Physiologically Based Pharmacokinetic Modeling of Metabolic Interactions between n-Hexane and Toluene in Humans

Xiaozhong YU¹, Gunnar JOHANSON², Gaku ICHIHARA¹, Eiji SHIBATA³,
Michihiro KAMIJIMA¹, Yuichiro ONO⁴ and Yasuhiro TAKEUCHI¹

¹Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine,

²Department of Occupational Medicine-Toxicology, National Institute for Working Life, S-171 84 Solna, Sweden,

³Department of Medical Technology, Nagoya University School of Medical Sciences and

⁴Department of Public Health, Fijita Health University School of Medicine

Abstract: Physiologically based Pharmacokinetic Modeling of Metabolic Interactions Between n-Hexane and Toluene in Humans: Xiaozhong Yu, *et al.* Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine—Some animal experiments have shown that mutual metabolic inhibition takes place between n-hexane and toluene, but we have found only one report dealing with their metabolic interaction at occupationally relevant exposure levels (Bælum *et al.* 1998). In order to evaluate the effect of dose-dependent metabolic interaction between toluene and n-hexane, especially in occupationally relevant exposure conditions such as relevant exposure levels, physical activities and exposure patterns, a physiologically based pharmacokinetic (PBPK) model for co-exposure to n-hexane and toluene was developed. The PBPK model for the binary co-exposure was established by initially validating or refining the existing PBPK models for n-hexane and toluene and then linking the individual solvent models via the hepatic metabolism terms. In reporting previous findings, noncompetitive inhibition was assumed and the inhibition constant of toluene on n-hexane biotransformation and that of n-hexane on toluene biotransformation used in simulation were 7.5, 30 μm , respectively, in previous data. According to the model, 8 h of constant exposure to 50 ppm n-hexane and 25, 50, 100 and 500 ppm toluene will cause about 7%, 18%, 62% and 96% decreases in the urinary excretion of 2,5-hexanedione (2,5-HD) and 4%, 10%, 25% and 30% increases in the n-hexane concentration in blood at the end of the fifth day of exposure simulated in a standard man at a 25 W work load. Simulations of co-exposure to 50 ppm n-hexane and 50 ppm toluene

in a standard man who inhaled 50 ppm n-hexane with 0 or 50 ppm toluene for 8 h at different work loads suggest that toluene causes a slight decrease in urinary 2,5-HD in the resting condition, a 17% decrease at 25 W, and a 41% decrease at 50 W work load. The simulations of co-exposure in different exposure patterns with the same time-weighted concentration (TWA) of 50 ppm, i.e. 50 ppm for 8 h, 100 ppm of 4 times for 1 h and 200 ppm of twice for 1 h, showed reductions in urinary 2,5-HD of 17%, 40% and 67%, respectively. These simulations suggest that co-exposure to n-hexane and toluene around 50 ppm (TWA) could affect urinary n-hexane metabolites to various degrees depending on the fluctuations in exposure concentrations and variety of work activities in the workplace.

(J Occup Health 1998; 40: 293–301)

Key words: n-Hexane, Toluene, Co-exposure, Metabolic interaction, PBPK model

Multiple exposure to solvents is often observed in workplaces^{1, 2}. Toxicokinetic interactions following multiple exposure to solvents have been studied in animals as well as in humans, volunteers and occupationally exposed subjects^{3–6}. Due to toxicokinetic interactions, the behaviour of the biological exposure indicators used to assess the degrees of exposure may vary significantly in relation to working conditions^{7, 8}. n-Hexane and toluene are common solvents in workplaces^{9, 10}. Both are metabolized by the cytochrome P450 system, mainly in the liver^{11–13}. In animal experiments, Iwata *et al.*^{3, 4} showed that simultaneous inhalation of 500 ppm n-hexane and 500 ppm toluene decreased the urinary excretion of n-hexane metabolites. The exposure levels play a major role in influencing the magnitude of the metabolic interference. In a study on

Received June 26, 1998; Accepted Aug 30, 1998

Correspondence to: X. Yu, Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

the interactions between toluene and benzene in which a multi-compartmental toxicokinetic simulation model was applied to a "standard male worker", Sato¹⁴⁾ concluded that at low exposure concentrations of benzene and toluene each solvent slightly inhibits the metabolism of the other. It is frequently argued that whenever multiple exposure in the workplace is within the occupational exposure limit values, interactions rarely occur, and that in these situations the levels of the biological indicators therefore do not undergo any particular changes compared to exposure to single substances, but several reports are in conflict with these arguments¹⁵⁻¹⁷⁾. Little is known with respect to the degree to which mutual interaction affects the toxicokinetic behavior of the n-hexane and toluene, especially in occupationally relevant exposure conditions.

