International Journal of Occupational Medicine and Environmental Health 2007;20(2):155–166
DOI 10.2478/v10001-007-0012-6

PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELING OF DURENE (1,2,3,5-TETRAMETHYLBENZENE) AND ISODURENE (1,2,4,5-TETRAMETHYLBENZENE) IN HUMANS

PIOTR JAŁOWIECKI[1,2] and BEATA JANASIK[1]

[1] Department of Biological Monitoring, Nofer Institute of Occupational Medicine
Łódź, Poland

[2] Faculty of Econometry and Informatics, Warsaw Agricultural University (SGGW)
Warszawa, Poland

Abstract

Objectives: Physiologically-based toxicokinetic (PB-TK) models are developed to simulate absorption, distribution and excretion of xenobiotics. PB-TK models consist of several groups of compartments, where tissues are grouped together according to physiological parameters (tissue blood flows, tissue group volumes) and physicochemical properties (partition coefficients, metabolic constants). Tetramethylbenzene (TETMB), a mixture of its three isomers: prenitene (1,2,3,4-TETMB), isodurene (1,2,3,5-TETMB), and durene (1,2,4,5-TETMB) is an essential component of numerous commercial preparations of organic solvents. The aim of the study was to develop the PB-TK model for two TETMB isomers, durene and isodurene, in humans. **Materials and Methods:** The assumed PB-TK model groups organs and tissues into five physiological compartments: fat tissue, muscles, organs, liver, and brain. The brain has been considered as a separate compartment due to the potential neurotoxicity of TETMB. Water/air, oil/air and blood/air partition coefficients for durene and isodurene were measured *in vitro.* Tissue/air partition coefficients were calculated from values of olive/air and water/air partition coefficients and the average fat and water content in different tissues. Tissue/blood partition coefficients were calculated as a tissue/air quotient and the blood/air partition coefficient measured *in vitro.* The Michaelis-Menten constant (K_M) values and maximum metabolism rate constant (V_{MAX}) for selected metabolites of durene and isodurene were obtained *in vitro* using microsomal fraction of the human liver. **Results:** The developed model was validated against experimental data obtained earlier as a result of an 8-h exposure of volunteers to durene and isodurene vapors of 10 and 25 mg/m^3. The prediction of both TETMB isomers concentration in blood as well as of the elimination rates of 2,4,5-TMBA and 2,3,5-TMBA were close to the results of experimental exposures. **Conclusions:** Simulations of one working week inhalation exposure to aromatic hydrocarbons indicate that the elaborated PB-TK model may be used to predict the chemical distribution in different body compartments, based on physicochemical properties.

Key words:
Tetramethylbenzene, Toxicokinetic, Physiologically-based toxicokinetic (PB-TK) model

INTRODUCTION

Physiologically-based toxicokinetic (PB-TK) models are developed to simulate absorption, distribution and excretion of xenobiotics. PB-TK models consist of several groups of compartments where tissues are grouped together according to physiological parameters (tissue blood flows, tissue group volumes) and physicochemical properties of the studied chemical (partition coefficients, metabolic constants).

Received: November 2, 2006. Accepted: March 19, 2007.
Address reprint requests to P. Jałowiecki PhD, Faculty of Econometry and Informatics Warsaw Agricultural University (SGGW), Nowoursynowska 166, 02-787 Warszawa, Poland (e-mail: pjalowiecki@mors.sggw.waw.pl).

Tetramethylbenzene (TETMB) in the form of its three isomers mixture: prenitene (1,2,3,4-TETMB), isodurene (1,2,3,5-TETMB), and durene (1,2,4,5-TETMB) is an essential component of numerous commercial preparations of organic solvents (Solvesso, Shellsol) used in different technical processes in many kinds of industry [1,2]. The aim of this study was to develop the PB-TK model for two TETMB isomers, durene and isodurene, in humans. The results of PB-TK modeling are the first data obtained for these compounds.

MATERIALS AND METHODS

Chemicals

Durene and isodurene (98% pure) were purchased from Sigma-Aldrich Co. (Poznań, Poland). NADPH was provided by Sigma-Aldrich Co. (Poznań, Poland). All other reagents and chemicals were obtained from commercial chemical corporations at the highest purity available.
2,4,5-TMBA (2,4,5-trimethylbenzoic acid) and 2,3,5-TMBA (2,3,5-trimethylbenzoic acid) (97% pure) were synthesized in the Technical University in Łódź, Poland.

Apparatus

Gas chromatographs HP 5890 Series II Plus and 6890 with a flame ionization detector were used. The capillary column used to analyze metabolites, durene and isodurene, was HP-5 (Crosslinked 50 m × 0.32 μm × 1.05 μm)

Volunteers and exposure conditions

The subjects were four male volunteers, aged 30–39 years, with no history of occupational exposure to durene and isodurene. The local Bioethical Committee approved the study protocol and the volunteers gave their written informed consent to participate in the study. They also underwent medical examinations before exposure to exclude health problems and were insured.
The experimental conditions were that of an exposure chamber where the subjects were exposed at rest to durene and isodurene. The capacity of the exposure chamber was 11.7 m^3 and air turnover rate was 350 m^3/h.
The subjects were exposed to durene and isodurene vapors of 10 and 25 mg/m^3 for 8 h. Capillary blood samples obtained from the finger tips were collected before the onset of exposure and in the chamber 15 and 30 min and 1, 2, 4 and 8 h after the beginning of exposure as well as 3, 6, 9, 15 and 30 min and 1, 2, 4, 6, 8, 25, 32, 49, 56 and 73 h after termination of exposure. Urine samples were collected before exposure, during the exposure, 2, 4, 6 and 8 h after the onset and 2, 4, 6, 8, 15, 19, 23, 27, 31, 39, 43, 47, 51, 55, 63, 67, 71, 75, 79, 83, 87 and 95 h after termination of exposure.

