toluene) following controlled administration [57]. Factors that contribute to a varying internal dose, given the same ambient toluene concentration, include the degree of exertion [8, 9, 64] and the use of personal protective equipment.

Biological indicators of exposure, such as parent toxicant levels in blood, metabolite levels in urine, or measurable biochemical changes related to exposure [2] generally account for these interexposure and interindividual differences. As such, in comparison with measured airborne levels, they provide an improved estimate of the toxicologically active dose. Moreover, biological indicators are often relatively time-insensitive [29] and, thus, can be measured at times convenient to the worker. These indicators can provide a cumulative measure of exposure but may not reflect acute exposure associated with toxicity. The ideal indicator would accurately reflect the target-tissue concentration – the brain and reproductive tissues for toluene – of the toxicologically active species (parent or metabolite) in a time frame related to the onset of the effect (acute, subchronic, or chronic). As esposure standards are lowered, nonoccupational contributions to potential biological indicators will be more likely to obscure work-related dosimetry [59]. Biological levels of a parent toxicant generally more closely reflect the absorbed dose than do the levels of primary or secondary metabolites, given potentially complex metabolite kinetics [62]. For toluene, however, the initial P450-mediated oxidation step appears to be slower than subsequent metabolic steps; thus, the rate of metabolite appearance in blood and urine closely reflects the level of exposure [60].

The three main goals for a biological indicator of exposure are an accurate measure of the absorbed dose; a rapid, noninvasive, relatively time-insensitive method of sampling; and a measurement without dietary, lifestyle, or endogenous sources of contamination. To understand the interindividual variability in internal dose following identical degrees of exposure and to identify the best indicator of toluene exposure we conducted 33 controlled episodes of human exposure to [$^1H_8$]-toluene and [$^2H_8$]-toluene. Exposure to the stable isotope-labeled form allowed toxicokinetic analysis in the presence of uncontrolled environmental exposure as well as the identification of background and endogenous levels of exposure to toluene and its metabolites. From 62% to 80% of absorbed toluene is metabolized to hippuric acid and about 1% is metabolized to the *o*-, *m*-, and p-cresols (Fig. 1)[29, 64]; the remaining dose is exhaled as toluene. As potential indicators of toluene exposure, we examined concentrations of toluene in blood and breath and of hippuric acid and *o*-, *m*-, and p-cresols in urine following exposure. After a determination as to which biological indicator provided the best measure of internal dose a physiologically based kinetic (PBK) model was used to simulate occupational conditions to determine an optimal sampling strategy. We then conducted a pilot field study, comparing airborne and biological samples from a worker exposed to toluene in a spray booth.

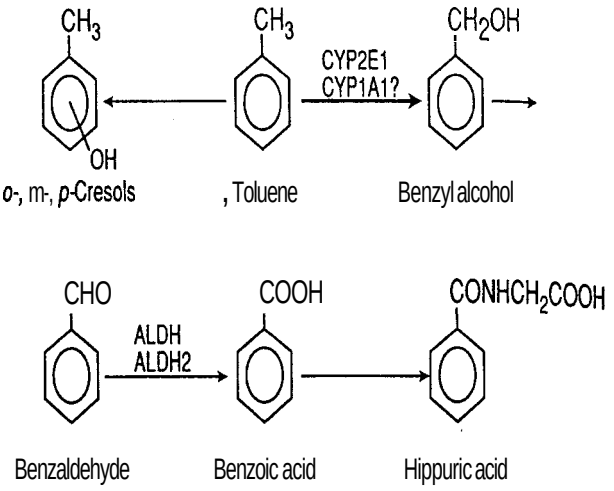

Fig. 1 Principal pathways of toluene metabolism in humans

Subjects and methods

Subjects

As previously described [57], 25 men aged 20–62 years were recruited and given a self-administered questionnaire to screen for occupational solvent exposure. In all, 5 of these subjects participated in 2 or 3 replicate exposures, with at least 2 peeks elapsing between exposures, for a total of 33 experiments. Weight (67–129 kg) and height (1.65–1.98 m) were measured, and the fraction of the body that was adipose tissue (0.10–0.39 kg/kg) was estimated by a skin-fold method using Lange calipers [21]. Alveolar ventilation rates (4.5–9.7 l/min), estimated as 70% of total ventilation were also measured [52]. This study was approved by the University of Washington Human Subjects Division, and all subjects provided informed written consent.

Exposure and sampling

Subjects inhaled 100 ppm of an approximately equimolar mixture of [$^1H_8$]-toluene and [$^2H_8$]-toluene for 2 h through a gated mouthpiece [52]. Inspired gas from each exposure was collected for off-line measurement of exact [$^1H_8$]- (43–71 ppm) and [$^2H_8$]-toluene (45–68 ppm) concentrations. Our resting conditions matched those used to associate exposure and toxicity for occupational standards [29].

One antecubital venous blood sample and one breath sample were taken prior to exposure for determination of preexposure [$^1H_8$]-toluene levels; 16 simultaneous blood and breath samples were taken after the end of exposure over the following 4 days at sampling intervals that varied from every 15 min immediately following exposure to every 12 h at times of ≥24 h after exposure. Blood samples were collected in 5-ml Vacutainer tubes containing citrate. Exhaled breath samples were collected in 20-l Tedlar plastic bags prefilled with 10 l of dry nitrogen and kept at 37 °C to prevent condensation. Urine samples were taken prior to, immediately

llowing, and at 4 and 8 h after exposure and then, with blood and reath samples, at times of ≥24 h after exposure.

hemical analyses

lood samples were analyzed in triplicate for [$^1H_8$]-toluene and $^1H_8$]-toluene by a headspace method [17] using gas chromatogra- ly with mass spectrometry detection in selected ion mode. Ap- arent low-level contamination of blood samples through contact ith Vacutainer vials or catheters compromised the [$^1H_8$]-toluene oncentrations in low-level blood samples; hence, the [$^1H_8$]-toluene lood data were not used. The contents of breath-sample bags were rawn through two-section (200 mg/100 mg) charcoal sorbent ubes using a calibrated personal sampling pump. The charcoal ections were then analyzed [70].