Physiologically based pharmacokinetic (PBPK) modeling has been used to study the extrapolation of biological data from animals to humans, variability of biological exposure indicators as well as the metabolic interactions between ethanol and trichloroethylene¹⁸⁾, toluene and m-xylene¹⁹⁾, 1,3-butadiene and styrene²⁰⁾, and ethanol and 2-butoxyethanol²¹⁾. As an effort to evaluate the effect of dose-dependent metabolic interaction between toluene and n-hexane, especially in occupationally relevant exposure conditions such as relevant exposure levels, and physical activities and exposure patterns, a physiologically based pharmacokinetic (PBPK) model for the co-exposure to n-hexane and toluene was developed.

Methods

Simulation Model

The pharmacokinetic simulation models for n-hexane and toluene were modified from Perbellini *et al.*^{22, 23)} and Ramsey and Anderson²⁴⁾. Each model is composed of five physiological compartments, as depicted in Fig. 1, including a lung compartment (LC), a vessel rich compartment (VRC), a vessel poor compartment (VPC), a fat tissue compartment (FC), and a liver tissue compartment (Liver). According to the studies on the metabolism of n-hexane^{11, 13)}, the metabolic pathway of n-hexane can be expressed briefly in two steps (Fig. 1). The first step is the

hydroxylation of n-hexane to hexanols, whereas the second step is assigned to the further metabolism of hexanols to 2,5-hexanedione (2,5-HD) and other metabolites. All metabolism and metabolic interactions were assumed to take place exclusively in the liver. The metabolisms of n-hexane to hexanols and of toluene to various metabolites were described by Michaelis-Menten kinetics. Mass balance was performed on each compartment, and the transfer of solvent throughout the system was expressed in the form of the simultaneous differential equations given in Table 1. This model is based on the assumptions that the solvent immediately

Fig. 1. Structure of PBPK model for combined inhalation exposure to n-hexane and toluene. LC: the lung compartment; VRC: the vessel-rich tissue compartment; VPC: the vessel-poor tissue compartment; FC: the fat compartment; Liver: the liver compartment; C: concentration in mg/l; L: Tissue/blood partition coefficient; Q: blood flow rate in L/min; subscripts alv and inh stand for alveolar air and inhaled air, respectively; c for cardiac output, l for lung, r for VRC, p for VPC, f for FC, h for liver, v for venous, art for arterial, hx for n-hexane, tol for toluene and 2,5-HD for 2,5-hexanedione. u_h is the conversion rate of n-hexane to hexanols. K_1 and K_2 represent the conversion of hexanols to 2,5-HD and other metabolites, respectively. K_3 describes the transfer of 2,5-HD to the water compartment and K_4 is the urinary excretion constant.

Table 1. Mass balance differential equations for n-hexane in the PBPK model. Corresponding equations for toluene can be obtained from equations 1–5.

n-Hexane in lung compartment	
$V_L \cdot dC_{art, hx}/dt = Q_{alv} \cdot C_{inh, hx} + \sum (Q_i \cdot C_{i, hx}/L_{i, hx})$	(1)
$+ Q_h \cdot C_{h, hx}/L_{h, hx} - Q_c \cdot C_{art, hx} - Q_{alv} \cdot C_{art, hx}/L_{b, hx}$	(2)
$C_{alv, hx} = C_{art, hx}/L_{b, hx}$	
n-Hexane in storage compartments	
$V_i \cdot dC_{i, hx}/dt = Q_i \cdot (C_{art, hx} - C_{i, hx}/L_{i, hx})$	(3)
$V_h \cdot dC_{h, hx}/dt = Q_h \cdot (C_{art, hx} - C_{h, hx}/L_{h, hx}) - v_{h, hx}$	(4)
Conversion of n-hexane to hexanols in liver compartment	
$v_{h, hx} = V_{max, hx} \cdot C_{h, hx} / ((K_m, hx + C_{h, hx}) (1 + C_{h, tol}/K_{i, tol}))$	(5)
2,5-Hexanedione in liver compartment	
$dA_{h, hd}/dt = K_1/(K_1 + K_2) \cdot v_{h, hx} - K_3 \cdot A_{h, hd}$	(6)
2,5-Hexanedione in water compartment	
$dA_{w, hd}/dt = K_3 \cdot A_{h, hd} - K_4 \cdot A_{w, hd}$	(7)
2,5-Hexanedione in urine	
$dA_{u, hd}/dt = K_4 \cdot A_{w, hd}$	(8)

Variables A: amount; V: volume of compartment in l. Subscripts b: blood/air; i: compartments r, f and p; u: urine; w: water compartment, Other symbols are explained in Fig. 1.

reaches a balance between alveolar air and arterial blood, and that the solvent in venous blood is in dynamic equilibrium with the corresponding tissue.

