Toxicokinetic Modeling of the Combined Exposure to Toluene and *n*-Hexane in Rats and Humans

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Abstract: Toxicokinetic Modeling of the Combined Exposure to Toluene and *n*-Hexane in Rats and Humans: Nabeel ALI, *et al.* Département de médecine du travail et d'hygiène du milieu, Faculté de médecine, Université de Montréal—We studied the toxicokinetic fate of toluene (TOL) and *n*-hexane (HEX) in rats exposed to mixtures of these solvents by inhalation during 4 hours. We developed and validated a physiologically based toxicokinetic (PBTk) model for this binary mixture in the rat, which after proper scaling, was used to predict the impact of combined exposure to TOL and HEX in humans. Simultaneous exposure to TOL (125–300 ppm) did not affect the kinetics of HEX (100 or 800 ppm) in blood in rats. Coexposure to HEX, however, increased the concentration of TOL in blood. Coexposure strongly reduced the urinary excretion of 2,5-hexanedione (HD) (up to 75% reduction for TOL 300 ppm + HEX 100 ppm) whereas the urinary excretion of HA and o-CR were reduced to a lesser extent (up to 28% reduction for TOL 200 ppm + HEX 100 ppm). Simulations of exposure to various TOL-HEX mixtures with the human PBTk model suggested that at concentration corresponding to their respective exposure limit values (TOL: 50 ppm; HEX: 50 ppm) coexposure during 7 hours would result in only slight effects on their respective kinetics. This study showed 1) that exposure indicators such as the blood concentration of unchanged TOL and HEX or the amount of their respective urinary metabolite(s) are not equally sensitive to the occurrence of a metabolic interaction and, 2) that such difference of sensitivity can be predicted a priori using a PBTk modeling approach.

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Key words: Toluene, *n*-Hexane, Coexposure, Toxicokinetic Modeling, Biological monitoring, Metabolic interaction

Toluene (TOL) and *n*-hexane (HEX) are used together in several industrial applications (e.g., adhesive compounds, and paints) and therefore workers are likely to be exposed simultaneously to these two solvents.

Both have neurotoxic properties. While TOL has been shown to exert its main toxic effect through perturbation of the central nervous system¹⁾, HEX rather affects the peripheral nervous system through the production of a toxic metabolite, namely 2,5-hexanedione (HD)²⁾.

Each is known to be mainly metabolized by different forms of cytochrome P-450, including CYP 2E1 and CYP 2B6^{3–8)}. Perbellini *et al.*⁹⁾ reported that a mutual non-competitive interference existed between TOL and HEX under *in vitro* conditions. In addition, it was shown that coexposure to TOL decreased the toxic effect of HEX on the peripheral nerve, an effect that has been ascribed to the decrease in the production of HD¹⁰⁾.

The objectives of the present study were 1) to investigate the toxicokinetic fate of TOL and HEX in rats exposed to mixtures of these solvents by inhalation and, 2) to verify whether such potential impact could be described/predicted using a physiologically based toxicokinetic (PBTk) modeling approach.

Materials and methods

Animals

Adult Sprague-Dawley male rats (Charles River Canada, St-Constant, Québec) weighing approximately 250–280 g were used in this study. Prior to use and during experiments, the animals were housed in a humidity- and temperature-controlled room with a 12-h light/dark cycle. The animals were not provided with food and water during inhalation exposures.

Chemicals

HEX (99%), TOL (99.8%) and HD (98%) were obtained from Aldrich Chemicals (Milwaukee, Wisc.) and were used as supplied.

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Inhalation exposures

Groups of 5 or 6 rats were exposed during 4 h (9.00 A.M.–13.00 P.M.) to TOL or HEX alone, or to several mixtures containing both solvents (Table 1). Exposures took place in a dynamic exposure chamber of 500-l (Unifab, Kalamazoo, Mich.) for which the airflow was set at approximately 300 l/min. Solvents were delivered with a high-pressure liquid chromatography (HPLC) pump (Varian Canada, Montréal) into the inlet ducting of the chamber where they were mixed with clean compressed air. Solvent concentration was monitored by injecting 1.0 ml of the chamber atmosphere into a gas chromatograph as previously described¹¹.

Determination on unchanged solvents in blood

The concentration of TOL and HEX in blood was measured at various time points (5, 30, 60, 90, 120 and 150 min) following the end of exposure. Determinations were made by head-space analysis using a Tekmar® head-space sampler and a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector according to a procedure described elsewhere¹¹. The blood analysis was conducted immediately after the blood sampling.