A previously described gas chromatography/electron-capture etection assay was used for determination of phenolic deuterated nd unsubstituted *o*-, *m*-, and *p*-cresols with a quantitation range of 1001–500 μg/ml (0.9–4,600 μ mol/l) [18]. Assays for hippuric acid ere performed on duplicate 1-ml aliquots of urine as previously escribed [60]. The appearance of metabolites in urine was expres- ed as excretion rates, obviating the need for creatinine correction.

hysiologically based model

{previously described semiempirical PBK model [57] (Fig. 2) was mplemented with SimuSolv software (Dow Chemical Co., Mid- and, Mich.) using subject-specific values for body weight, the ad- ose tissue fraction, the blood/air partition coefficient, the xposure concentration, and the alveolar ventilation rate. Values or fractional tissue compartment volumes, fractional blood flows, issue/blood partition coefficients, and hepatic metabolic constants $V_{max\text{-}h}$ and K_m were taken from literature sources (Table 1). Car- iac output (CO) and, therefore, all blood flows were scaled to body weight)$^{0.74}$ [20, 31, 51, 61]. The fractional blood flow to the dipose tissue (Qa/co) and the maximal rate of extrahepatic me- abolism ($V_{max\text{-}x}$) were fitted [57] to each of the [$^1H_8$]- and [$^2H_8$]- oluene data sets.

The PBK model was previously found to describe an average of 1% (range 75–96%) of data variability in the 33 exposures [57]. he model was also used to estimate background levels of envi- onmental exposure to [$^1H_8$]-toluene [59] and to compare the tox- okinetics of [$^1H_8$]- and [$^2H_8$]-toluene using blood and breath oncentrations [60]. In the present study the PBK model was used o simulate a 40-h work week to determine optimal times for ampling of toluene breath levels as indicators of exposure.

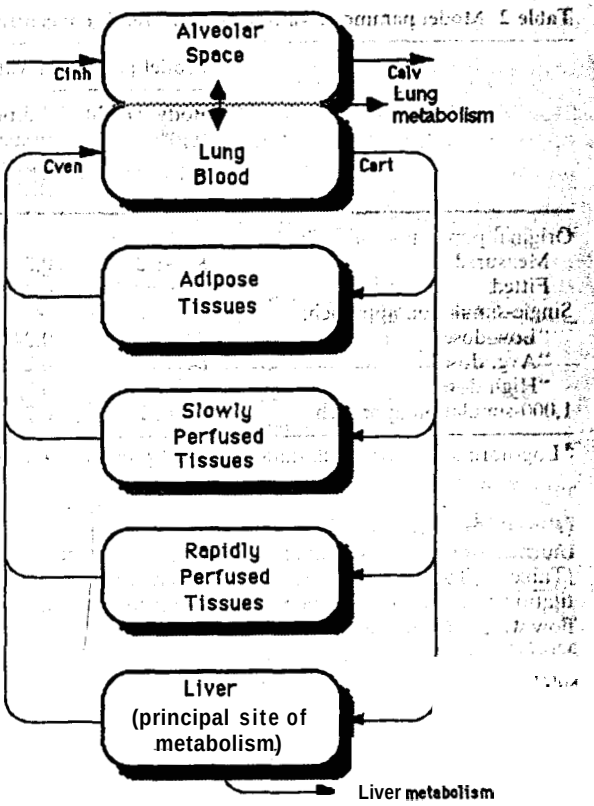

Fig. 2 Diagram of the physiologically based kinetic (PBK) model for toluene disposition, where C_{inh} represents the inhaled toluene concentration, C_{alv} represents the concentration in alveolar air, C_{ven} represents the venous blood concentration, and C_{art} represents the arterial blood concentration

Simulating interindividual ranges of internal exposure

We employed two approaches to simulate physiologic conditions that would represent the range of toluene body burdens found in workers exposed to the same occupational limit concentration. Both of these approaches were based on the observed distributions of anthropometric parameters in our subjects. Visual examination of histograms and Kolmogorov-Smirnov and chi-square tests

Table 1 Values of parameters sed in the physiologic model[a]

Parameter	Tissue group			
	Slowly perfused	Rapidly perfused	Liver	Adipose
Volume (V, 1)	$0.95BW - V^b_{adipose}$	$0.05BW - V^b_{liver}$	$0.023BW^b$	0.10–0.39 BW (measured)
Blood flow (Q, I/h)	$0.24Q_{co} - Q^c_{adipose}$	$0.76Q_{co} - Q^c_{liver}$	$0.27Q^b_{co}$	0.06-0.18 Qco (fitted to data)
K_p	1.54[d]	4.64[d]	4.64[d]	55.9[e]
K_m (μmol/l)	–	–	5.97[c]	–
V_{max} (μmol/h)	–	–	52.1 $BW^{0.7}$ [c, f, g]	–

[a] *BW* represents body weight (kg) and Q_{co} represents cardiac output (l/h) = 12.92 $BW^{0.74}$ as based on similar scaling of cardiac output and the alveolar ventilation rate [68] and measurements of the ventilation rate in our subjects. K_p represents the tissue/blood partition coefficient
[b] From Rowland and Tozer [62]
[c] From Tardif et al. [68]
[d] From Gargas et al. [26]
[e] From Pierce et al. [58]
[f] Allometric scaling from Mordenti [51]
[g] The maximal extrahepatic rate of metabolism was varied within a range of 0–70% of V_{max} to describe the data

Table 2 Model parameter values used in breath concentration simulations

	Model parameter values (mean ± SD)					
	Body weight (kg)[a]	Adipose tissue fraction (kg/kg)	Alveolar ventilation rate (l/min)	Blood/air coefficient[a]	Extrahepatic metabolism rate (μmol h^{-1} kg)	Adipose blood flow (l/l cardiac output)
Original population (n = 26):						
Measured	84/÷1.2	0.24 ± 0.078	6.0 ± 1.0	20/÷1.5	–	–
Fitted	–	–	–	–	5.9 ± 4.5	0.10 ± 0.029
Single-simulation approach:						
"Low dose"	84	0.24	3.9	20	15	0.10
"Avg. dose"	84	0.24	6.0	20	5.9	0.10
"High dose"	84	0.24	8.1	20	0	0.10
1,000-simulation approach	84/÷1.2	0.23 ± 0.078	6.0 ± 1.1	19/÷1.5	6.7 ± 3.7	0.11 ± 0.038

[a] Log-normally distributed; data represent geometric mean values /÷ geometric SD

($P < 0.05$) were used to determine the best distributional fit (normal, log-normal, or gamma) for each of the model parameters (Table 2). The values for the adipose tissue fraction, alveolar ventilation rate. rate of extrahepatic metabolism, and adipose blood flow were consistent with normal distribution; body weight and the blood/air partition coefficient were best described by log-normal distribution in our subjects.

