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Biological Monitoring of Exposure to Organic Solvent Vapors I. A Physiological Simulation Model of m-Xylene Pharmacokinetics in Man

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Abstract: A physiological pharmacokinetic model of the transfer of organic solvents in the human body was developed. The model was comprised of seven compartments, i.e., lungs, vessel-rich tissues, vessel-poor tissues, muscles, fat tissues, gastrointestinal tissues, and liver, each being connected to the others by blood flow. The transfer of organic solvents was expressed by simultaneous differential equations, which were then solved numerically by a personal computer using a simple spreadsheet program. m-Xylene was used as the representative organic solvent. Partition coefficients of m-xylene between blood and air and between body tissue and blood were experimentally determined with blood and tissues of rats. The metabolic constants (Vmax and Km) of m-xylene metabolism and the excretion rate constant of m-methyl hippuric acid (m-MHA) were also determined using rats. These animal data were scaled up and used as the simulation parameters for humans. The results of the simulation of human exposure to m-xylene were essentially in agreement with human experimental data.

Key words: Physiologically based pharmacokinetic model, m-Xylene, m-Methyl hippuric acid, Michaelis-Menten type metabolism, Animal scale-up

INTRODUCTION

Xylene is a clear, colorless, aromatic liquid. This chemical is widely used as a solvent for paints, glues, printing inks, pesticides and adhesives, and as a component of industrial and household products[1]. The technical grade of mixed xylenes is a mixture of all three xylene isomers (o-, m-, p-) and varying amounts of ethylbenzene, in which m-xylene is the major component[2].

Recently, biological monitoring (biomonitoring) has been introduced in the field of industrial hygiene. Biological monitoring of the work environment is based on two concepts, biological exposure monitoring and biological effect monitoring[3]. Biological exposure monitoring is designed to estimate the magnitude of exposure to chemicals or their metabolites by means of their assay in biological samples, blood, urine, or expired air. In this sense, biological monitoring complements environmental monitoring, i.e., measuring the concentrations of chemicals in the work environment.

Biological effect monitoring is designed to predict the early health effects of chemical exposure through analysis of biological samples. Measurement of δ-aminolevulinic acid in urine or δ-aminolevulinic dehydratase in erythrocytes after exposure to lead, and measurement of β_2-microglobulin in urine after exposure to cadmium are examples of biological effect monitoring.

However, biological monitoring of organic solvents means only biological exposure monitoring, i.e., estimation of the level of exposure,

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since no appropriate markers are available for early assessment of their health effects.

Basic knowledge about absorption, distribution, metabolism, and excretion of the chemical is essential for interpretation of the values obtained by biological exposure monitoring. However, to date there have been only a limited number of reports[4–10] concerning the pharmacokinetics of xylene in humans.

Understanding of the pharmacokinetics of organic solvents is facilitated by the use of physiological models[11–14]. This method enables us to analyze the pharmacokinetic processes of solvents in humans by incorporating both human physiological parameters (alveolar ventilation, cardiac output, tissue volume, tissue blood flow, etc.) and physicochemical or biochemical properties of the chemical (blood/air partition coefficient, tissue/blood partition coefficients, metabolic constants, etc.). The present study was intended to develop a physiological simulation model of m-xylene pharmacokinetics in humans.

Methods

1. Physiological pharmacokinetic model for m-xylene

Our model is based on the general assumption that the body is composed of several tissue groups connected to each other via the circulation. The tissue volume and the blood flow are equivalent to those in the living body, hence the model is called "physiological".