Determination of urinary metabolites

Urine was collected during a period of 24 h beginning at the onset of inhalation exposure. Non-conjugated and total HD were measured by gas-chromatography¹². The content of hippuric acid (HA) was measured by HPLC according to the method proposed by Poggi *et al.*¹³ The concentration of o-cresol (o-CR) was determined by gas chromatography with flame ionization detection according to a method developed by Truchon *et al.*¹⁴

PBTK modeling of single and combined exposures in rats

A PBTK model was constructed for each solvent individually. Each PBTK model consisted of four compartments as described by Ramsey and Andersen (1984)¹⁵: liver, fat, slowly perfused tissues, richly perfused tissues (Fig. 1). The individual models were developed by obtaining the physiological, physicochemical and biochemical parameters in the literature (Table 2). Metabolism was limited to the liver compartment and described as a saturable process. Metabolic constants of HEX (Vmax: 1.35 mg/kg/hr; Km: 0.4 mg/l) were obtained by optimization and correspond to the values that best describe the blood concentration-time course curves for HEX obtained following exposure of rats to 100, 200 and 800 ppm of HEX (Fig. 2). The PBTK model for the binary mixture of TOL and HEX consisted of two individual models linked via the metabolism term (Fig. 1) which was modified to test various mechanisms of metabolic interaction. This was achieved by modifying the basic saturable hepatic

Table 1. Summary of the various treatments administered in this study

Group no. ^a	TOLUENE (ppm)	n-HEXANE (ppm)
Single exposures		
1	0	100
2	0	200
3	0	800
4	125	0
5	200	0
6	300	0
Mixed exposures		
7	125	100
8	200	100
9	300	100
10	100	800

^a Animals were exposed by inhalation during 4 h.

metabolism term to accommodate three types of metabolic inhibition mechanisms, namely, competitive, non-competitive and uncompetitive¹⁶. Urinary excretion of HD in the rat was modeled by incorporating the excretion constants used by Yu *et al.*²² to describe such excretion in humans, except that the percentage of HEX metabolized converted to hexanols in rats had to be set at 21%. Urinary excretion of o-CR was described assuming that 0.7% of toluene metabolized is converted to o-CR and that o-CR half-life in urine is 3.5 h²⁸.

PBTK modeling of TOL-HEX interaction in humans

The structure of the PBTK model used to describe the kinetics of TOL and HEX in humans was identical to the one developed for the rat. The numerical values of the species-specific model parameters, however, were replaced with those for humans. The approach involved (i) substituting rat physiological parameters and blood: air partition coefficients with those for human, (ii) scaling the Vmax on the basis of body weight^{0.75}, and (iii) keeping all other model parameters species-invariant. The human and rat PBTK model for TOL have been used in previous studies^{16, 17}. The human PBTK model for HEX allowed a good description of the concentration of unchanged HEX measured in venous blood of volunteers exposed to 100 and 200 ppm of HEX during 4 h (data not shown)¹⁸. In addition, the model successfully predicted the alveolar concentration of HEX (experimental: ~ 41 ppm; predicted: 43 ppm) in volunteers exposed to 60 ppm of HEX during 15.5 min¹⁹. The rat and human interaction PBTK models were used to simulate the impact of simultaneous exposure to TOL and HEX on their respective kinetics in both species.

Fig. 1. Physiologically based toxicokinetic (PBTK) model for the mixture of TOL and HEX. CV_{hex} and CV_{tol} refer to the venous blood leaving the liver compartment; Ki_{th} and Ki_{ht} refer to constants describing uncompetitive inhibition of the metabolism of one solvent by the other. RAM refers to rate of TOL or HEX metabolism in the liver; Vmax and Km refer to the maximal velocity of metabolism and Michaelis constant.

Model simulation

The algebraic and differential equations describing the toxicokinetics of TOL and HEX were written as a program and solved with a commercially available software, namely ACSL (Advanced Continuous Simulation Language; Mitchell and Gauthier Associates, Huntsville, AL). Optimizations were performed using ACSL Optimize v.2.1. Program (Mitchell and Gauthier Associates, Huntsville, AL).

Statistics

Differences between various treatments on the blood concentration of TOL and HEX and on the amount of metabolites excreted in urine were tested using one-way ANOVA or Student-*t* test. The level of significance was set at 0.05.

Results

Single exposure to HEX

Figure 2 depicts the venous blood concentration of unchanged HEX obtained in rats exposed during 4 h to either 100, 200 or 800 ppm of HEX. It shows that the PBTK model for the rat allows a good description of the blood kinetics of HEX under these exposure conditions. The best fit of experimental data was achieved when the values of Vmax and Km were set at 1.35 mg/kg/hr and 0.4 mg/l, respectively.