In our initial, "single-simulation" approach the model parameter that most affected the absorbed dose, i.e., the ventilation rate, and the elimination parameter that varied most among the 26 subjects, i.e., the extrahepatic rate of metabolism, were assigned specific values to represent different workers. The choice of extrahepatic metabolism as a varied parameter was guided by information that although the liver is the principal site of metabolism, the hepatic blood flow is evidently the limiting factor in this process [57]. Because this flow was scaled to (body weight)^{74}, much less interindividual variability in hepatic as compared with extrahepatic elimination was observed [57]. We then assigned parameter values to create theoretical "low-dose," "average-dose," and "high-dose" workers as follows: low dose – 2.5th percentile ventilation rate and 97.5th percentile extrahepatic metabolism rate; average dose – average rates of ventilation and extrahepatic metabolism; and high dose – 97.5th percentile ventilation rate and 2.5th percentile extrahepatic metabolism rate. These simulations also used the average values recorded for body weight, the adipose tissue fraction, the blood/air partition coefficient, and the fractional blood flow to adipose tissue in our subjects (Table 2) [57]. This approach was similar to that of Droz and Guillemin [19] in simulating the effects of interindividual, intraday, and interday factors on solvent breath concentrations.

The second, "Monte Carlo" approach utilized the distributions and covariances for six model parameters – the four measured parameters (body weight, adiposity, breathing rate, and blood/air partition coefficient) and the two fit parameters (rate of extrahepatic metabolism and adipose blood flow). We used the "standard two-stage" approach [15] to determine the values for the model parameter means (arithmetic mean of the 26 available individual parameter sets) and covariances (determined as the sample covariance among the individual parameter sets). Although it has been shown that this approach yields an overestimate of the population covariance [15], it was adopted because we felt the need to be conservative and because of its intrinsic simplicity.

From the sample means and covariances we simulated 1,000 sets of the 6 parameters using the corresponding normal and log-normal distributions. None of the 1,000 simulations was discarded a posteriori, apart from those that yielded negative parameter values. The means, variances, and distributional shapes of these parameter sets were found to be very close to the measured and fitted values (Table 2). The largest deviation was observed in the rate of extrahepatic metabolism, whose simulation mean was 14% higher than the original value. This difference was due to the exclusion of values of less than zero, produced in part by the 76% coefficient of variation in the original values for this parameter. However, the original and simulation values were both best fit with normal distributions and were not different ($P > 0.05$) as determined using a t-test.

The sets of parameters were used to generate blood and breath concentration curves for toluene that would result from occupational exposure to simulated workers. We then defined a low-dose worker as having the 2.5th percentile toluene blood and breath concentrations, the average-dose worker as having the mean values, and the high-dose worker as having the 97.5th percentile values. Occupational exposure in both approaches was simulated using a constant 50-ppm level for 4 h of exposure, 1 h with no exposure, and another 4 h of exposure over a 5-day work week.

Field study

To examine the value and practicality of breath sampling in a field exposure setting we measured breathing-zone and exhaled breath levels of toluene from a spray-booth worker over a 10-h Monday shift. Work activities included spray application of lacquer and hand application of stains and of a lacquer thinner to wooden surfaces, with subsequent equipment cleanup using paint thinner. One of us (R.L.D.) held the tip of a Miran 1B infrared spectrophotometer probe (Foxboro, Mass.) with a 10-m flexible sampling tube within 1 m of the worker so as to monitor continuously the toluene levels in air near the subject. During breath sample collections, potential contributions of toluene in workspace air were obviated by inhalation through a half-face organic cartridge respirator, where exhaled breath traveled through a short rubber hose to each collection bag. In this way, 20-l breath samples were taken before exposure, prior to and immediately following two breaks after work, twice during the evening hours, and once prior to the next work shift. An additional set of breath samples was taken from the start of exposure to 32 h postexposure for R.L.D., who did not use a respirator. Samples were analyzed as described for the controlled exposures. Because the worker had exertion-level changes, intermittently used a half-face respirator and ventilation fans, occasionally left the room, and took work breaks, it was expected that breath'sampling would provide a better estimate of the absorbed dose than would the breathing-zone measurements.

Results

The [$^2H_8$]-toluene blood data were previously presented in the ,development of a subject-specific modeling approach [57], and the [$^1H_8$]- and [$^2H_8$]-toluene levels in alveolar breath were published in support of a model-estimated background environmental concentration of

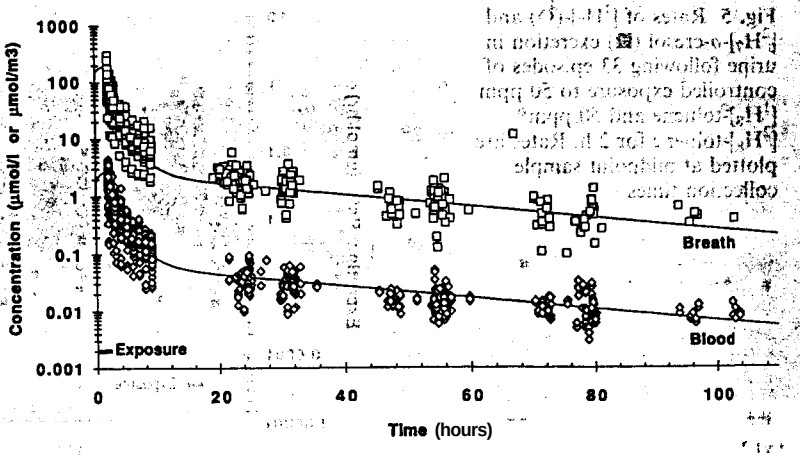

Fig. 3 Measured alveolar breath (□, µmol/m³) and blood (◇, µmol/l) concentrations of [$^1H_8$]-toluene in 33 episodes of exposure to 50 ppm for 2 h at rest and model-predicted concentrations (–) recorded for a subject with average parameter values

[$^1H_8$]-toluene [59]. Portions (0–40 h) of the urinary hippuric acid and cresol data were presented in a comparison of the toxicokinetics of [$^1H_8$]- and [$^2H_8$]-toluene [60]. New data presented herein include additional metabolite data, new modeling simulations, and field study results. Both published and new data are presented in new figures below to support the determination of a biological indicator of toluene exposure.

