

# A human physiological model describing acetone kinetics in blood and breath during various levels of physical exercise

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## Abstract

Physiologically based toxicokinetic (PBTK) modeling of human experimental data suggests difficulties to simultaneously describe the time courses of inhaled polar solvents in blood and breath, especially if exposures occur during physical exercise. We attribute this to the washin–washout effect in the airways. The aim was to develop a PBTK-model that explains the behavior of acetone in blood and exhaled air at different levels of physical exercise. The model includes exchange of inhaled solvent vapor with the blood flow via the mucosa and separate compartments to describe working and resting muscles. The developed model was contrasted to a traditional PBTK-model where the conducting airways were regarded as an inert tube. Our model predictions agrees well with experimentally observed acetone levels in both arterial blood and end- and mixed-exhaled air from 26 inhalation experiments conducted with 18 human volunteers at 0, 50, 100 and 150 W workload. In contrast, the inert-tube model was unable to describe the data. The developed model is to our knowledge the first which explains the toxicokinetics of acetone at such various levels of physical exercise. It may be useful in breath monitoring and to obtain more accurate estimates of absorbed dose during inhalation of polar volatiles.

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## 1. Introduction

One of the goals of using physiologically based toxicokinetic (PBTK) modeling is to provide an estimate of target dose which relates to the toxic outcome. This is fairly straight forward when dealing with the inhalation of non-polar volatile substances. In these models the conducting airways of the respiratory tract are assumed

to act as an inert tube, which function merely to conduct the vapor to the alveolae where the exchange of solvent between air and body takes place. Modeling of polar volatile compounds is more problematic. Polar solvents are highly water soluble and are absorbed and subsequently desorbed by the mucous membrane lining the respiratory tract during inhalation and exhalation. This so-called washin–washout effect results in a lower uptake of hydrophilic solvent compared to what would be expected from its blood to air partition coefficient (Johanson, 1991; Johanson and Filser, 1992). Furthermore, pre-alveolar uptake may be of importance. In rodents, it has been demonstrated that polar solvents can be transferred to the systemic blood stream via the

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mucosa and epithelial lining in the nose (Morris and Cavanagh, 1986, 1987; Morris et al., 1986).

There have been several attempts to describe the washin–washout of hydrophilic solvents in PBTK models. The complexity of the descriptions range from simple fractional uptake models (Fisher et al., 2000; Johanson, 1986) to a multi-compartmental model describing the kinetics in various regions of the respiratory tract during cyclic breathing (Johanson, 1991).

Most physiologically based models of inhaled substances focus on describing the toxicokinetics during rest conditions. However, the changes in physiology occurring during physical exercise, such as increased ventilation and increased and redirected cardiac output, offer an opportunity to examine and understand the exchange processes that determine absorption, desorption and distribution of volatiles. A large number of organic solvents have indeed been studied experimentally with respect to inhalation kinetics in humans, including for example acetone, *n*-butanol, methylene chloride, styrene, tetrachloroethylene, toluene, 1,1,1-trichloroethane, trichloroethylene and xylene (see review by Löf and Johanson, 1998).

The objective of this study was to develop a human PBTK-model able to explain the toxicokinetics of an inhaled polar volatile substance in arterial blood, end- and mixed-exhaled air at various workloads. To evaluate the influence of the washin–washout process on the uptake of polar solvents, the performance of the developed model was contrasted to a traditional PBTK-model where the lungs were regarded as an inert tube. Acetone was chosen as a model substance as we have access to large sets of human experimental data for all three end-points. The major exposure route is through inhalation, but acetone is also taken up via the skin (Fukabori et al., 1979). In addition, small amounts of acetone are formed in the body as by-products of fat metabolism. Acetone is primarily cleared by metabolism and to a small extent via exhalation and urinary excretion.

## 2. Material and methods

### 2.1. Experimental data

Two sets of human experimental data collected at our laboratory (formerly located at the Swedish National Institute for Working Life and its predecessors) were used to validate and compare the PBTK models.

A series of 16 controlled exposures to acetone (Wigaeus et al., 1981) in eight male volunteers were used as a starting point. The exposures lasted 2 h and were carried out on two different occasions separated by at least 2 months. On the first occasion

all eight volunteers were exposed to 1309 mg/m<sup>3</sup> acetone at rest for 120 min (series 1). On the second occasion, four volunteers were exposed to 712 mg/m<sup>3</sup> at rest for 30 min followed by light physical exercise (50 W) on a bicycle ergometer for 90 min (series 2a). The remaining four volunteers were exposed to 737 mg/m<sup>3</sup> during stepwise increases in workload from rest to 50, 100 and 150 W (30 + 30 + 30 + 30 min) (series 2b). Arterial blood and end-exhaled air were sampled during exposure and up to 20 h post-exposure. In addition, the pulmonary ventilation rate was monitored.

The second data set included 10 male volunteers exposed to approximately 554 mg/m<sup>3</sup> acetone for 2 h during light physical exercise (50 W) on a bicycle ergometer (Ernstgård et al., 1999). Arterial blood and mixed exhaled air were sampled during exposure and up to 4 h post-exposure. The pulmonary ventilation rate was monitored during the exposure.