Combined exposure to TOL and HEX

Unchanged solvents in blood

Simultaneous exposure to TOL does not affect the kinetics of unchanged HEX in blood of rats exposed to 100 or 800 ppm of HEX (Fig 3A, B). Conversely, the

Table 2. Physiological parameters, partition coefficients and metabolic constants used in the PBTK models for toluene and *n*-hexane

Parameters	Values	
	Rat ^a	Human ^b
Alveolar air ventilation (liters/h/kg)	15.0	18.0
Cardiac output (liters/h/kg)	15.0	18.0
Fraction of cardiac output corresponding to each compartment		
Fat	0.09	0.05
Slowly perfused tissues	0.15	0.25
Richly perfused tissues	0.51	0.44
Liver	0.25	0.26
Fraction of body weight corresponding to each compartment		
Fat	0.09	0.19
Slowly perfused tissues	0.72	0.62
Richly perfused tissues	0.05	0.05
Liver	0.049	0.026
Partition coefficients	HEX ^d	TOL ^d
Blood: air (rat)	2.29	18.0
Blood: air (human)	0.80 ^c	15.6 ^f
Fat: air	159.0	1021.0
Slowly perfused tissues: air	2.9	27.7
Richly perfused tissues: air	5.2	83.6
Liver: air	5.2	83.6
Metabolic constants		
V _{max} (mg/h/kg)	1.35 ^e	4.80 ^a
K _m (mg/liter)	0.40 ^e	0.55 ^a

^a From Tardif *et al.* (1993)¹⁶. ^b From Arms and Travis (1988)²³ and Kaneko *et al.* (1981)²⁴.

^c From Perbellini *et al.* (1985)²⁶. ^d From Gargas *et al.* (1989)²⁵. ^e This study. ^f Sato (1991)²⁷.

Fig. 2. Comparison of PBTK model simulations (lines) of venous blood concentrations of HEX with experimental data (symbols) obtained in rats exposed for 4 h to 3 concentrations of HEX. Each data point represents the mean ($\pm$ SD) of 5 rats.

Fig. 3. Comparison of PBTK model simulations (lines) of venous blood concentrations of HEX with experimental data (symbols) obtained in rats exposed for 4 h to TOL and HEX singly or in combination. Each data point represents the mean ($\pm$ SD) of 5 rats.

Fig. 4. Comparison of PBTK model simulations (lines) of venous blood concentrations of TOL with experimental data (symbols) obtained in rats exposed for 4 h to TOL and HEX singly or in combination. Each data point represents the mean ($\pm$ SD) of 5 rats.

concentration of TOL in blood of rats exposed to 100 or 200 ppm of TOL is increased by co-exposure to (100 or 800 ppm) HEX. These results are in accordance with the PBTK model predictions (Fig. 4A, B).

Excretion of urinary metabolites

Coexposure to TOL strongly reduces the urinary excretion of HD (Fig. 5). The reduction of unconjugated

(free) HD is 60%, 66% and 74% when exposure concentration of TOL is 125, 200 or 300 ppm, respectively. For total HD, the corresponding values are 54%, 74% and 78%. On the other hand, as depicted in Figure 6, the urinary excretion of HA and o-CR is also reduced but to a lesser extent (10% to 28%) (not statistically significant) as a result of coexposure.

Fig. 5. Unjugated and total amount of HD excreted in urine during 24 h in rats exposed to HEX alone and to combinations with TOL. Each data represents the mean ($\pm$ SD) of 6 rats (*: $P \leq 0.05$).

Fig. 6. Amount of HA and o-CR excreted in urine during 24 h in rats exposed to TOL alone and to combinations with HEX. Each data represents the mean ($\pm$ SD) of 6 rats (A: TOL 125 ppm + HEX 100 ppm; B: TOL 200 ppm + HEX 100 ppm; C: TOL 300 ppm + HEX 100 ppm).

The extent of reduction of the amount of HEX and TOL metabolized provided by the PBTK model for the binary mixture is close to what was observed experimentally in rats (Table 3).