Figure 1 shows a physiological pharmacokinetic model for m-xylene. With appropriate modifications, it can be applied to other organic solvents. The model consists of 7 compartments[15]: the lung compartment (LC) composed of the lung tissue, arterial blood, and one-third of the tidal volume[16], the vessel-rich compartment (VRC) composed of the brain, heart, kidneys and glandular tissues, the vessel-poor compartment (VPC) composed of tissues including the red bone marrow, the muscle compartment (MC) composed of mus-

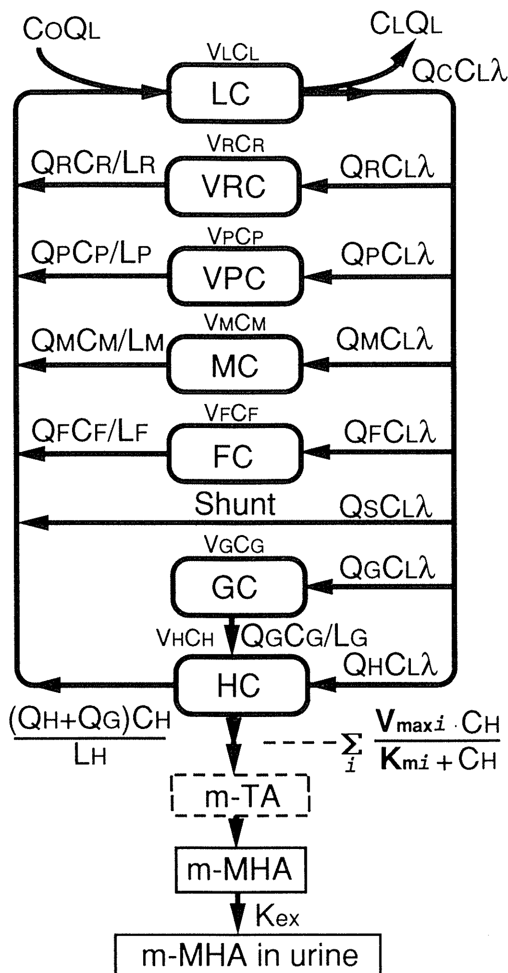

Fig. 1. A physiological pharmacokinetic model for the transfer of m-xylene. LC, lung compartment; VRC, vessel-rich tissue compartment; VPC, vessel-poor tissue compartment; MC, muscle compartment; FC, fat compartment; GC, gastrointestinal compartment; HC, liver compartment. C, concentration in mmol/*l*; V, volume of compartment in *l*; Q, flow in *l*/min; L, tissue/blood partition coefficient; λ, blood/air partition coefficient. Subscript O stands for inhaled air; C for cardiac output; L for LC; R for VRC; P for VPC; M for MC; F for FC; G for GC; H for HC. m-TA, m-toluic acid; m-MHA, m-methyl hippuric acid; Kex, excretion rate constant of m-MHA in min^{-1}.

cles and skin, the fat compartment (FC) composed of adipose tissues and yellow bone marrow, the gastrointestinal compartment (GC) composed of the portal system excluding the liver, and the hepatic compartment (HC). The muscles and skin are regarded as an individual compartment because the blood flow in these tissues changes with body movements. "Shunt" means arteriovenous shunt. These 7 compartments are connected by the blood flow system.

Several assumptions were needed to simulate the pharmacokinetics of m-xylene. First, respiration was regarded as a continuous rather than a periodic process. Next, the solvent concentration in the blood flowing out of a given compartment (venous blood) was regarded as equilibrated with the concentration in that compartment. For example, when the solvent concentration in a compartment (i) is defined as Ci, the solvent concentration in the venous blood is assumed to be Ci/Li, where Li is the tissue/blood partition coefficient for that compartment. Also, all processes other than metabolism in the liver were represented by linear expressions. No metabolism was assumed to take place except in the liver, and elimination of unchanged solvent was assumed to occur only via expired air.

The metabolism of m-xylene in the liver was expressed as a Michaelis-Menten process, i.e., $v = V_{max} \cdot C_H/(K_m+C_H)$, where v is the rate of metabolism and C_H the intrahepatic concentration of m-xylene.