Model Parameters

Model parameters are listed in Table 2. Values for the volume and blood flow in individual compartments were assigned according to literature data^{21, 25}. The blood: air and tissue: blood coefficients of n-hexane were taken from Perbellini *et al.*²⁶, and the blood: air coefficient of toluene for humans was from Fiserova-Bergerova²⁷. The human tissue: blood coefficients were assumed to be the same as those of rats and shown by Gargas *et al.*²⁸ Values for maximum metabolic rate (V_{max}) and Michaelis's constant (K_m) for n-hexane were taken from Perbellini *et al.*²³ The V_{max} value for toluene was scaled from animal experiments according to the formula $4.8 \cdot (\text{weight})^{0.75}$ for humans, whereas K_m was assumed to be the same across species¹⁹. The conversion of hexanols to 2,5-HD and to other metabolites is defined by the rate constants K_1 and K_2 , respectively. The rate constant K_3 describes the transfer of 2,5-HD to the water compartment and K_4 is the urinary excretion rate constant. Urinary metabolites account for about 15% of the n-hexane absorbed via

Table 2. Parameters used in the PBPK model for n-hexane and toluene

Compartment	Tissue Volume ^a (l)	Blood Flow ^c (l/min)			Tissue/Blood Partition Coefficient	
		Rest	25W	50	n-Hexane	Toluene
Lung (L)	V_L^b	Q_c	Q_c	Q_c		
VRC	0.0371Wt	0.51 Q_c	0.32 Q_c	0.27 Q_c	5 ^d	4.64 ^e
VPC	0.6100Wt	0.19 Q_c	0.41 Q_c	0.51 Q_c	6.2 ^d	1.54 ^e
FC	0.2310Wt	0.05 Q_c	0.06 Q_c	0.06 Q_c	130 ^d	56.7 ^e
LIVER	0.0314Wt	0.25 Q_c	0.21 Q_c	0.16 Q_c	6.5 ^d	4.64 ^e
Parameter						
Cardiac output (Q_c) (l/min)		5.8	7.55	10.05		
Alveolar ventilation (l/min)		5.8	9	18		
Blood/air partition coefficient					0.80 ^d	16.3 ^h
V_{max} (mg/min)					0.50 ^e	1.94 ⁱ
K_m (mg/l)					0.587 ^e	0.55 ⁱ
$K_1/(K_1 + K_2)$					0.055	
K_3 (min ⁻¹)					0.003 ^e	
K_4 (min ⁻¹)					0.0009 ^e	
Inhibitory constant of toluene in relation to n-hexane ($K_{i, tol}$) (μM)					7.5 ^f	
Inhibitory constant of n-hexane in relation to toluene ($K_{i, hx}$) (μM)						30 ^f
Urine output (Q_u) (l/min)		$3.08 \cdot 10^{-5} \cdot \text{wt}^{0.82j}$				
Body weight (Wt)		70 kg				

^a From Davis and Mapleson²⁵. ^b V_L = Volume of lung (0.6 l) + volume of arterial blood (1.4 l) + FRC (2.7 l)/blood: air partition coefficient²². ^c From Johanson *et al.*²¹ ^{d, e, f} From Perbellini *et al.*^{6, 23, 26} ^g From Gargas *et al.*²⁸ ^h From Fiserova-Bergerova²⁷. ⁱ From Tardif *et al.*¹⁹ ^j From Adolph³⁸.

Fig. 2. Venous blood concentration of n-hexane during and after exposure to 100 or 200 ppm n-hexane for 4 h as suggested by the PBPK model. Spotted data are from Veulemans *et al.*³³⁾

inhalation²⁹⁾. The average excretion of 2,5-HD in workers exposed to 100 ppm n-hexane for 8 h was found to be 2.8 mg in 24 h³⁰⁾. The estimated uptake of n-hexane by the workers exposed to 100 ppm for 8 h is about 338 mg, assuming an alveolar ventilation of 10 l/min, and an alveolar retention of 20%³¹⁾, so that about 0.83% of absorbed n-hexane can be expected to be excreted as 2,5-HD, corresponding to about 5.5% of urinary n-hexane metabolites. Therefore, the value for $K_1/(K_1 + K_2)$ was set at 0.055.