Discussion

The present study indicates that combined inhalation exposure to TOL and HEX resulted in toxicokinetic interference probably as a result of mutual metabolic interaction between them. Perbellini *et al.*⁹⁾ have reported that simultaneous oral administration of TOL and HEX resulted in metabolic interference; combined exposure decreased the urinary excretion of HEX metabolites

whereas that of TOL was not modified. These authors suggested that the ability of TOL to interfere with HEX metabolism might explain the observations made by Takeuchi *et al.*¹⁰⁾ who reported that rats treated with HEX-TOL mixture showed slighter neurotoxic effect compared to rats which received HEX only. Likewise, Iwata *et al.*¹²⁾ also reported that inhalation coexposure to high concentrations TOL (1,000 ppm) and HEX (1,000 ppm) during 8h caused an important reduction of the production of HEX metabolites, including HD. More recently, Baclum *et al.*²⁰⁾ reported the results of a human study suggesting that metabolic interactions are likely to occur between TOL, HEX and trichloroethylene at

Table 3. Excreted amount of 2,5-hexanedione (HD) and o-cresol in urine measured in rats compared to that predicted by the PBTK model following exposure to various mixtures of TOL and HEX

Treatments	Measured Amount ($\mu\text{g}/24\text{ h}$)	Predicted Amount ($\mu\text{g}/24\text{ h}$)
2,5-Hexanedione ¹⁾		
HEX 100 ppm	151.1 $\pm$ 19.8 (100%)	141.7 (100%)
HEX 100 ppm + TOL 125 ppm	69.0 $\pm$ 10.7 (46%)	59.6 (42%)
HEX 100 ppm + TOL 200 ppm	38.9 $\pm$ 9.6 (26%)	50.7 (36%)
HEX 100 ppm + TOL 300 ppm	33.5 $\pm$ 7.5 (22%)	38.9 (28%)
o-Cresol		
TOL 125 ppm	34.6 $\pm$ 17.8	45.2
TOL 200 ppm	60.1 $\pm$ 9.6	65.9
TOL 300 ppm	86.1 $\pm$ 8.5	85.6
TOL 125 ppm + HEX 100 ppm	28.1 $\pm$ 6.1 (81%) ²⁾	34.4 (76%)
TOL 200 ppm + HEX 100 ppm	43.6 $\pm$ 14.0 (72%)	53.4 (81%)
TOL 300 ppm + HEX 100 ppm	77.5 $\pm$ 24.1 (90%)	68.4 (80%)

¹⁾ Total 2,5-Hexanedione: free + conjugated. ²⁾ Percentage of the excretion that resulted from single exposure to toluene.

Table 4. Metabolic inhibition constants (Ki) according to various mechanisms of metabolic inhibition among toluene and n-hexane

Inhibitor	Substrate	Ki (mg/liter)		
		C ^a	NC ^b	UC ^c
TOL	HEX	1.11	2.08	0.69
HEX	TOL	0.059	0.28	0.22

^a C: competitive, ^b NC: non-competitive, ^c UC: uncompetitive.

concentrations corresponding to their inhalation exposure limits.

PBTK modeling provided a good description of the blood kinetics of both solvents in rats exposed to various mixtures. Such a good fit was achieved whatever the mechanism of inhibition tested (i.e., competitive, non-competitive, uncompetitive) simply by adjusting by optimization the value of the inhibition constants (Table 4). Based on these values, non-competitive or uncompetitive inhibition appeared to be a plausible mechanism of interaction.

This study shows that exposure indicators such as the blood concentration of unchanged solvent or the amount of a urinary metabolite are not equally sensitive to the occurrence of a metabolic interaction. Indeed, exposure to mixtures increased the blood concentration of TOL and did not affect significantly the production of TOL metabolites. On the other hand, although the blood concentration of HEX remained unchanged, exposures

to mixtures caused a dramatic reduction of the urinary excretion of HD. Such difference in indicator sensitivity has recently been reported for another mixture containing *m*-xylene and methylchloroform²¹⁾.

The human PBTK model was used to simulate exposure to various TOL-HEX mixtures. Figures 7 show that at concentrations corresponding to their respective exposure limit values (TOL: 50 ppm; HEX: 50 ppm) coexposure during 7 h results in only slight effects on their respective kinetics. Figure 8 shows that human exposure to mixtures of TOL and HEX, at concentrations corresponding to those tested in the present study with rats, would result in significant reduction of urinary excretion of TOL and HEX metabolites. However, at their respective exposure limit such reduction would be considerably less. In this regard, recently Yu *et al.*²²⁾, using a PBTK modeling approach, showed that human exposure to mixtures of TOL and HEX, at concentrations corresponding to their respective exposure limits, could affect the urinary excretion of HD, in a dose-dependant fashion. The slight reduction predicted in their study is in accordance with the observation made in the present study showing that urinary HD is more sensitive to TOL exposure than unchanged HEX in blood.

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Fig. 7. Blood concentrations of HEX and TOL predicted by the PBTK model (uncompetitive inhibition) in a man (body weight: 70 kg) exposed to various concentrations of TOL and HEX, alone or in combination during 7 h.

Fig. 8. Model predictions of the percentage metabolized [(amount metabolized/amount inhaled) $\times$ 100] at the end of 7 hr-exposure to various mixtures, in a man (body weight: 70 kg).

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