Blood and breath concentrations of [$^1H_8$]- and [$^2H_8$]-toluene

The 33 episodes of exposure resulted in a 10-fold interindividual range in [$^2H_8$]-toluene blood and breath concentrations (Fig. 3). These data provided time-concentration profiles uncontaminated by background sources, and visual examination suggested three phases of exponential decline.

Urinary excretion rates of [$^1H_9$]- and [$^2H_5$]-hippuric acid

We found significant preexposure levels of [$^1H_9$]-hippuric acid, about a 10-fold interindividual difference in [$^2H_5$]-hippuric acid excretion rates, and a slightly larger difference in [$^1H_9$]-hippuric acid rates (Fig. 4). Although a peak and washout trend in the [$^1H_9$]-hippuric acid excretion rate was observed in the first 15 h post-exposure, a pronounced effect of the background rate was evident from pre-exposure samples and from the leveling off of the excretion rate after 20 h. The initial IO-fold difference between the [$^1H_9$]- and [$^2H_5$]-hippuric acid rates became larger with increasing time postexposure, providing further evidence of the profound effect of background levels of this metabolite on postexposure measurements.

Urinary excretion rates of [$^1H_8$]- and [$^2H_7$]-*o*-, *m*-, and p-cresols

[$^1H_8$]-Cresol levels determined in preexposure urine samples were 0.211 ± 0.32, 0.500 ± 0.66, and 333 ± 290 µmol/l (mean ± **SD**, n = 38) for the *o*-, *m*-, and p-cresols, respectively. The o-cresol excretion rates showed about a 10-fold range of interindividual differences (Fig. 5) and revealed much more overlap between native and deuterated metabolites than was observed for hippuric acid (Fig. 4). However, background excretion rates of [$^1H_7$]-*o*-cresol were within 1 order of magnitude

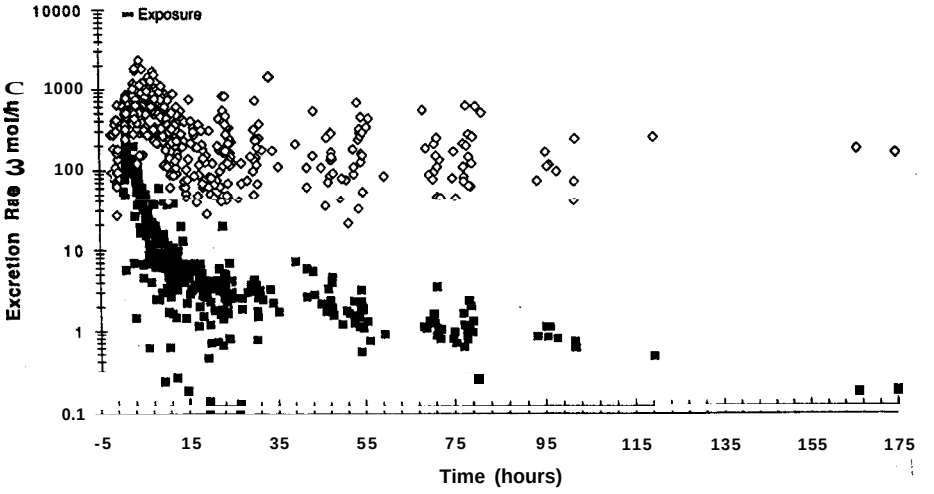

Fig. 4 Rates of [$^1H_9$]-(◇) and [$^2H_5$]-hippuric acid (■) excretion in urine following 33 episodes of controlled exposure to 50 ppm [$^1H_8$]-toluene and 50 ppm [$^2H_8$]-toluene for 2 h. Rates are plotted at midpoint of sample collection times

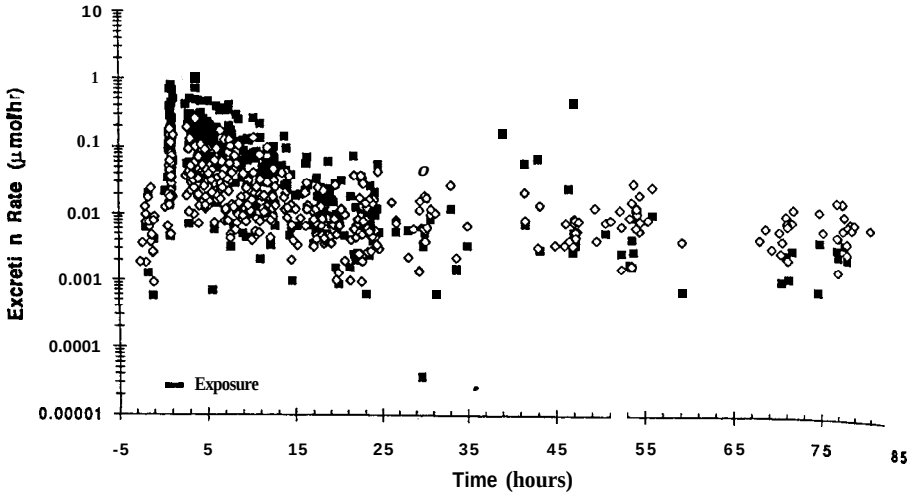

Fig. 5 Rates of [$^1H_8$]-(◇) **and** [$^2H_7$]-*o*-cresol (■) **excretion** in **urine following 33 episodes** of **controlled exposure to 50 ppni** [$^1H_8$]-toluene **and 50 ppm** [$^2H_8$]-toluene for 2 h. **Rates are plotted at midpoint sample collection times**

of the post-exposure rates, providing evidence of background contributions to the observed rates, particularly at times exceeding 10 h.

The 10-fold higher peak rate of [$^2H_8$]- versus [$^1H_7$]-*m*-cresol excretion was indicative of a metabolic isotope effect (Fig. *6)* [60]. **A** steeper postexposure slope for the labeled metabolite and a relative constant rate for the native metabolite clearly demonstrated background contributions. For [$^1H_8$]-*p*-cresol, background contributions were so substantial that the effects from the controlled [$^1H_8$]-toluene exposure were not evident (Fig. 7). **As** seen with the hippuric acid and o-cresol data, the interindividual excretion rates for the *m*- and p-cresol deuterated isomers demonstrated about a 10-fold range, whereas the native isomer ranges were somewhat larger.