Modeling of metabolic interaction

Noncompetitive metabolic interaction between n-hexane and toluene was assumed in view of the findings of Perbellini *et al.*⁶⁾ It was further assumed that n-hexane and toluene mutually interfered with each other in metabolism and that toluene affects the first step in n-hexane metabolism. The above assumptions enabled us to express the rate of n-hexane metabolism under the influence of simultaneous exposure to toluene by means of the equation

$$v_{h,hx} = (V_{max} \cdot C_{h,hx}) / ((K_m + C_{h,hx}) \cdot (1 + C_{h,tol}/K_{i,tol}))$$

where V_{max} and K_m stand for the metabolic constants of n-hexane, $C_{h,hx}$, $C_{h,tol}$ the hepatic concentrations of n-hexane and toluene, respectively, and $K_{i,tol}$ the n-hexane inhibitory constant of toluene. A similar equation may be used for the metabolism of toluene under the influence of n-hexane. The complete differential equations for the liver compartment are given in Table 1. Solutions to the sets of simultaneous differential rate equations were

Fig. 3. Simulated pharmacokinetic behavior of toluene in blood and alveolar air during and after exposure to 300 mg/m³ (80 ppm) toluene for 120 min at rest or in 50 W work load condition. Spotted data are from Carlsson's human volunteer studies³⁴⁾.

obtained by numeral integration with a spread sheet macro written in Microsoft Excel (version 5.0)³²⁾.

Results

Validation of PBPK model

The behavior of venous blood concentrations of n-hexane during and after human exposure to 100 ppm (360 mg/m³) or 200 ppm (720 mg/m³) for 4 h in the resting condition with average lung ventilation of 10.4 l/min reported by Veulemans *et al.*³³⁾ were well simulated in our PBPK model for n-hexane (Fig. 2). Furthermore, the calculated venous blood concentration and alveolar air concentration of toluene during and after 120 min exposure to 300 mg/m³ ($\approx$ 80 ppm) at rest or with a 50 W work load were in general agreement with the experimental data reported by Carlsson³⁴⁾ (Fig. 3), so it appears that our model is suitable for simulating the behavior of n-hexane and toluene in human single exposure.

Influence of toluene on n-hexane toxicokinetics

The effects of toluene + n-hexane co-exposure on n-hexane levels in venous blood were simulated in a standard man who inhaled either 50 ppm (A) or 500 ppm (B) n-hexane together with 0, 25, 50, 100, or 500 ppm toluene during very light physical exercise (25 W) for 8 h a day. The results are shown in Fig. 4. At 50 ppm n-hexane alone, the concentration in blood reached nearly 0.09 mg/l at the end of the workshift without any notable changes during one week of exposure (Fig. 4A). Co-exposure to 25 ppm toluene gave only a 4% increase in

Fig. 4. PBPK predicted effect of co-exposure to n-hexane and toluene on the n-hexane concentration in venous blood. A standard man (70 kg) inhaled 50 ppm (A) or 500 ppm (B) n-hexane with 25, 50, 100 or 500 ppm toluene for 8 h per day during light work (25 W).

blood n-hexane levels, whereas 50, 100 and 500 ppm toluene caused marked increases in blood n-hexane by 10%, 25% and 30%, respectively. At the 500 ppm n-hexane level, co-exposure to 25, 50, 100 and 500 ppm toluene caused blood n-hexane increases by 6%, 7%, 8% and 8%, respectively, as compared with single exposure (Fig. 4B).

Simulated patterns of the urinary concentrations of 2,5-HD are shown in Fig. 5. According to our model, urinary 2,5-HD reached a maximum of about 1.8 mg/l after 8 h exposure on the first day. At repeated daily 8-h exposure, 2,5-HD in urine increased gradually to a maximum of about 2.8 mg/l on the fifth day. Upon co-exposure to 50 ppm n-hexane and 25 ppm toluene, there was a 7% decrease in urinary 2,5-HD, but co-exposure to 50 or 100 ppm toluene reduced urinary 2,5-HD by 18% and 62% and 500 ppm toluene almost completely depressed it (96%) (Fig. 5A). The corresponding reductions in urinary 2,5-HD at 500 ppm n-hexane were 18%, 37%, 70% and 95% (Fig. 5B).