Determination of individual parameters

Michaelis-Menten constant (Km) and maximum metabolism rate constant (V max): Incubation procedures

Microsomes from human livers were obtained from GENTEST ABD Bioscences Company (USA) (cytochrome P450 = 0.8 mg/ml and total protein 20 mg/ml). Reaction solution contained a microsomal fraction (5 μl, 0.1 mg of protein), phosphate buffer (1.0M pH 7.4) $MgCl_2$ (1.6 mM) and NADPH (0.8 mM) in a final volume of 2 ml. The reaction was initiated by adding 100 μl of polyethylene 600 (PEG/H_2O, 1:1 v/v) with durene and isodurene in final concentration levels of 5, 10, 15 mM/l. The vials were closed and the mixture was incubated at 37°C for 90 and 120 min for durene and isodurene, respectively, with constant shaking. The reaction time was empirically determined. Reactions were stopped by addition of 0.5 ml HCl (6N).

2,4,5- and 2,3,5-TMBA in microsomal fraction mixture

Determination of 2,4,5- and 2,3,5-TMBA were performed by gas chromatography (HP5890, FID detector) using the method described for metabolites. After termination of incubation of the mixture (2 ml), 0.2 g of NaCl was added. Then extraction of metabolites was carried out twice with 7.5 ml of ethyl ether for 10 min. Organic layer was evaporated to dryness. The residue was silylated for 30 min in 70°C with 0.2 ml of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Next, ethyl ether (1.5 ml) was added and aliquot (1 ml) was used for injection into the gas chromatograph.

Urine 2,4,5- and 2,3,5-TMBA

2,4,5- and 2,3,5-TMBA concentrations were determined by gas chromatography (HP5890 Series II Plus, FID, HP-5 50 m × 0.32 μm × 1.05 μm) in the following operation conditions: carrier gas (helium) flow, 30 ml/min; hydrogen, 30 ml/min; air, 300 ml/min; injector, 200°C; and detector, 220°C.

The internal standard solution (ß-naftol c = 80 mg/l) in the volume of 0.5 ml was added to a glass vial and evaporated under a stream of air. Then 2 ml of urine was added and hydrolyzed in water bath with 2 ml of 11 mol NaOH at 100°C. After hydrolysis 5 ml of 6nH_2SO_4 was added. After saturation with 0.4 g of NaCl, metabolites were extracted with 10 ml of ethyl ether for 10 min. Organic layer was evaporated to dryness. The residue was silylated for 15 min with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Then 1.5 ml of ethyl ether was added and 1ml of aliquot was used for injection into the gas chromatograph [3].

Blood durene and isodurene

Durene and isodurene in blood were determined by gas chromatography (HP6890, FID, HP-5 50 m × 0.32 μm × × 1.05 μm) with the headspace technique in the following operation conditions: carrier gas (helium) flow, 30 ml/min; hydrogen, 30 ml/min; air, 300 ml/min; injector, 170°C; and detector, 220°C.

Capillary blood (0.1 ml) and 0.2 ml of the internal standard solution (chlorobenzene) were added to glass vials (2 ml) and closed with teflon plugs. Solutions were shaken for 30 min in a water bath at 70°C, then 1ml of the gas phase was sampled with a gastight syringe and injected into the chromatograph.

The analytical parameters for the aforesaid procedures are presented in Table 1.

Partition coefficients

Water/air, olive/air and blood/air partition coefficients for durene and isodurene were obtained *in vitro* according to the method described by Gargas [4] and Sato [5]. Tissue/air partition coefficients were calculated from the values of olive/air and water/air partition coefficients and the average fat and water contents defined in different tissues [6–8]. Tissue/blood partition coefficients were calculated as the tissue/air quotient and the blood/air partition coefficient measured *in vitro.*

Model structure

PB-TK models for durene and isodurene were developed in two steps. First, the preparation of a general model, which assumed an inhalation way of absorption, and contained a number of body compartments. Second, the preparation of models for durene and isodurene by ascribing the experimentally obtained and calculated values of partition coefficients and metabolic constants for TETMB isomers and its selected metabolites. In the assumed PB-TK model, organs and tissues are grouped together into six compartments according to blood flow and fat content. The model also contains three auxiliary modules for inhalation, absorption, transport and TETMB distribution via blood. Physiological compartments comprise: fat tissue, muscles, organs, liver, and brain. The auxiliary modules are lungs, arterial, and venous blood (Fig. 1). The lung module includes lung tissues and heart. The brain was considered as a separate compartment due to the potential neurotoxicity of tetramethylbenzene.

Inhalation absorption, penetration from lungs to blood, and excretion of the parent TETMB with exhaled air are the responsibility of the lung module. Change of TETMB

Table. 1. Analytical parameters of the methods

Compounds	Concentration range	Imprecision RSD ±%	Limit of detection (μg/l)
Isodurene (blood)	50–200 μg/l	5.0	27.0
2,4,5-Dimethylbenzoic acid (urine)	10–800 mg/l	5.9	0.74 mg/l
Durene (blood)	50–200 μg/l	6.8	30.0
2,3,5-Dimethylbenzoic acid (urine)	10–800 mg/l	1.7	1.06 mg/l

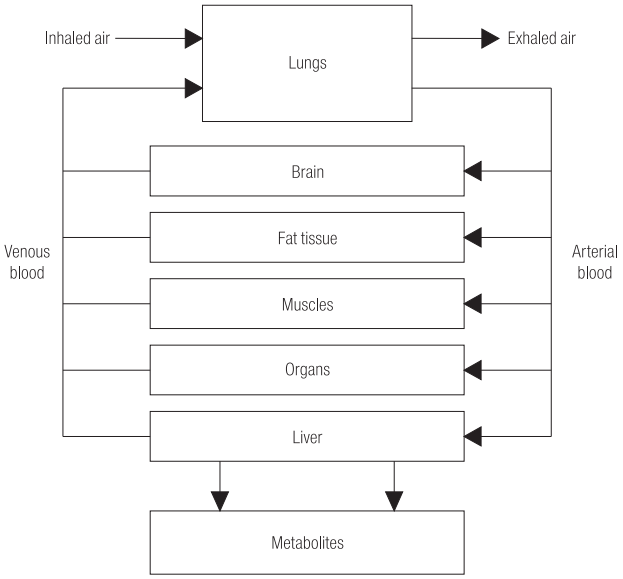

Fig. 1. The structure of the main physiologically-based toxicokinetic (PB-TK) model used in durene and isodurene simulations.