Based on these assumptions, the following differential equations were obtained by applying the law of mass action to the balance of the compound in each compartment. In the lung compartment,

$$V_L \frac{dC_L}{dt} = Q_L C_O + Q_R C_R/L_R + Q_P C_P/L_P + Q_M C_M/L_M + Q_F C_F/L_F + (Q_G+Q_H)C_H/L_H - (Q_C-Q_S)\lambda C_L - Q_L C_L, \quad (1)$$

in the hepatic compartment,

$$V_H \frac{dC_H}{dt} = Q_H \lambda C_L + Q_G C_G/L_G - (Q_G+Q_H)C_H/L_H - V_{max} \cdot C_H/(K_m+C_H), \quad (2)$$

in the other compartments,

$$V_i \frac{dC_i}{dt} = Q_i \lambda C_L - Q_i C_i/L_i, \quad (3)$$

where i = R, P, M, F, G (For symbols, see the legend for Fig. 1).

An outline of the metabolic pathway of m-xylene[17] is shown at the bottom of Fig. 1. m-Xylene is metabolized primarily to m-toluic acid (m-TA), which is then conjugated with glycine and excreted as m-methyl hippuric acid (m-MHA) in the urine. The pathway from m-xylene to 2,4-xylenol accounts for only 1–2% of the total amount metabolized[7], and this pathway was ignored to simplify the model. Also, as mentioned later, conversion of m-TA to m-MHA was considered not to regulate the metabolic processes. In addition, transport of the metabolites was assumed to occur within a single compartment.

When the amount of m-MHA present in the body t min after the beginning of m-xylene inhalation is expressed as X_{MHA}, the following equation can be derived.

$$\frac{dX_{MHA}}{dt} = V_{max} \cdot C_H/(K_m+C_H) - K_{ex}X_{MHA}. \quad (4)$$

Let the amount of m-MHA excreted in urine by t min after the beginning of inhalation be U_{MHA}, then

$$\frac{dU_{MHA}}{dt} = K_{ex}X_{MHA}. \quad (5)$$

Although the differential equations (1)-(5) cannot be solved analytically, computer-assisted numerical analysis is possible. We solved these equations by Euler's method using a spreadsheet program[15]. Parameters used in the model (blood flow, tissue volume, partition coefficients, metabolic constant, initial concentration) are given in the form of a table (Model description) and shown in Fig. 2. The blood flow at rest or during exercise can be entered

MODEL DESCRIPTION					
Volume (% BW)	Lambda (tissue/blood)	Blood flow (% Qc) (rest)	(light)	InitC	Compartment
2	-	100	204.807692	0	lung
3	4.42	37.9	27.1	0	richly
8.5	2.01	6.3	3.6	0	poorly
41.5	3.01	11.4	31.8	0	muscle
21.1	77.84	5.3	7.7	0	fat
-	-	15.1	16.4	0	shunt
1.9	4.67	17.1	9.5	0	gi
2.3	3.02	6.9	3.8	0	liver
BW (kg)	(blood/air)	Qc (l)	Qc (l)		
70	26.4	5.79242393	10.4		
Km (mmol/l)	Kex (min^-1)	Km (mmol/l)			
0.33	0.012	0.033			
Vmax (mmol/min)		Vmax (mmol/min)			
0.21818505		0.02727313			

CONTROL PANEL			
start	mindt	incr	interval
0	0.01	1.02	30

SCHEDULE			
stop	conc	maxdt	activity
30	0.0020486	0.5	rest
60	0	0.5	rest
240	0.0040972	0.5	rest
300	0	0.5	light
420	0.0030729	0.5	light
480	0.00040972	0.5	light
1440	0	0.5	rest