The simulated results of the effects of toluene on urinary 2,5-HD at different work loads are shown in Fig. 6. A standard man (70 kg) inhaled 50 ppm n-hexane with 0 or 50 ppm toluene for 8 h at 0, 25 or 50 W work load. The percentages of reduction in urinary 2,5-HD were considerably different, so that there were 8%, 17% and 41% reductions under 0, 25 and 50 W work load conditions, respectively, as compared with exposure to n-hexane only.

The effect of different patterns of co-exposure to toluene and n-hexane on n-hexane metabolites in the urine was studied by simulating a standard man (70 kg) inhaling 50 ppm n-hexane together with 0 or 50 ppm toluene, expressed as time-weighted averages for 8 h at a 25 W work load. The exposure patterns were: (A) continuous co-exposure to 50 ppm n-hexane and 50 ppm toluene for 8 h, (B) four 1-h co-exposures to 100 ppm n-hexane and 100 ppm toluene during the day, and (C) two 1-h co-exposures to 200 ppm n-hexane and 200 ppm toluene. The results showed that urinary excretions of 2,5-HD were remarkably different for the different exposure patterns. There were 17%, 40% and 67% decreases in urinary 2,5-HD in patterns A, B and C respectively, as compared to n-hexane alone (Fig. 7).

Influence of n-hexane on toluene toxicokinetics

Changes in the toluene concentration in mixed venous blood after co-exposure to toluene and n-hexane are shown in Fig. 8. Toluene in blood increased in a non-linear fashion upon co-exposure to toluene and n-hexane, so that 50 ppm toluene plus 25 and 50 ppm n-hexane caused 2% and 6% increases in blood toluene. Co-exposure to 100 and 500 ppm n-hexane, on the other hand, caused relative high increases in toluene in blood of 22% and 124%, respectively (Fig. 8A). Co-exposure to 500 ppm toluene and n-hexane (25, 50, 100, 500 ppm) induced less dramatic increases in toluene in blood (2%, 4%, 6% and 11%) (Fig. 8B).

Fig. 5. PBPK predicted effect of co-exposure to n-hexane and toluene on urinary excretion of 2,5-hexanedione. A standard man (70 kg) inhaled 50 ppm (A) or 500 ppm (B) n-hexane with 25, 50, 100 or 500 ppm toluene for 8 h a day at 25 W work load.

Fig. 6. Comparison of the effect of co-exposure to n-hexane and toluene on urinary excretion of 2,5-hexanedione in different physical activity as suggested by the PBPK model. A standard man (70 kg) inhaled 50 ppm n-hexane with 0 or 50 ppm toluene for 8 h a day at 0, 25, or 50 W work load.

Fig. 7. Predicted effect of co-exposure to n-hexane and toluene on n-hexane metabolites in urine within different exposure scenarios, all with equal time-weighted averages of 50 ppm. All scenarios are 8-h exposures of a standard man (70 kg) during light work (25 W). Scenarios: (A) Continuous exposure to 50 ppm n-hexane and 0 or 50 ppm toluene. (B) Four 1-h exposures to 100 ppm n-hexane and 0 or 100 ppm toluene. (C) Two 1-h exposures to 200 ppm n-hexane and 0 or 200 ppm toluene.

Discussion

The PBPK model for n-hexane was modified from the one presented by Perbellini²²⁾, and the results for simulation of the kinetics of n-hexane and 2,5-HD were similar to those already reported^{22, 23)}. In addition, the present model for toluene, which was scaled from a rat model, seems suitable to describe the toxicokinetics of

toluene, as suggested by comparing computer simulations with human volunteer experimental data³⁴⁾.

There are few experimental observations as to whether co-exposure to toluene and n-hexane has any significant effect on the n-hexane concentration in human blood or urinary 2,5-HD. A field survey by Saito and Shibata³⁵⁾

Fig. 8. PBPK predicted effect of co-exposure to n-hexane and toluene on toluene concentration in venous blood. A standard man (70 kg) inhaled 50 ppm (A) or 500 ppm (B) toluene with 25, 50, 100 or 500 ppm n-hexane for 8 h a day at 25 W work load.