concentration in arterial blood is described by differential equation (1).

$$\frac{dC_{Blood}}{dt} = \frac{Q_{Alveolar} \times \left(C_{Air} - \frac{C_{Blood}}{P_{Blood/Air}} \right) + \sum_{Tissue} Q_{Tissue} \times \frac{C_{Tissue}}{PC_{Tissue/Blood}} - Q_{Blood} \times C_{Blood}}{V_{Blood}} \quad (1)$$

Fat tissue, muscles, organs, and brain compartments absorb TETMB and accumulate in tissue groups. Change of tetramethylbenzene concentration in these compartments is described by differential equation (2).

$$\frac{dC_{Tissue}}{dt} = \frac{Q_{Tissue} \times \left(C_{Blood} - \frac{C_{Tissue}}{P_{Tissue/Blood}} \right)}{V_{Tissue}} \quad (2)$$

Liver is a compartment, in which metabolic transformations of TETMB and elimination of its metabolites with urine take place. Change in TETMB concentration in this compartment is described by differential equation (3). All symbols used in equations (1), (2), and (3) are presented in Table 2.

$$\frac{dC_{Liver}}{dt} = \frac{Q_{Liver} \times \left(C_{Blood} - \frac{C_{Liver}}{PC_{Liver/Blood}} \right) - \frac{V_{Max} \times C_{Liver}}{K_M + C_{Liver}}}{V_{Liver}} \quad (3)$$

Table 2. Symbols used in mass-balance differential equations of the PB-TK model

C_{Air}	—	TETMB concentration in inhaled air
C_{Blood}	—	TETMB concentration in blood
C_{Tissue}	—	TETMB concentration in compartment (fat, muscles, organs, brain)
C_{Liver}	—	TETMB concentration in liver compartment
$Q_{Alveolar}$	—	Lungs alveolar ventilation
Q_{Blood}	—	Cardiac output
Q_{Tissue}	—	Blood flow to compartment (fat, muscles, organs, brain)
Q_{Liver}	—	Blood flow to liver compartment
V_{Blood}	—	Lungs compartment volume
V_{Tissue}	—	Compartment (fat, muscles, organs, brain) volume
V_{Liver}	—	Liver compartment volume
$PC_{Blood/Air}$	—	Blood/air partition coefficient
$PC_{Tissue/Blood}$	—	Compartment/blood partition coefficient
$PC_{Liver/Blood}$	—	Liver/blood partition coefficient
K_M	—	Michaelis-Menten constant for metabolism
V_{Max}	—	Maximum rate of metabolism

Simulation conditions

Three kinds of model simulations were performed. The first simulation described the dynamics of durene and isodurene concentrations in blood and excretion of their metabolites in urine after an 8-h inhalation exposure at concentrations of 10 and 25 mg/m^3.

The second simulation described the dynamics of the same parameters within the weekly work cycle of 5 working days (8 h/day of exposure and 2 days free of exposure). The aim of these simulations was to assess possible accumulation of durene and isodurene as well as selected metabolites in the body. The third simulation grouped computer simulations of selected aromatic hydrocarbons (toluene, xylene, trimethylbenzene, tetramethylbenzene) with different quantities of methyl groups with the aim to predict their levels in the central nervous system (CNS).

Software

The described PB-TK model was written, compiled, and tested using SSPA Simnon (ver. 3.0) simulation program. To provide a comparative prediction, the model was transferred and simulated using Berkeley Madonna (ver. 8.0.1) simulation software.

RESULTS

Tissue/blood partition coefficients were calculated as the quotient of tissue/air and blood/air partition coefficient measured *in vitro* (Table 3).

Table 3. Values of partition coefficients for durene and isodurene

Partition coefficient	Durene	Isodurene
Blood/air	206.00	152.00
Brain/blood	40.15	29.31
Fat tissue/blood	311.89	227.56
Muscles/blood	27.39	20.00
Organs/blood	21.91	16.00
Liver/blood	19.73	14.41

Model parameters

In the PB-TK model for durene and isodurene, different physiological parameters of the human body were involved: alveolar lungs ventilation, cardiac output, blood flows in all compartments, and volumes of all compartments. The values of physiological parameters in the model were calculated for adult males (average parameters of volunteers: height, 180 cm and weight, 80 kg) (Table 4).

Values of metabolic parameters: Michaelis-Menten constant (K_M) and maximum metabolism rate constant (V_{MAX}) for 2,4,5-TMBA (selected metabolite of durene) and 2,3,5-TMBA (selected metabolite of isodurene) were obtained *in vitro* using microsomal fraction of human liver according to the procedure described by Ligocka et al. [9, point 2.1].