RESULTS									
time	C0	Cl	Cr	Cp	Cm	Cf	Cg	Ch	MHA
0	0.002	0	0	0	0	0	0	0	0
0.42	0.002	4E-05	0.0002	1E-05	6E-06	5E-06	0.0002	5E-05	1E-08
1.18	0.002	8E-05	0.0014	9E-05	3E-05	3E-05	0.001	0.0003	7E-07
2.6	0.002	1E-04	0.004	0.0003	0.0001	1E-04	0.0031	0.0007	1E-05
5.22	0.002	0.0001	0.0081	0.0007	0.0003	0.0003	0.0066	0.0012	1E-04
10.1	0.002	0.0001	0.0133	0.0016	0.0006	0.0006	0.0119	0.002	0.0007
19	0.002	0.0002	0.0183	0.0032	0.0014	0.0014	0.018	0.0029	0.0037
30.2	0.002	0.0002	0.0211	0.005	0.0025	0.0025	0.0216	0.0035	0.0124
30.3	0	0.0002	0.0211	0.0051	0.0025	0.0025	0.0217	0.0035	0.0125
30.8	0	0.0001	0.0207	0.0051	0.0025	0.0025	0.0215	0.0034	0.013
31.7	0	0.0001	0.0194	0.0051	0.0026	0.0026	0.0205	0.0032	0.014
33.4	0	9E-05	0.0167	0.0051	0.0026	0.0027	0.0185	0.0028	0.0159
36.4	0	7E-05	0.0126	0.005	0.0027	0.0028	0.0149	0.0022	0.0196
42.1	0	5E-05	0.0079	0.0047	0.0028	0.003	0.01	0.0015	0.027
52.6	0	3E-05	0.0041	0.0039	0.0028	0.0032	0.0052	0.0008	0.0413
60.5	0	2E-05	0.003	0.0034	0.0027	0.0033	0.0036	0.0005	0.0519
60.6	0.0041	6E-05	0.0031	0.0034	0.0027	0.0033	0.0037	0.0006	0.0521
61.2	0.0041	0.0001	0.0043	0.0034	0.0028	0.0033	0.0045	0.0008	0.0529
62.3	0.0041	0.0002	0.0079	0.0036	0.0028	0.0034	0.0072	0.0014	0.0543

Fig. 2. Spreadsheet: "Model description," "Control panel," "Schedule," and "Results".

into this spreadsheet. These values are regarded as defaults until new values are entered. The spreadsheet includes a "Control panel" and a "Schedule" for setting values such as exposure concentration, exposure time, and differential interval (dt), which control the calculations. The results are provided as a table in "Results" (Fig. 2). The differential equations were solved with gradual increases in the differential interval (dt) to save time. In general, changes in the concentration in each compartment are large at the beginning of the simulation, requiring a small dt. However, as the simulation progresses, the changes in the concentration decrease to nearly zero (steady state), allowing step-wise increases in dt.

2. Simulation parameters

Parameters including the tissue volumes, blood flows, partition coefficients, metabolic constants (Vmax and Km) and excretion rate constant are needed to run this model. The volume and the blood flow were calculated for each compartment from the values of Davis and Mapleson[18]. The partition coefficients of m-xylene, Vmax and Km of m-xylene metabolism, and the rate constant of urinary m-MHA excretion were determined by the following experiments. The animal experiments were performed in accordance with Guidelines for Animal Experiments, Yamanashi Medical College.

1) Partition coefficients of m-xylene

The tissue/air partition coefficients were determined using tissues from adult male Wistar rats according to the method of Sato and Nakajima[19]. Tissue specimens were prepared according to Sato *et al.*[11].

2) Vmax and Km

The metabolic constant of m-xylene was determined using hepatic microsomes of adult male Wistar rats by measuring the rate of 3-methyl benzyl alcohol (MBA) formation. The microsomes were prepared according to Sato and Nakajima[20].