indicated a slight decrease in urinary 2,5-HD in workers simultaneously exposed to n-hexane and toluene at levels as low as 10–40 ppm (TWA). Cardona *et al.* also observed a tendency to a decrease in urinary 2,5-HD in workplaces³⁶. Bælum¹⁵ *et al.* reported their recent study on the mutual metabolic interaction between toluene, trichloroethylene and n-hexane. Eight health male volunteers were exposed to combinations of toluene (1.5 or 4 mg/min), trichloroethylene (1.5 or 4 mg/min) and n-hexane (0.3 or 1.0 mg/min) for 60 min. The effects of the above dose rates to the liver are comparable with those obtained by inhalation of concentrations at the actual Danish occupational exposure limits (50 ppm for toluene, 30 ppm for trichloroethylene and 50 ppm for n-hexane). There was no effect of either toluene or trichloroethylene co-exposure on the excretion of 2,5-HD during a 240 min period after co-exposure, but co-exposure to n-hexane and toluene at a high rate decreased the hippuric acid excretion by 18%. The extent of hepatic metabolism of solvents *in vivo* during combined exposure is determined by the relation between the intrinsic metabolic clearances (CL_i) (In the case of noncompetitive inhibition, $CL_i = (V_{max} / ((K_m + C_{h,a}) (1 + C_{h,b}/K_i)))$, where $C_{h,a}$ and $C_{h,b}$ are the hepatic concentrations of substances A and B, respectively) and the hepatic blood flow³⁷. When CL_i is much larger than hepatic blood flow, the blood flow rate limits the metabolism. Conversely, when CL_i is considerably smaller than hepatic blood flow, the

enzyme capacity becomes the rate limiting factor. The liver has a high capacity for metabolism of many organic solvents. Thus, at low exposure levels, blood flow limits the metabolism and metabolic interactions will not modify the overall clearance, neither the toxicokinetics or biological exposure indicators, to any significant extent. At high exposure levels, on the other hand, where metabolism approaches saturation, enzyme capacity becomes rate limiting, and interactions will affect the overall clearance more clearly. This suggests that there is a “threshold” level below which metabolic interaction is negligible. Obviously, it is of importance to identify such “thresholds” in the interpretation of biological monitoring data. Thus, for example, in controlled laboratory studies in volunteers Tardif *et al.*⁸ observed a slowdown in toluene metabolism manifested by an increased concentration of toluene in blood and reduced urinary excretion of hippuric acid during 4 h co-exposure to 95 ppm toluene and 80 ppm xylene, but no changes in urinary metabolites were seen during 7 h exposure to 50 ppm toluene and 40 ppm xylene.

The present PBPK simulations suggested that during combined exposure to 50 ppm n-hexane and 25 ppm toluene, the rate limiting step of metabolism is liver blood flow rather than intrinsic metabolic capacity. The effects of metabolic interactions on urinary 2,5-HD might be under the intra- or extra-individual variations, and therefore their interactions were hardly detected in

experiments or field studies. Nevertheless, during co-exposure to 50 ppm n-hexane and 50 ppm toluene the intrinsic metabolic capacity became critical, and the effects of metabolic interactions on urinary 2,5-HD could not be neglected, but there are still other factors affecting the degree of metabolic interaction during n-hexane and toluene co-exposure. Physical activity results in dramatic changes in alveolar ventilation, cardiac output and distribution of arterial blood, thus causing profound changes in the absorption and distribution of solvents. Our PBPK model therefore suggested that the metabolic interactions between 50 ppm n-hexane and 50 ppm toluene were not so dramatic in the resting condition, but became noticeable as work intensity increased. In addition, there are great fluctuations in exposure levels in workplaces. Our simulations with different exposure patterns suggest that the urinary excretion of 2,5-HD decreases noticeably by about 67% after two 1-h co-exposure to 200 ppm n-hexane and 200 ppm toluene but only marginally (17%) at continuous 50 ppm co-exposure for 8 h. Considering great fluctuations in exposure concentrations and a variety of work activities in actual workplaces, co-exposure to n-hexane and toluene could reduce urinary 2,5-HD even at concentrations lower than occupational exposure limits. These factors should be considered when the binary metabolites are used for biological exposure indices for n-hexane and toluene under co-exposure conditions.

It should be mentioned that in our simulations inhibition constants (K_i) were derived from rat experiments *in vitro*. Obviously, species differences between rats and man exist. In addition, several problems still remain when trying to extrapolate results for animals to man, short term *in vivo* and *in vitro* experiments to long term exposure conditions. The simulations presented here, however, suggest that dose-dependent interactions of n-hexane and toluene could occur in occupationally relevant exposure conditions. Such knowledge is valuable for biological monitoring as well as for risk assessment in combined exposure to n-hexane and toluene.