Table 4. The values of physiological parameters in main PB-TK model (at rest)

Parameter	Value
Alveolar lungs ventilation	543.0 dm^3/h
Cardiac output	321.0 dm^3/h
Blood flow to brain	45.6 dm^3/h
Blood flow to fat tissue	15.6 dm^3/h
Blood flow to muscles	65.4 dm^3/h
Blood flow to organs	99.6 dm^3/h
Blood flow to liver	94.8 dm^3/h
Heart and lungs volume	1.37 dm^3
Brain volume	1.5 dm^3
Fat tissue volume	12.2 dm^3
Muscles volume	39.8 dm^3
Organs volume	1.23 dm^3
Liver volume	3.9 dm^3

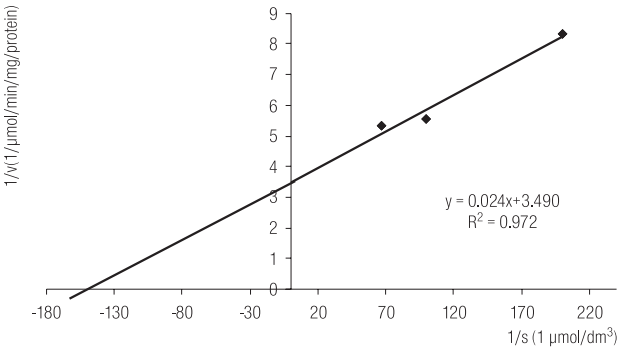

Fig. 2. Lineweaver-Burke curves of *in vitro* metabolites of durene 2,4,5-trimethylbenzoic acid hydroxylation.

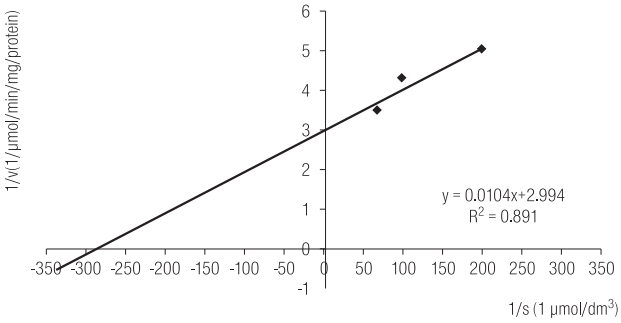

Fig. 3. Lineweaver-Burke curves of *in vitro* metabolites of isodurene 2,3,5-trimethylbenzoic acid hydroxylation.

The experimentally obtained Lineweaver-Burke curves are presented in Figures 2 and 3.

Kinetic parameters for 2,4,5-TMBA, K_M = 6.09 µmol/dm^3; V_{MAX} = 2.20 µmol/min and 2,3,5-TMBA: K_M = 3.89 µmol/dm^3; V_{MAX} = 3.0 µmol /min were calculated from Lineweaver-Burke curves (Figs. 2 and 3).

Comparison of computer simulation results with experimental data for durene and isodurene

The 8-h computer-simulated inhalations of durene and isodurene in concentrations of 25 mg/m^3 were prepared with use of the described PB-TK model. The results of simulated durene and isodurene elimination from blood as well as their selected metabolites (2,4,5-TMBA and 2,3,5-TMBA, respectively) in urine were compared

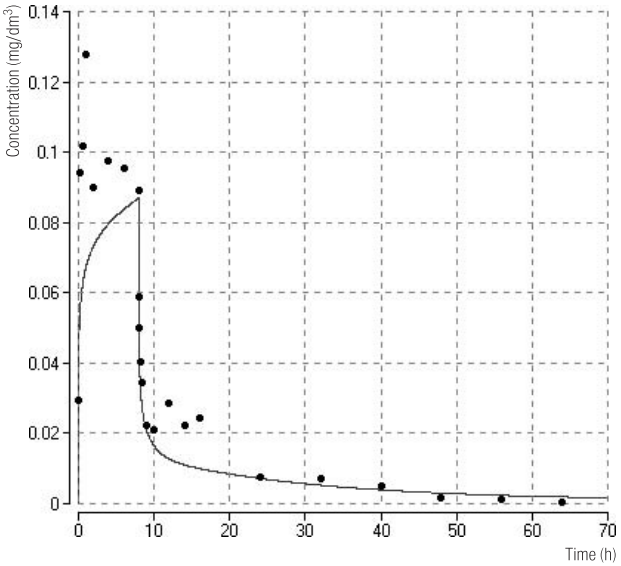

Fig. 4. Experimental (points) and predicted (line) durene concentrations in capillary blood during and after an 8-h inhalation exposure to 25 mg/m^3 of durene.

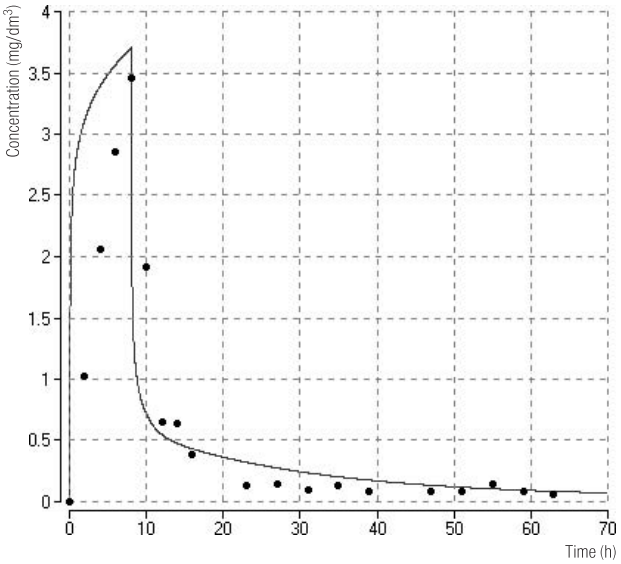

Fig. 5. Experimental (points) and predicted (line) 2,4,5-TMBA elimination rates in urine during and after an 8-h inhalation exposure to 10 mg/m^3 of durene.