The reaction mixture (0.5 m*l*) contained 0.75 mg microsomal protein, 1 mM NADP, 20 mM glucose-6-phosphate (G-6-P), 2 units G-6-P dehydrogenase, and 50 mM K/K-phosphate buffer. The reaction was initiated by adding m-xylene and was stopped after 10 min by adding 0.1 m*l* each of 15% $ZnSO_4$ and saturated $Ba(OH)_2$. The mixture was then centrifuged at 3,000 rpm for 15 min. The supernatant (20 μl) was analyzed for MBA by high-performance liquid chromatography (HPLC). The HPLC operating conditions were: Column, Hitachi ODS, 4.6 mm $\Phi \times 150$ mm; mobile phase, 30% acetonitrile; flow rate, 1 m*l*/min; detection wavelength, 220 nm. Under these conditions, the production of MBA linearly increased with the microsomal protein level up to 1.0 mg and with the incubation time up to 10 min.

3) Rate constant of m-MHA excretion (Kex)

m-TA and m-MHA were administered at 0.04 mmol/rat via the tail vein to adult male Wistar rats. Urine samples were collected at predetermined intervals after administration, and the urinary m-MHA concentration was measured according to the method of Takeuchi *et al.*[21]. The urine was diluted with distilled water to 100 m*l*, and was centrifuged at 3,000 rpm for 5 min. The supernatant (20 μl) was analyzed for m-MHA by HPLC. The HPLC operating conditions were: Column, Hitachi ODS, 4.6 mm $\Phi \times 150$ mm; mobile phase, acetonitrile: distilled water: acetic acid: β-cyclodextrine = 100: 900: 15: 15; flow rate, 1 m*l*/min; detection wavelength, 228 nm.

Results

1. Partition coefficients of m-xylene

The tissue/air partition coefficients of rats obtained from this experiment are shown in Table 1.

The tissue/blood partition coefficients for humans were calculated as the rat tissue/air partition coefficients divided by the human blood/air partition coefficient (Table 2). The value reported by Sato and Nakajima[19] was used as the human blood/air partition coef-

Table 1. Partition coefficients of m-xylene. Experimental results determined using rats.

Tissue	Tissue/air (Mean±SD)	Tissue/blood
Lung	108 ± 24.1	2.70
Brain	107 ± 12.6	2.68
Heart	76.5± 21.0	1.92
Kidney	151 ± 5.61	3.78
Testis	53.2± 16.9	1.33
Muscle	79.7± 20.2	1.99
Fat	2050 ±459	51.5
Intestine	129 ± 2.72	3.23
Spleen	58.3± 17.0	1.46
Liver	79.9± 9.42	2.00
Blood	39.9± 7.18	—

ficient.

2. Vmax and Km

This experiment indicated the presence of two different pairs of Vmax and Km ($Vmax_1 = 0.6\times10^{-3}$ mmol/liver/min, Km_1 = 0.033 mmol/*l*; $Vmax_2 = 4.8\times10^{-3}$ mmol/liver/min, Km_2 = 0.330 mmol/*l*) at high and low substrate concentrations, respectively (Fig. 3). These two pairs of Vmax and Km values were used in our simulation study. The Vmax derived from rats was corrected for body surface area, $(\text{body weight})^{0.7}$, while the Km was used directly.

3. Rate constant of m-MHA excretion (Kex)

The excretion rate constant was determined from the slope of the cumulative excretion curve (Fig. 4). There was no significant difference between the m-MHA excretion rates determined after administration of m-MHA and m-TA, a finding which suggests that conversion of m-TA to m-MHA does not regulate the rate of m-MHA excretion. On the basis of this experiment, the urinary excretion rate constant (Kex) of m-MHA was determined to be 0.012 min^{-1}. This value was used in the simulation study as the value for humans.

4. Agreement between the simulated and human experimental data

Table 2 summarizes the basic parameters used in the simulation. Simulations were performed for a man weighing 70 kg who inhaled

Table 2. Simulation parameters for m-xylene pharmacokinetics in man.