Acknowledgment: This work was supported in part by a Grant-in-aid for Research (No. 08770269) from the Japanese Ministry of Education, Science, Sports and Culture and by a Grant from the Hori Information Promotion Foundation.

References

- Ikeda M, Kasahara M. n-Hexane and benzene contents in gasoline for industrial purpose. *Ind Health* 1986; 24: 63-66.
- Inoue T, Takeuchi Y, Hisanaga N, Ono Y, Iwata M, Ogata M, Saito K, Sakurai H, Hara I, Matsushita T and Ikeda M. A nationwide survey on organic solvent components in various solvent products. Part 1 Homogeneous products such as thinners, degreasers and reagents. *Ind Health* 1983; 21: 175-183.
- Iwata M, Takeuchi Y, Hisanaga N, Ono Y. Changes of n-hexane neurotoxicity and its urinary metabolites by long term co-exposure with MEK or toluene. *Int Arch Occup Environ Health* 1984; 54: 273-281.
- Iwata M, Takeuchi Y, Hisanaga N, Ono Y. Changes of n-hexane metabolites in urine of rats exposed to various concentrations of n-hexane and to its mixture with toluene or MEK. *Int Arch Occup Environ Health* 1983; 53: 1-8.
- Tardif R, Goyal R, Brodeur J. Effect of simultaneous exposure to toluene and xylene on their respective biological exposure indices in human. *Int Arch Occup Environ Health* 1991; 63: 279-284.
- Perbellini L, Leone R, Fracasso ME, Brugnone F, Venturini MS. Metabolic interaction between n-hexane and toluene in vivo and in vitro. *Int Arch Occup Environ Health* 1982; 50: 351-358.
- Shibata E, Huang J, Ono Y, Hisanaga N, Iwata M, Saito I, and Takeuchi Y. Changes in urinary n-hexane metabolites by co-exposure to various concentrations of methyl ethyl ketone and fixed n-hexane levels. *Arch Toxicol* 1990; 64: 165-168.
- Tardif R, Goyal R, and Brodeur J. Assessment of occupational health risk from multiple exposure: review of industrial solvent interaction and implication for biological monitoring of exposure. *Toxicol Ind Health* 1992; 8: 37-52.
- Iwata M, Takeuchi Y, Hisanaga N, Ono Y. A study on biological monitoring of n-hexane exposure. *Int Arch Occup Environ Health* 1983; 51: 253-260.
- Kumai M, Koizumi A, Saito K, Sakurai H, Inoue T, Takeuchi Y, Ogata M, Matsushita T and Ikeda M. A nationwide survey on organic solvent components in various solvent products. Part 2 Heterogeneous products such as paints, inks and adhesives. *Ind Health* 1983; 21: 185-197.
- Morohashi K, Sadano H, Okada Y, Omura T. Position specificity in n-hexane hydroxylation by two forms of cytochrome P 450 in rat liver microsomes. *J Biochem Tokyo* 1983; 93: 413-419.
- Nakajima T, Wang RS. Induction of cytochrome P450 by toluene. *Int J Biochem* 1994; 26: 1330-1340.
- Toftgard R, Haaparanta T, Eng L, Halpert J. Rat lung and liver microsomal cytochrome P 450 isozymes involved in the hydroxylation of n-hexane. *Biochem Pharmacol* 1986; 35: 3733-3738.
- Sato A. The effect of environmental factors on the pharmacokinetic behaviour of organic solvent vapours. *Ann Occup Hyg* 1991; 35: 525-541.
- Bælum J, Mølhav L, Hansén SH, Væth M (1998) Metabolic interaction between toluene, trichloroethylene and n-hexane in humans. *Scand J Work Environ Health* 24: 30-37.
- Wallen M, Holm S, Byfalt Nordqvist B. Coexposure to toluene and p-xylene in man: uptake and elimination. *Brit J Ind Med* 1985; 42: 111-116.
- Jacobowski M, kostrezewski P. Excretion of Methylbenzoic acid in urine a result of single and combined exposure to m-xylene. *Pol J Occup Med* 1986; 2: 238-247.