Simulation of workplace exposure in individuals

The predicted alveolar breath levels determined in the low-, average-, and high-dose "workers" from the single-simulation approach are presented as curves in Fig. 8. The corresponding pre-final-shift toluene breath concentrations were 10, 27, and 47 µmol/m^3 and the postexposure breath levels were 150, 325, and 500 µmol m^3, respectively. The Monte Carlo approach resulted in pre- and postexposure breath levels that were log-normally distributed. The pre-final-shift breath concentrations recorded for the low-dose, average-dose, and high-dose workers using this approach were 7.3, **23,** and 73 µmol/m^3 and the postexposure breath levels were 120, 310, and 810 µmol/m^3, respectively (Fig. 8).

Field study

The average breathing-zone concentration of toluene measured during the 10-h field study was **5** ppm, with the peak being 110 ppm (Fig. 9). The breath concentrations recorded for the two subjects overlapped and showed a similar decline postexposure. There was a decline in breath levels for subject 1 during both breaks and for subject **2** during the first break. The breath level of toluene noted for subject **2** actually increased over the period **of** the second break, which was likely due to his

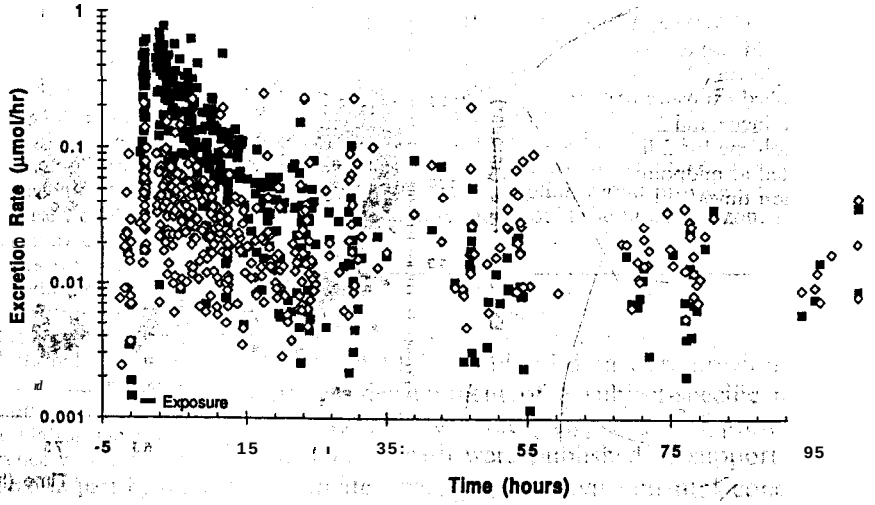

Fig. 6 Rates of [$^1H_8$]-(◇) **and** [$^2H_7$]-*m*-cresol (■) **excretion in urine following 33 episodes** of **controlled exposure to 50 ppm** [$^1H_8$]-toluene **and 50 ppm** [$^2H_8$]-toluene **for 2 h. Rates are plotted at midpoint sample collection times**

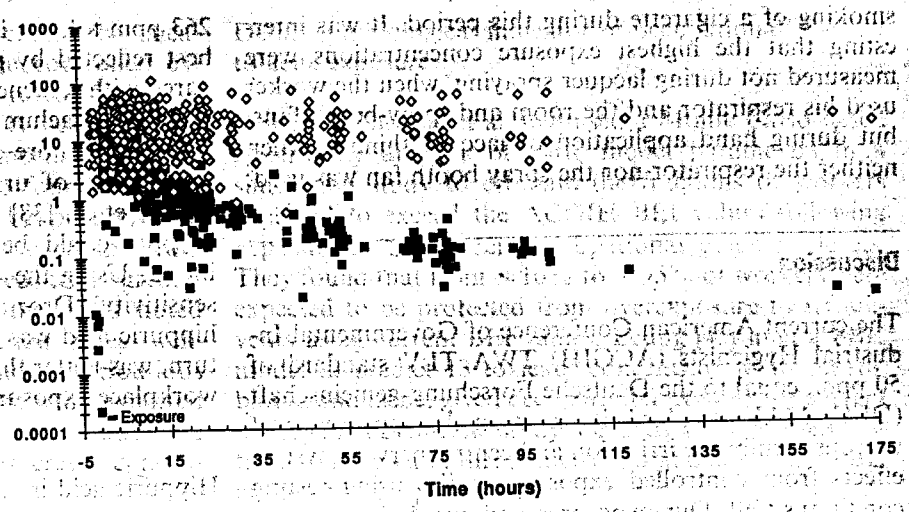

Fig. 7 Rates of [$^1H_8$]-(◇) and [$^2H_7$]-*p*-cresol (■) excretion in urine following 33 episodes of controlled exposure to 50 ppm [$^1H_8$]-toluene and 50 ppm [$^2H_8$]-toluene. Rates are plotted at midpoint sample collection times

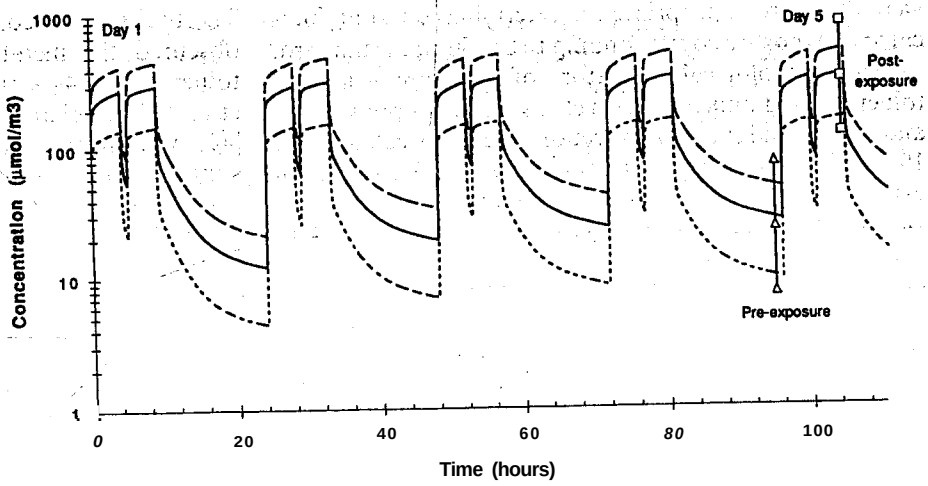

Fig. 8 Model-predicted levels of toluene in alveolar breath for the high-dose (——), average-dose (–), and low-dose (---) workers under conditions of a 50-ppm level of exposure for 4 h, followed by a 1-h break, followed by 4 h of exposure over a 5-day period. The *vertical lines* indicate the high-dose (97.5th percentile), average-dose (geometric mean), and low-dose (2.5th percentile) preexposure (A) and postexposure (B) alveolar breath levels found in the Monte Carlo simulations