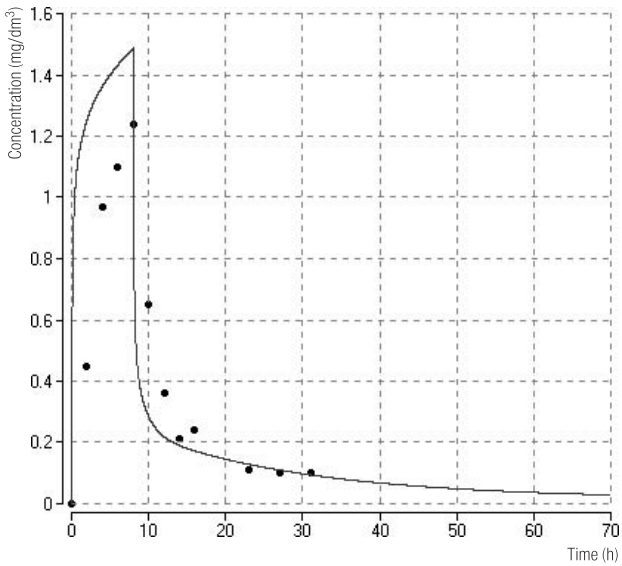

Fig. 6. Experimental (points) and predicted (line) 2,4,5-TMBA elimination rates in urine during and after an 8-h inhalation exposure to 25 mg/m^3 of durene.

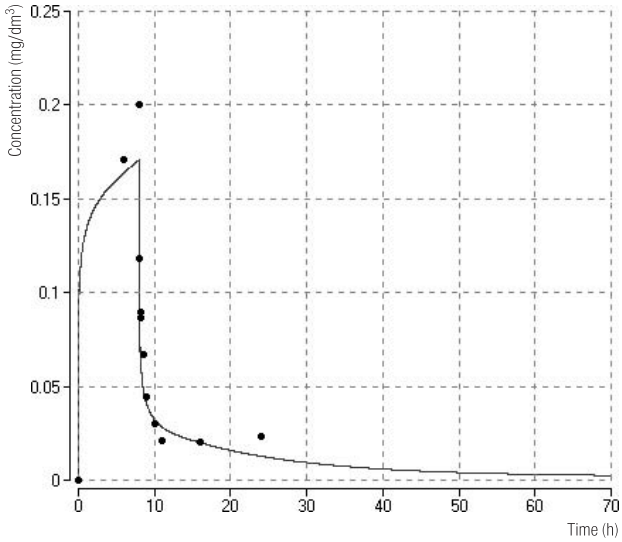

Fig. 7. Experimental (points) and predicted (line) isodurene concentrations in capillary blood during and after an 8-h inhalation exposure to 25 mg/m^3 of isodurene.

with the data obtained from an 8-hour experimental inhalation of volunteers (Figs. 4–9).

Assessment of possible accumulation of durene, isodurene, and trimethylbenzoic acids

The one week computer-simulated inhalations of durene and isodurene (25 mg/m^3) were performed using the described PB-TK models. The results of simulated durene and isodurene elimination from blood as well as their selected metabolites (2,4,5-TMBA and 2,3,5-TMBA, respectively) in urine were used to assess a possible accumulation of these chemicals in the body (Figs. 10–13).

The accumulation tendency may be described by comparing blood concentration of parent TETMB or the rate of

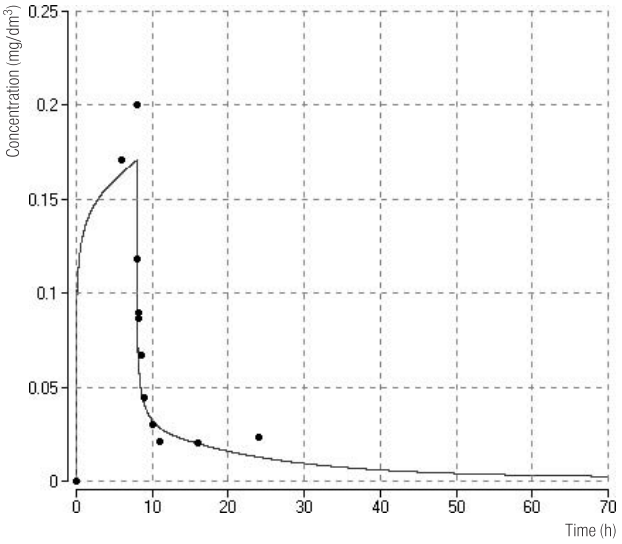

Fig. 8. Experimental (points) and predicted (line) 2,3,5-TMBA elimination rates in urine during and after an 8-h inhalation exposure to 10 mg/m^3 of isodurene.

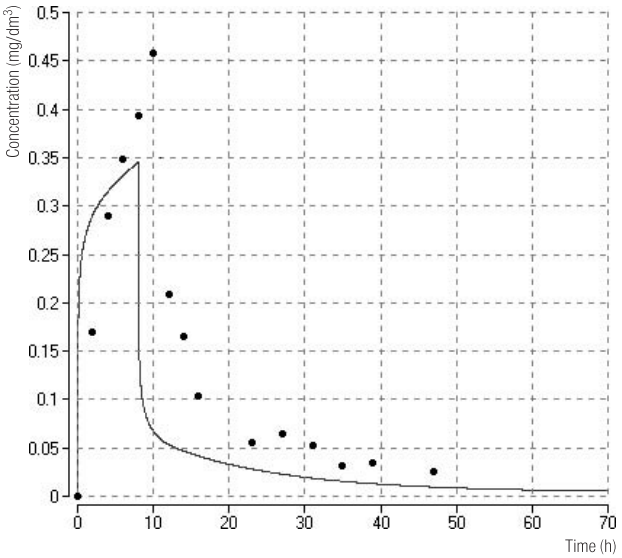

Fig. 9. Experimental (points) and predicted (line) 2,3,5-TMBA elimination rates in urine during and after an 8-h inhalation exposure to 25 mg/m^3 of isodurene.