- 18) Sato A, Endoh K, Kaneko T, Johanson G. Effects of consumption of ethanol on the biological monitoring of exposure to organic solvent vapours: simulation study with trichloroethylene. *Brit J Ind Med* 1991; 48: 548–556.
- 19) Tardif R, Lapare S, Krishnan K, and Brodeur J. Physiologically based modelling of the toxicokinetic interaction between toluene and m-xylene in the rat. *Toxicol Appl Pharmacol* 1993; 120: 266–273.
- 20) Filser JG, Johanson G, Kessler W, Kreuzer PE, Stei P, Baur C, Csanady GA. A pharmacokinetic model to describe toxicokinetic interactions between 1,3-butadiene and styrene in rats: predictions for human exposure. *IARC Sci Publ* 1993; 127: 65–78.
- 21) Johanson G. Physiologically based pharmacokinetic modeling of inhaled 2-butoxyethanol in man. *Toxicol Lett* 1986; 34: 23–31.
- 22) Perbellini L, Mozzo P, Brugnone F, Zedde. Physiologicomathematical model for studying human exposure to organic solvents: kinetics of blood/tissue n-hexane concentrations and of 2,5-hexanedione in urine. *Brit J Ind Med* 1986; 43: 760–768.
- 23) Perbellini L, Mozzo P, Olivato D, Brugnone F. "Dynamic" biological exposure indexes for n-hexane and 2,5-hexanedione, suggested by a physiologically based pharmacokinetic model. *Am Ind Hyg Assoc J* 1990; 51: 356–362.
- 24) Ramsey JC, Andersen ME. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol Appl Pharmacol* 1984; 73: 159–175.
- 25) Davis MR, Mapleson WW. Structure and quantification of physiological model of the distribution of injected agents and inhaled anaesthetics. *Brit J Anaesth* 1981; 53: 399–405.
- 26) Perbellini L, Brugnone F, Caretta D, Maranelli G. Partition coefficient of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. *Brit J Ind Med* 1985; 42: 162–167.
- 27) Fiserova-Bergerova. Gases and their solubility: a review of fundamentals. In: Fiserova-Bergerova, eds. *Modeling of inhalation exposure to vapor; uptake, distribution and elimination Vol I*. Florida: CRC Press, Inc. Boca Raton. 1992: 3–28.
- 28) Gargas ML, Burgess RJ, Voisard DE, Cason GH, and Andersen ME. Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. *Toxicol Appl Pharmacol* 1989; 98: 87–99.
- 29) Mutti A, Falzoi M, Lucertini S, Arfini G, Zignani M, Lombardi S, Francini I. n-Hexane metabolism in occupational exposure. *Brit J Ind Med* 1984; 41: 533–538.
- 30) Perbellini L, Bartolucci GB, Brugnone F, De Rosa E, Valentini F. 2,5-esandione nel controllo biologico dell'esposizione professionale a n-esano. *Med Lav* 1985; 76: 35–43.
- 31) Perbellini L, Brugnone F, Mozzo P, De Rosa E, Bartolucci GB, Facinni G. Toxicokinetic aspects of n-hexane and 2,5-hexanedione in the biomonitoring of occupational exposure to n-hexane. *Ann Am Conf Ind Hyg* 1985; 12: 357–364.
- 32) Johanson G, Näslund PH. Spreadsheet programming: a new approach in physiologically based modelling of solvent toxicokinetics. *Toxicol Lett* 1988; 41: 115–127.
- 33) Veulemans H, Van Vlem E, Janssens H, Masschelein R, Leplat A. Experimental human exposure to n-hexane. Study of the respiratory uptake and elimination, and of n-hexane concentrations in peripheral venous blood. *Int Arch Occup Environ Health* 1982; 49: 251–263.
- 34) Carlsson A. Exposure to toluene, uptake, distribution and elimination in man. *Scand J Work Environ Health* 1982; 8: 43–55.
- 35) Saito I, Shibata E, Hisanaga N, Takeuchi Y, Huang J. Relationship between n-hexane and urinary 2,5-hexanedione of workers in shoe and bag factories. *Jpn J Ind Health* 1992; 34: 831.
- 36) Cardona A, Marhuenda D, Marti J, Brugnone F, Roel J, Perbellini L. Biological monitoring of occupational exposure to n-hexane by measurement of urinary 2,5-hexanedione. *Int Arch Occup Environ Health* 1993; 65: 71–74.
- 37) Sato A, Johanson G. Physiological factors affecting the behaviour of organic solvent vapors in humans. In: Cheremisinoff PN, eds. *Encyclopedia of environmental control technology*. Vol 7. High hazard pollutants. Gulf Publishing Company, Houston, Texas, 1995: 53–80.
- 38) Adolph EF. Quantitative relations in the physiological constitutions of mammals. *Sci* 1949; 109: 579–585.