Compartment	Volume[a)], *l*	Blood flow[a)], *l*/min	Partition coefficient[b)] (tissue/blood)
Lung (LC)	V_L[c)]	Qc	4.09
Vessel-rich (VRC)	0.030BW[d)]	0.379Qc	4.42
Vessel-poor (VPC)	0.085BW	0.063Qc	2.01
Muscle (MC)	0.415BW	0.114Qc	3.01
Fat (FC)	0.211BW	0.053Qc	77.8
Gastrointestinal (GC)	0.019BW	0.171Qc	4.67
Hepatic (HC)	0.023BW	0.069Qc	3.02
Shunt	—	0.151Qc	—
Blood/air partition coefficient (λ)[e)]		26.4	
Cardiac output (Qc)[a)], *l*/min		$0.296\ (BW)^{0.7}$	
Vmax[b)f)], mmol/min	$Vmax_1$ $1.394\times10^{-3}(BW)^{0.7}$		$Vmax_2$ $1.115\times10^{-2}(BW)^{0.7}$
Km[b)], mmol/*l*	Km_1 0.033		Km_2 0.330
Kex[b)], min^{-1}		0.012	
Q_L		Q_C	

a) Reference 18.
b) Experimentally determined.
c) V_L = Functional residual capacity + 1/3 of tidal volume + volume of arterial blood × λ + volume of lung tissue × lung/air partition coefficient (Reference 16).
d) Body weight in kg.
e) Reference 19.
f) Extrapolated from rat data as follows: (Vmax of rats) × (BW of humans/BW of rats)$^{0.7}$.

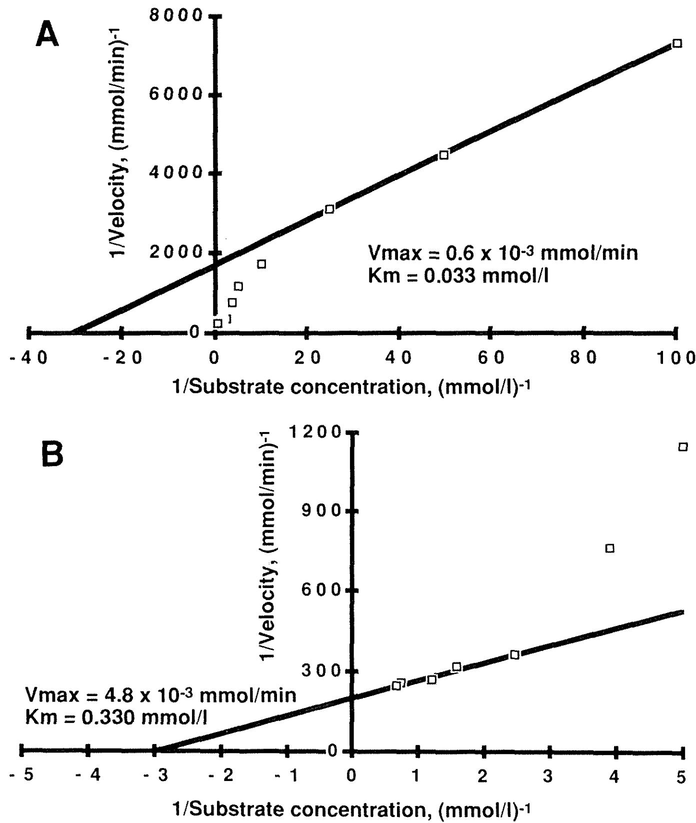

Fig. 3. Double reciprocal plots of metabolic velocity against substrate concentration. A, Vmax and Km at low substrate concentrations. B, Vmax and Km at high substrate concentrations.

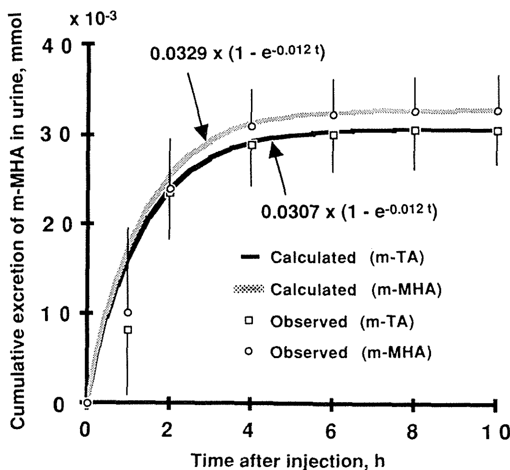

Fig. 4. m-MHA excretion after i.v. injection of m-TA and m-MHA.