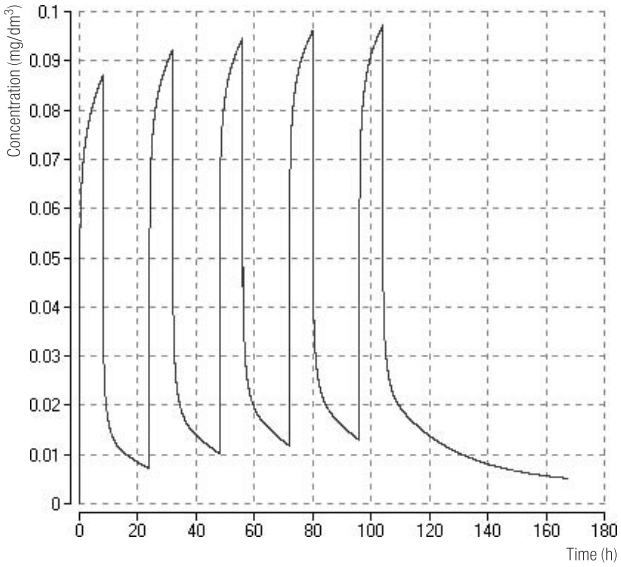

Fig. 10. Durene concentration in blood during exposure to 25 mg/m^3 of durene in the weekly work cycle.

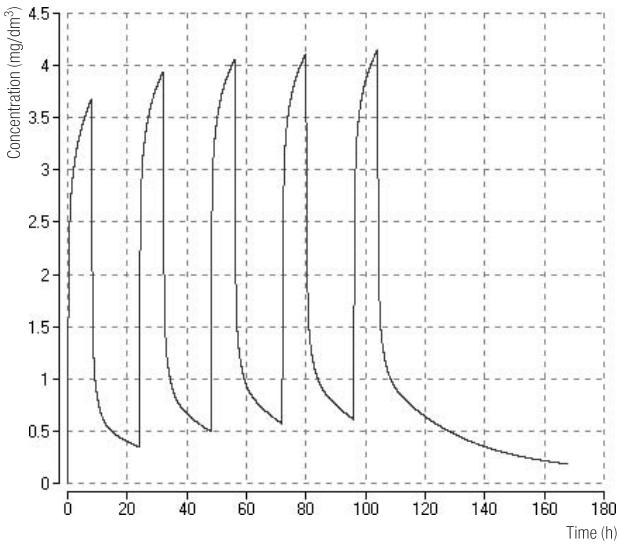

Fig. 11. The rate of 2,4,5-TMBA elimination in urine during exposure to 25 mg/m^3 of durene in the weekly work cycle.

changes in excretion of its metabolites in urine one and five days after the exposure cycle [10,11]. This quotient is presented in equation (4).

$$\frac{\mu_\infty}{\mu_1} = \frac{\sum_{i=1} \mu_i \times L_i}{\sum_{i=1}^{n} \mu_i} \qquad (4)$$

Li and μi parameters from equation (4) may be determined from equation (5), which describes the kinetics of parent TETMB elimination from blood or its metabolites in urine, according to a multi-compartment model [10,11].

$$\frac{dC}{dt} = \sum_{i=1}^{n} \mu_i \times e^{-k_i \times t} \quad \text{where} \quad L_i = \frac{1}{1 - e^{-24 \times k_i}} \qquad (5)$$

The increase in the rate of the metabolites elimination amounted to 11% for 2,4,5-TMBA, and to 7% for 2,3,5-TMBA at the end of the fifth day as compared to the first day of exposure.

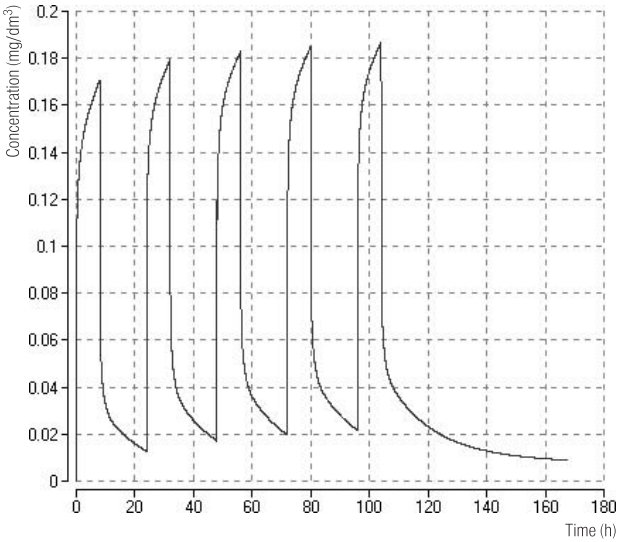

Fig. 12. Isodurene concentration in blood during exposure to 25 mg/m^3 of isodurene in the weekly work cycle.

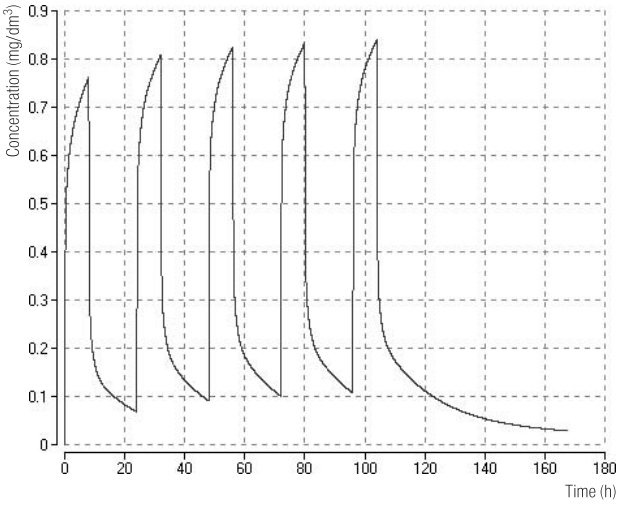

Fig. 13. The rate of 2,3,5-TMBA elimination in urine during exposure to 25 mg/m^3 of isodurene in the full weekly work cycle.

The increase in concentrations of unchanged durene and isodurene in blood amounted to 12% and 8%, respectively.