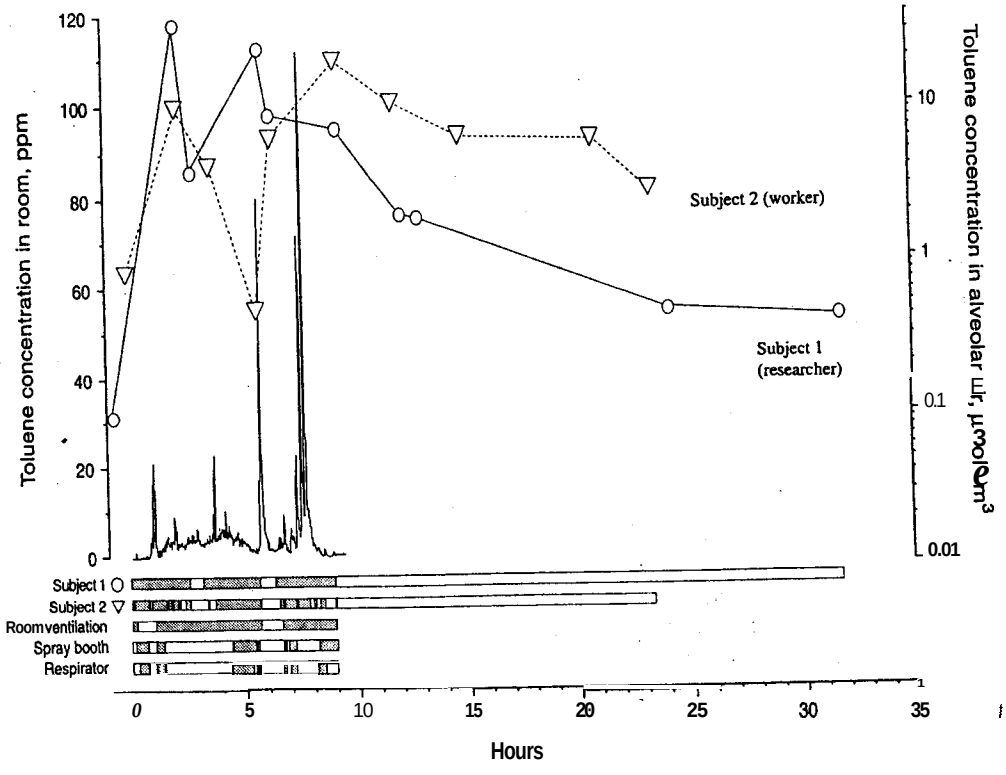

Fig. 9 Measured concentrations in air (– , ppm) and alveolar breath (○, subject 1, R.L.D.; ▽, subject 2, worker; µmol/m^3) as recorded from a 10-h shift in a spray booth and at 25 h postexposure. The *shaded portions of bars below the plot* indicate when subjects were in the room, when the room and/or booth ventilation was Wive, and when a respirator was used by the worker

smoking of a cigarette during this period. It was interesting that the highest exposure concentrations were measured not during lacquer spraying, when the worker used his respirator and the room and spray-booth fans, but during hand application of lacquer thinner, when neither the respirator nor the spray booth fan was used.

Discussion

The current American Conference of Governmental Industrial Hygienists (ACGIH) TWA TLV standard of 50 ppm, equal to the Deutsche Forschungsgemeinschaft (DFG) **MAK** standard, is based on the appearance of mucous membrane irritation and central nervous system effects from controlled exposure studies using resting conditions [29]. Our exposures and simulations at rest were therefore appropriate for determination of an indicator of exposure corresponding to the 50-ppm standard.

All six biological indicators of exposure – labeled toluene blood and breath levels as well as hippuric acid and *o-*, *m-*, and p-cresol excretion rates – revealed about 10-fold interindividual ranges among the 33 episodes of exposure to 50 ppm. Caperos found about a 3-fold variability in breath levels following the exposure of 17 men for 8 h to 100 ppm toluene (cited in Droz and Guillemin [19]). The similarity in indicator ranges was consistent with the expectation that oxidation, not phase II metabolism or urinary excretion, would be the rate-limiting step in toluene elimination. In addition to these indicators, measurement of toluene in urine may be a promising measure of cumulative exposure [30], and a preliminary report has found some utility of the minor urinary metabolite S-p-toluylmercapturic acid [4].

Toluene in blood and breath

Although invasive, sampling of toluene blood levels both postexposure [3] and pre-final-shift exposure [30] provides accurate assessments of exposure. The observed parallel decline in breath and blood [$^2H_8$]-toluene levels reflected the expected rapid equilibrium across these two fluids (Fig. 3). Breath levels from the controlled exposure did not reach measured background levels of 138–764 nmol/m^3 [11, 59] until 240 h postexposure (Fig. 3). This observation was confirmed by comparison of measured blood levels (Fig. 3) with average background concentrations of 3–16 nmol/l [6, 11, 12, 22, 25, 59, 71]. In contrast, background levels of hippuric acid and p-cresol were prominent throughout the pre- and postexposure periods (Figs. 4, 7), and background levels of o- and m-cresols prominently contributed to measurements made at 220 h (Figs. 5, 6). Monster et al. [50] found that alveolar air concentrations 8 h following occupational exposure provided a better correlation ($r = 0.99$) with personal air concentrations than toluene or hippuric acid in urine. Foo et al. [23] found that exposures to 8-h **TWA** TLV values of 1.6–263 ppm toluene in a speaker-manufacturing plant were best reflected by postexposure breath samples as compared with toluene in finger-prick blood or hippuric acid in urine. Baelum [8] determined that toluene breath levels were more closely correlated with exposure than were levels of urinary hippuric acid or o-cresol, and Kawai et al. [38] found that occupational exposure of ≥4 ppm could be detected from levels of toluene in blood. Using the concept of "validity" (specificity plus sensitivity), Droz and Guillemin [19] found that urinary hippuric acid was better than breath toluene, which, in turn, was better than urinary o-cresol, as an indicator of workplace exposure.