100 ppm m-xylene for 6 hours (9:00–12:00 and 13:00–16:00) in accordance with the report of Riihimäki *et al.*[7]. The blood concentration was expressed as the concentration in the blood flowing out of VRC.

The values resulting from this simulation were in general agreement with the human experimental data reported by Riihimäki *et al.* (Fig. 5).

Discussion

The greatest advantage of a physiological model is that experimental data obtained from animals can be extrapolated to humans[14]. The volume of each tissue is convertible between animals and humans using the tissue-body

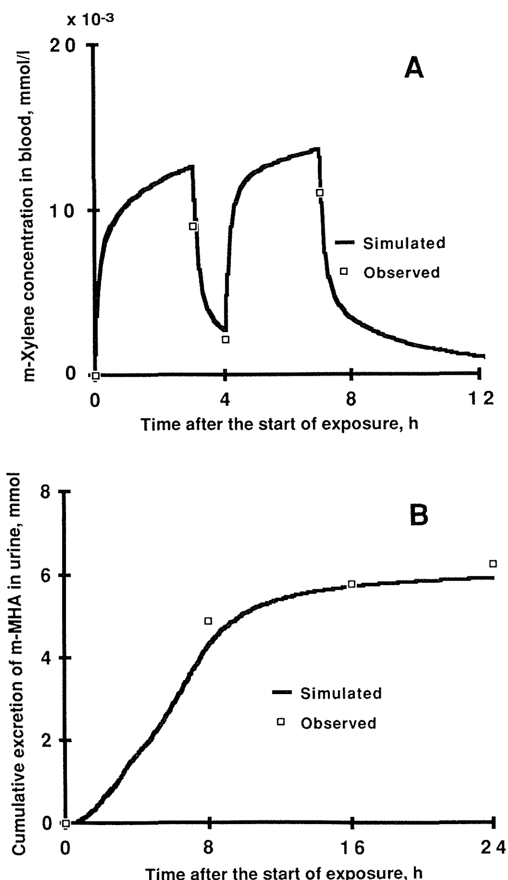

Fig. 5. Comparison between experimentally observed and simulated m-xylene pharmacokinetics in humans. The experimental data were adapted from Reference 7. A, m-xylene concentration in blood. B, m-MHA excretion in urine.

weight ratio and the blood flow from the body surface area. The metabolic constant in humans can also be estimated from the values in small animals, because the Michaelis constant (Km) is considered to be the same in both small animals and humans, and the maximum velocity (Vmax) is assumed to be proportional to the body surface area. However, since there is no appropriate method to extrapolate the urinary excretion rate constant, it is assumed that the rate constant is the same in humans and animals.

In our present model, the volume and the blood flow were calculated for each compartment from the values in the literature[18]. The partition coefficients, metabolic constants, and the rate constant of urinary m-MHA excretion were determined from animal experiments. The results of the simulation using these parameters were in general agreement with human experimental data (Fig. 5). Therefore, this model is appropriate for predicting the pharmacokinetic behavior of m-xylene in humans.

Knowledge of toxicokinetics of chemicals is the basic requirement for understanding the relationship between external and internal doses. The physiologically based pharmacokinetic model presented here can be used to gain insight into the kinetic behavior of organic solvents in humans.

Factors such as body build, physical exercise, etc. can alter the toxicokinetic profiles, and thus the relationship between external and internal doses. Physiologically based pharmacokinetic models provide us with particularly useful information in this regard. Application of our model to elucidate some kinetic aspects of human exposure to organic solvent vapors will be discussed in detail in the accompanying paper[22].

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