Computer simulation of aromatic hydrocarbons in the brain

The central nervous system is a target system for many aromatic hydrocarbons [12–15]. Thus, the described PB-TK model was used to compare the distribution of aromatic hydrocarbons with an increasing number of methyl groups in the human body. For this purpose, one-working-week simulations of inhalation exposures to toluene, xylene,

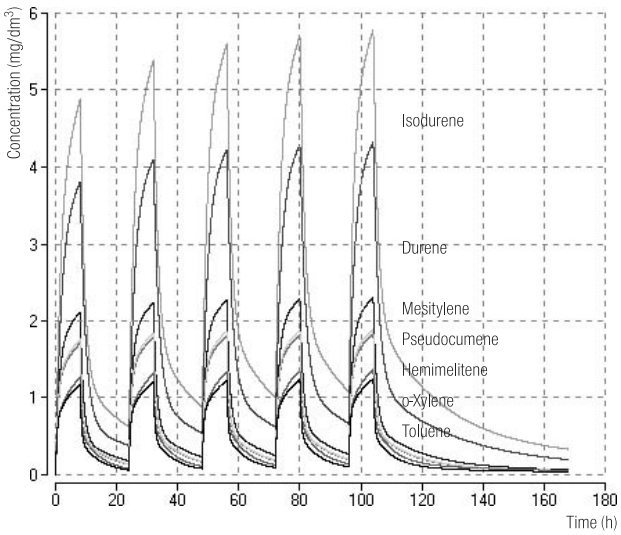

Fig. 14. Comparison of predicted aromatic hydrocarbon concentrations in the brain resulting from one-working-week simulations of inhalation exposure to the concentration of 25 mg/m^3.

trimethylbenzene, and tetramethylbenzene in concentrations of 25 mg/m^3 were assumed. To conduct these simulations, the toxicokinetic data known from the literature and obtained in this study, were used [4,5,16].

The obtained results indicate a possible influence of the number of methyl groups and resulting solubility in lipids on the CNS concentration of aromatic hydrocarbons. At the end of the fifth day of exposure, their predicted brain concentrations were as follows: toluene — 1.24 mg/dm^3, o-xylene — 1.37 mg/dm^3, hemimelitene — 1.84 mg/dm^3, pseudocumene — 1.89 mg/dm^3, mesitylene — 2.30 mg/dm^3, durene — 4.32 mg/dm^3, and isodurene — 5.89 mg/dm^3 (Fig. 14).

DISCUSSION

The main aim of our study was to develop, elaborate, and verify the PB-TK model for durene and isodurene. In the course of its development, SSPA Simnon, and Berkeley Madonna simulation software were used. The detailed description of methodology of the PB-TK model construction was presented earlier [17–19].

The values of physiological parameters (alveolar lungs ventilation, cardiac output, blood flow to selected compartments) and their volumes were calculated based on the literature data for men with height of 180 cm, and weight of 80 kg (average parameters of volunteers) [7,20–22].

So far the values of liquid phase/air partition coefficients for durene, and isodurene as well as for K_M and V_{MAX} have not been published. Partition coefficients between air and blood, olive oil, and 0.9% NaCl values were obtained by means of the headspace technique according to Gargas [4] and Sato [5]. The values of tissue/blood partition coefficients for durene and isodurene were calculated, based on experimentally determined liquid phase/air partition coefficients values, and according to known percentage of lipid and water fractions in different groups of tissues [6,8,23,24].

The results concerning the elimination of unchanged durene and isodurene from blood, and excretion of their selected metabolites, 2,4,5-TMBA and 2,3,5-TMBA, in urine resulted from the study in a group of volunteers. Inhalation exposures to durene and isodurene were conducted in concentrations significantly lower (10 and 25 mg/m^3) than the Polish occupational exposure limit (OEL) value for trimethylbenzene (100 mg/m^3).

The values of metabolic parameters, Michaelis-Menten constant and maximum metabolism rate for selected durene and isodurene metabolites, 2,4,5-TMBA, and 2,3,5-TMBA, were determined using a microsomal fraction of the human liver, and calculations of kinetic parameters of metabolic reactions using the Lineaver-Burke graphical methods [9,25,26].

The construction of the PB-TK model for durene and isodurene was based on the determination and calculation of the values of parameters presented above. The next step was to validate this model against experimental data obtained earlier in the study of volunteers exposed to durene and isodurene in concentrations of 25 mg/m^3. The predicted rates of blood concentration of both TETMB isomers and elimination of its metabolites, 2,4,5-TMBA, and 2,3,5-TMBA, were very similar to those of experimental exposures.

To evaluate the influence of durene and isodurene accumulation in the body on possible results of biomonitoring of exposure, simulations of inhalation exposure during one-weekly-work cycle (5 working days, 8 h/day) were performed. The results of predictions indicated about 10% increase in blood concentrations of unchanged compound and elimination of metabolites in urine at the end of the fifth day. This means that in practice accumulation will not exert a significant effect on the possibility of evaluation of internal exposure on the basis of determination of unchanged compounds in blood or metabolites in urine in samples collected any day of exposure [27].

The elaborated PB-TK model may be used to predict distribution of volatile organic compounds (VOCs) in different body compartments, based on physicochemical properties. The relationship between the amount of methyl groups in benzene ring of aromatic hydrocarbons and CNS concentration was confirmed.

The predicted data on a possible accumulation of aromatic hydrocarbons related to the number of methyl groups are in agreement with the experimental data on the effect of aromatic hydrocarbons on CNS in rats [14,28,29]. The results of the 4-h inhalation exposures to TETMB isomers [14], xylene [28,29], and toluene indicated a significantly stronger neurotoxic effect for trimethylbenzene isomers, than for toluene, and xylene. The neurotoxic effect of xylene was stronger than that of toluene. The values of EC_{50} for TMB (trimethylbenzene) isomers were similar: 3779 mg/m^3 for hemimelitene, 4693 mg/m^3 for pseudocumene, and 4738 mg/m^3 for mesitylene [14]. The EC_{50} value for xylene was about two times higher (8658 mg/m^3) and for toluene about four times higher (15 262 mg/m^3) than for trimethylbenzene isomers [28,29].