Hippuric acid in urine

The background contributions of urinary hippuric acid obscured the metabolite elimination profile of [$^1H_8$]-toluene (Fig. 4), consistent with the findings of Hjelm et al. [27]. Similar results were reported by Kawai et al. [41], who found that hippuric acid levels in urine resulting from 8 h of occupational exposure could be distinguished from background levels only at exposure levels of 230 ppm, and by Inoue et al. [34], who found this threshold to be 120 ppm. In examining toluene poisonings, Meulenbelt et al. [48] found that exposure levels of less than 800 ppm could not be accurately assessed using hippuric acid. These observations are in accord with the expected dietary contributions of benzoic acid (in fruits, vegetables, and food preservatives) and benzoic acid precursors (in prunes, cranberries, and plums) to hippuric acid levels in urine [29].

Our observed postexposure hippuric acid excretion rates (Fig. 4) were consistent with previous rates of 260 and 375 μmol/h [27] obtained by scaling of exposure concentrations. Observed background production rates of 235 ± 158 μmol/h (mean ± SD, range 26–649 μmol/h; Fig. 4) were higher than the rate of 60 μmol/h found by Kawamoto et al. [43] (assuming urine production of 1 ml/min) but were similar to the rates of 215–645 μmol/h reported from other previous studies [29] (using a creatinine production rate of 76 mg/h for our subjects of average 86.3 kg weight and 34 years of age [62]). Angerer and Kramer [3] have measured background contributions of up to 1,220 μmol/h. The ACGIH biological exposure index (BEI) value of 1.6 g hippuric acid/g creatinine in an end-shift urine sample now being adopted [30] corresponds to a production rate of 690 μmol/h (assuming creatinine production of 76 mg/h). Such a standard is likely to be compromised by substantial background levels of 60–1,220 μmol/h (9–177%).

o-, m-, and p-Cresols in urine

Background levels of o-cresol were consistent with earlier measurements of about 0.013 [43] and about

0.023 μmol/h [34] and partially obscured the measurement of levels associated with the controlled exposure (Fig. 5). Previous research has revealed that an exposure level of ≥200 ppm toluene is necessary for the use of urinary *o*-cresol as an exposure indicator [34], whereas other investigators have found a linear relationship between *o*-cresol levels and exposure to up to 120 ppm [48]. The rates of *m*-cresol excretion from the controlled exposures were clouded to a greater degree by background contributions than was noted for *o*-cresol (Fig. 6). *p*-Cresol excretion rates were completely dominated by background contributions (Fig. 7), a finding that is in accord with the expected contributions of food constituents and products of amino acid metabolism to measured *p*-cresol concentrations.

o-Cresol was the most promising indicator of exposure among the metabolites because of its relatively lower background contributions (Fig. 5), which were consistent with its expected absence in urine from unexposed subjects [64]. This finding was consistent with an occupational study by Angerer and Krämer [3] and with the ACGIH listing of *o*-cresol as a potential BEI [30]. The peak levels we recorded after 2 h of exposure were about 10-fold lower than the DFG occupational standard of 3 mg/l (about 1.6 μmol/h; Fig. 5). Other investigators have shown that *o*-cresol is a better indicator of toluene exposure than is hippuric acid in glue-sniffers [75]. In contrast, Inoue et al. [34], who also found that smoking and drinking lowered *o*-cresol levels in urine, concluded that urinary hippuric acid was more sensitive to the toluene dose than was *o*-cresol in toluene-exposed workers. Nise [54] found that neither hippuric acid, which was influenced by alcohol consumption, nor *o*-cresol, which was affected by smoking, provided a suitable indicator of occupational exposure. Thus, endogenous and dietary sources as well as smoking and drinking habits render urinary metabolite levels following occupational exposure to toluene difficult to interpret.

ntraday and interday exposure variability

simplified exposure scenario (8 h at the TWA TLV) used in our simulations does not reflect the complexities of occupational exposure. In a series of simulation studies, Droz and Guillemin [19] found that intraday variations in solvent *exposure (i.e., exposure in the morning or afternoon only) resulted in an 80% difference in postexposure breath levels but in only a 5% difference in levels taken before the next shift. In contrast, they found that interday variations (i.e., no exposure or exposure at 2x the TLV value on the previous day) resulted in just a 5% difference in postexposure levels and in a 25% difference in preshift levels. Therefore, for accommodation of large intra- and interday exposure fluctuations, both post- and preexposure breath samples are necessary.

Defining a biological indicator of occupational toluene exposure

Recently, Thomas et al. [69] used a Monte Carlo-based uncertainty analysis of PBK model parameters in a simulation study to estimate the fractions of workers expected to exceed the ACGIH BEI values following exposure at the current occupational guideline levels. They found that from < 10% to > 95% of workers were expected to be protected from overexposure to six solvents using current BEI values. Leung [45] used a PBK model to predict 13 toxicant concentrations in breath, blood, and urine, given occupational exposure at current limits. In the current study we used the observed distributions of six anthropometric parameters and a PBK model to determine pre- and postexposure toluene breath levels that would protect 97.5% of workers from exposure at levels exceeding 50 ppm.

The single-simulation approach predicted an "average-dose" alveolar breath concentration of 27 μmol/m^3 (Fig. 8) and a venous blood level of 0.059 mg/l prior to the 5th working day, reflecting a blood/breath ratio of about 20 [58]. The Monte Carlo approach resulted in a mean breath level of 23 μmol/m^3 and a blood level of 0.050 mg/l. Both blood concentrations are in the midst of values determined from previous occupational exposure studies adjusted to 50 ppm exposure (Table 3) and are equal to the ACGIH BEI value of 0.05 mg toluene/l in blood now being adopted [30]. The model-predicted postshift breath levels of 325 (single-simulation) and 310 μmol/m^3 (Monte Carlo) corresponded to blood levels of 0.72 and 0.68 mg/l, respectively, which similarly lie within the range of values found by previous investigators (Table 3) and below the current DFG BAT value of 1 mg/l [16].