The results of the study confirm the usefulness of the described PB-TK model in predicting the body toxicokinetics, and it may also be useful in biological monitoring to predict toxicity levels in selected organs and tissues.

REFERENCES

1. Czerski B, Kostrzewski P. *Benzene, Indene, Naphthalene, Diphenyl and Fluorene alkyl derivatives as potential sources of occupational and environmental exposure.* Med Pr 1995;4:359–68 [in Polish].
2. Wesołowski W, Gromiec JP. *Occupational exposure in Polish paint and lacquer industry*. Int J Occup Med Environ Health 1997;10:79–88.
3. Kostrzewski P, Wiaderna-Brycht A, Czerski B. *Determination of dimethylbenzoic acids isomers in urine by gas chromatography.* Med Pr 1994;45:37–43 [in Polish].

4. Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME. *Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues*. Toxicol Appl Pharmacol 1989;98:87–99.
5. Sato A, Nakajima T. *Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil*. Brit J Ind Med 1979;36:231–4.
6. Falk A, Gullstrand E, Lof A, Wigaeus-Hjelm E. *Liquid/air partition coefficients of four terpenes*. Br J Ind Med 1990;47:62–4.
7. Fiserova-Bergerova V. *Gases and their solubility. A review of fundamentals*. In: Fiserova-Bergerova V, editor. *Modeling of Inhalation Exposure to Vapors: Uptake, Distribution and Elimination.* Vol. I. Boca Raton: CRC Press; 1983. p.3–28.
8. Poulin P, Krishnan K. *A mechanistic algorithm for predicting blood: air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin*. Toxicol Appl Pharmacol 1996;136:131–7.
9. Ligocka D, Lison D, Haufroid V. *Contribution of CYP2E1 to N-methyl-2-pyrrolidone metabolism*. Arch Toxicol 2003;77:261–6.
10. Gromiec JP, Piotrowski JK. *Urinary mandelic acid as an exposure test for ethylbenzene*. Int Arch Occup Environ Health 1984;55:61–72.
11. Wieczorek H, Piotrowski JK. *Kinetic interpretation of the exposure test for styrene*. Int Arch Occup Environ Health 1988;61:107–13.
12. Browing E. *Toxicity and Metabolism of Industrial Solvents*. New York: Elsevier Publishing; 1965. p.111–9.
13. Korsak Z, Świercz R, Rydzyński K. *Toxic effects of acute inhalation exposure to 1,2,4–trimethylbenzene (pseudocumene) in experimental animals*. Int J Occup Med Environ Health 1995;8(4):331–7.
14. Korsak Z, Rydzyński K. *Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimelitene) in rats*. Int J Occup Med Environ Health 1996;9:341–9.
15. Korsak Z, Majcherek W, Rydzyński K. *Toxic effects of acute inhalation exposure to 1,2,4,5–tetramethylbenzene (durene) in experimental animals*. Int J Occup Med Environ Health 1998;11(3):267–72.
16. Meulenberg CJW, Vijverberg HPM. *Empirical relations predicting human and rat tissue: air partition coefficients of volatile organic compounds*. Toxicol Appl Pharmacol 2000;165:206–16.
17. Jałowiecki P, Kostrzewski P. *Specific nature of the software used to construct simulations: physiologically-based toxicokinetic models.* Med Pr 2001;52(5):361–8 [in Polish].
18. Kostrzewski P. *Simulation compartment and physiological models used in pharmaco- and toxicokinetics.* Med Pr 1998;49(1):83–92 [in Polish].
19. Kostrzewski P, Jałowiecki P. *Application of physiologically-based toxicokinetic models for toxicokinetic studies.* Med Pr 2000;51(5):447–56 [in Polish].
20. Arms AD, Travis CC. *Reference Physiological Parameters in Pharmacokinetic Modeling*. Washington: US Environmental Protection Agency; 1988.
21. Renwick AG. *Pharmacokinetics in toxicology*. In: Hayes AW, editor. *Principles and Methods of toxicology*. New York: Raven Press; 1982. p. 659–710.
22. Travis CC, Hattemer-Frey HA. *Physiological Pharmacokinetic Models*. In: Krewski D, Franklin C, editors. *Statistics in Toxicology*. Ch. 9. New York: Gordon and Breach Science Publishers; 1991. p. 161–90.
23. Fiserova-Bergerova V. *Physiological models for pulmonary administration and elimination of inert vapors and gases.* In: Fiserova-Bergerova V, editor. *Modelling of Inhalation Exposure to Vapors: Uptake, Distribution and Elimination.* Vol. I. Boca Raton: CRC Press;1983. p. 73–100.
24. Poulin P, Krishnan K. *A tissue composition-based algorithm for predicting tissue: air partition coefficients of organic chemicals*. Toxicol Appl Pharmacol 1996;136:126–30.
25. Knaak JB, Al-Bayati MA, Raabe OG, Blancato JN. *Development of in vitro V_{max} and K_m values for the metabolism of isofenphos by P-450 liver enzymes in animals and humans*. Toxicol Appl Pharmacol 1993;120:106–13.
26. Reitz RH, Mendrala AL, Guengerich FP. *In vitro metabolism of methylene chloride in human and animal tissues: Use in physiologically-based pharmacokinetic models*. Toxicol Appl Pharmacol 1989;97:230–46.
27. Dutkiewicz T, Piotrowski JK, Kęsy-Dąbrowska. *Chemical examinations of biological materials in industrial toxicology.* Warszawa: PZWL; 1964 [in Polish].

28. Korsak Z, Sokal J, Dedyk A, Tomas T, Jędrychowski R. *Toxic effects of combined exposure to toluene and xylene in animals*. I. *Acute inhalation study*. Pol J Occup Med 1988;1:45–50.

29. Korsak Z, Świercz R, Jędrychowski R. *Effects of acute combined exposure to n-butyl albohol and m-xylene*. Pol J Occup Med Environ Health 1993;6:35–41.