Table 3 Pre-final-shift and end-of-shift toluene blood concentrations obtained in simulations (Fig. 8) and previous occupational studies. Blood concentrations corresponding to an 8-h period of 50-ppm exposure were determined from regression equations provided by the authors or from data obtained in each study

Pre-final-shift conc. (mg/l)	Reference	Postshift conc. (mg/l)	Reference
0.03	[10][a]	0.18	[38]
0.045	[56]	0.25	[39]
0.05	Monte Carlo approach	0.46	[40]
0.05	[12]	0.52	[14]
0.059	Single-simulation approach	0.54	[10]
0.07	[55]	0.55	[55]
0.09	[36]	0.60	[5]
0.17	[23]	0.67	[24]
		0.68	Monte Carlo approach
		0.69	[12]
		0.72	Single-simulation approach
		0.73	[23]
		0.96	[13]

[a] Simulation study

The single-simulation approach allowed us to define three hypothetical workers to represent the majority of the general working population by characterizing the crucial parameters of ventilation and extrahepatic metabolism rates. However, this approach did not include the complex interplay that could exist among the parameters of the nonlinear PBK model. Specifically, the high-, average- and low-dose workers were assumed to have the same values for body weight, adiposity, blood/air partition coefficient, and adipose blood flow (Table 2). In contrast, the Monte Carlo approach generated a large number of parameter sets that were based on the original sample covariance and, thus, included the dependencies among all 6 of the parameters observed in the 26 subjects. Despite the different approaches, the "low-dose" (2.5th percentile) pre- and postshift toluene breath indicators of exposure were within 15% (Fig. 8). A different approach to this problem would have been to perform a full-fledged population analysis on the available data sets using other available methods [15]. This would have allowed the determination of all model parameters at once from all the available data and, thus, would reconstruct a more reliable picture of the population variability than would the standard two-stage approach we employed. Such an investigation is currently under way.

Although we attempted to include wide ranges of age (20–62 years). weight (67–129 kg), and adiposity (0.10–0.39 kg/kg), all of our subjects were nonsmoking Caucasian males. Use of such a proscribed study group underestimates the true toxicokinetic diversity of the general population. However, the development of ACGIH BEI values is almost always based upon similarly homogeneous controlled exposure and worker populations (where the "healthy worker effect" may also be operative) [29]. Our conservative approaches to include a greater diversity of anthropometric values were used to address this limitation. Other investigators have examined the effects of smoking, drinking, and ethnicity on toluene kinetics. Although smokers have higher blood [7, 11, 74] and alveolar breath [11] levels of toluene, consistent with our field study observation, there is also some evidence for a slightly higher clearance in smokers [27]. Numerous studies have documented different confounding effects of smoking and drinking on urinary *o*-cresol and hippuric acid levels [33, 34, 42, 54]. Inoue et al. [32] have found possible differences in toluene metabolism based on ethnic differences in Chinese, Turkish, and Japanese solvent workers, and Jang et al. [37] have found higher than expected toluene blood levels and lower than expected urinary hippuric acid levels in Korean workers. There is also some evidence that P450 1A1 and ADLH2 genotypes affect toluene kinetics [42].

Because the narcotic and irritating effects of solvent exposure which are the basis for the current ACGIH 50-ppm occupational standard for toluene [29], are proximately related to the inhaled concentration, an end-of-shift breath sample can be useful in the protection of workers. To reflect the most recent exposure, however, this sample must be taken immediately (within 5 min) following exposure – immediate postexposure breath concentrations declined with a model-determined half-life of 10 min (Fig. 8). As a postexposure sample, an alveolar (or end-exhaled) breath concentration of 150 μmol/m^3 was expected to correspond to an average inhaled concentration of 50 ppm for an 8-h period of exposure in the low-dose worker (Fig. 8). In comparison, adjustment of the previous "semiquantitative" ACGIH BEI value of 40 ppm in breath [29] to a 50-ppm exposure level gave a postexposure breath level of 816 μmol/m^3. A second, less time-sensitive sample taken prior to the final shift was found to be indicative of the cumulative workplace exposure over the previous several days. Because the preshift levels demonstrated an increase of 100% as compared with 6.8% in the postshift levels over the work week (Fig. 8), a preshift indicator of average work-week exposure would be most useful when assessed prior to the final day. The previous ACGIH BEI preshift value adjusted to a 50-ppm level of exposure was 20.4 μmol/m^3 [29]; our analysis found that an indicator of 10 μmol/m^3 would reflect an average exposure level of 50 ppm in the low-dose worker (Fig. 8).

An alveolar breath concentration of 10 μmol/m^3 is 13–71 times higher than the levels of 0.14–0.76 μmol/m^3 measured in nonoccupationally exposed groups [11, 59]. Although breath samples are more bulky to transpon than urine or blood samples and may incur fears of alcohol measurement, they can be taken even in a contaminated environment and can be corrected for different breathing styles using CO_2 adjustment. In contrast to blood sampling, breath collection is noninvasive and requires no specialized training. Unlike urine samples, breath samples can be collected at any time and in virtually any environment and provide a simpler biological matrix than blood or urine for analysis. The ACGIH BEI end-of-shift value of 1.6 g hippuric acid g creatinine in urine being adopted is likely to be confounded by background contributions of 9–94%. The BEI value of 0.05 mg toluene/l blood prior to the last shift that is currently being adopted would be considered protective of about half of the working population on the basis of our analysis.

The breath-sampling method was effective and practical in assessing the internal dose of the spray-booth worker and investigator. Whereas the average breathing-zone toluene concentration was 5 ppm, the breath levels reflected the complex, effects of the exertion rate, personal protective equipment, and work breaks on the absorbed dose. Although taken after only a single 10-h shift, both the peak breath concentration and the end-of-shift level were below the suggested 150-μmol/m^3 guideline reflecting a 50-ppm level of exposure for the low-dose worker (Fig. 9). Similarly, the pre-second-shift level was below the suggested 10-μmol/m^3 guideline.

A useful biological indicator of chemical exposure provides an accurate reflection of concentrations at a target tissue site in a time frame reflective of exposure and relevant to the potential appearance of toxicity.

Such a measure should also be rapid, noninvasive, and relatively time-insensitive and should not be contaminated by environmental or endogenous sources. Toluene breath sampling fits these requirements. After examination of the distribution of anthropometric characteristics affecting toluene toxicokinetics in our 33 episodes of controlled exposure, the PBK model was used to estimate alveolar breath concentrations reflective of exposure to 50 ppm toluene for 8 h. Breath levels of ≤10 μmol/m^3 pre-final-shift exposure and ≤150 μmol/m^3 postexposure were reflective of exposure levels of ≤50 ppm in the majority of the population.

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