### 2.2. Inert-tube PBTK-model

The inert-tube PBTK-model was based on a typical model for inhaled solvent, with compartments for arterial blood (including lungs), richly perfused tissue, adipose tissue, liver and muscles and skin (Fig. 1). The richly perfused tissue compartment included the brain, kidneys and other tissues (Droz, 1992).

The standard model was modified to account for the effect of physical exercise and for the formation of endogenous acetone. To reflect the increased blood flow to leg muscle during bicycle ergometer exercise, a compartment for working muscle was added (Johanson and Näslund, 1988). Further, production of acetone was included in the liver compartment to uphold the endogenous acetone concentration.

### 2.3. Washin–washout PBTK-model

To account for the washin–washout effect in the upper respiratory tract, the arterial blood compartment of the inert-tube model was divided in four parts: bronchioles, mucosa, alveolae and arterial blood. Further, the model allows acetone to be exchanged not only between the air in the alveolae and arterial blood but also between the air in the bronchioles and the mucosa, and between the mucosa and arterial blood (Fig. 1). The rate of the exchange between bronchiolar air and the mucous membrane was assumed equivalent to the alveolar ventilation rate, and the rate of the transfer between mucosa and blood was set to equal the cardiac output.

In both models, acetone is eliminated by exhalation and metabolism. Metabolism was assumed to occur in the liver by a single pathway following Michaelis–Menten kinetics. As urinary excretion accounts for only 1% of the total elimination (Simonsen, 1986), it was neglected.

### 2.4. Model parameters

The inert-tube and the washin–washout models are identical with respect to parameter values, except for those describing

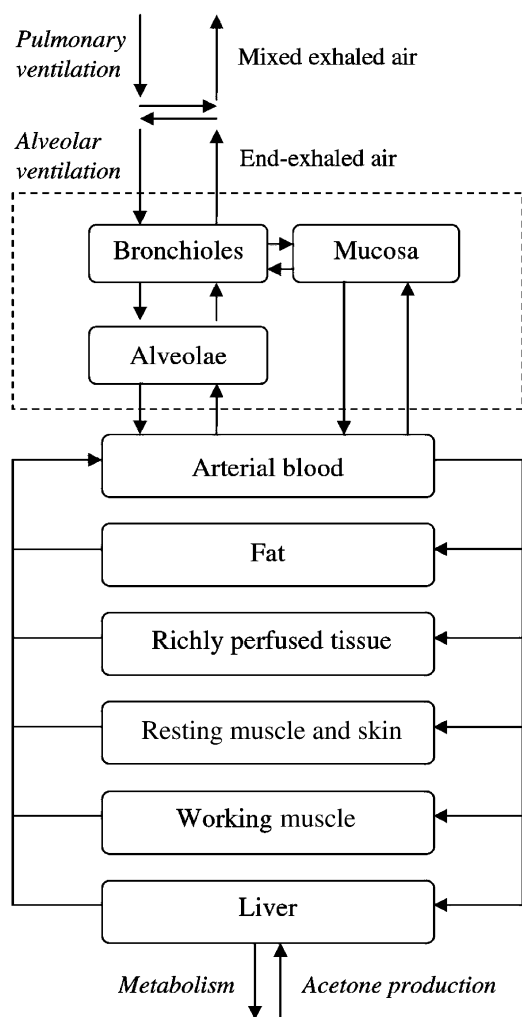


Fig. 1. Structure of the physiologically based toxicokinetic (PBTK) model for acetone. The washin–washout of solvent vapor in the upper respiratory tract is represented by the dotted box. Removing the compartments within the dotted box gives the structure of the inert-tube PBTK-model.

the respiratory tract. The arterial blood compartment of the inert-tube model includes not only the volume of arterial blood but also volumes for lung tissue and alveolar blood (Table 3). The bronchiolar, alveolar and arterial blood compartment volumes of the washin–washout model are found in Tables 1 and 3. The volume of the mucous membrane (Table 1) was calculated from a thickness of 10  $\mu\text{m}$  (Schlesinger, 1990) and a wall area of 5270  $\text{cm}^2$  (Johanson, 1991). For both models, the remaining compartment volumes are calculated as fractions of lean body mass (Table 3).

Average values for body weight (bw) and height (ht) were calculated from the individual values in the raw data. The average body weights were 71, 69 and 73 kg and the average heights were 181, 177 and 186 cm for the volunteers in series 1, 2a

Table 1

Workload independent PBTK-model parameters

Parameter	Abbreviation	Value
Partition coefficients		
Blood:air <sup>a</sup>	$P_{b:a}$	196
Richly perfused tissue:air <sup>b</sup>	$P_{rpt:a}$	137
Muscle:air <sup>a</sup>	$P_{m:a}$	151
Fat:air <sup>a</sup>	$P_{fat:a}$	86
Water:air <sup>b</sup>	$P_{w:a}$	263
Liver:air <sup>c</sup>	$P_{liv:a}$	113
Metabolic parameters		
Maximal rate of metabolism ( $\text{mg/kg}^{0.75}/\text{h}$ ) <sup>d</sup>	$V_{\text{max}}$	18.6
Michaelis–Menten constant ( $\text{mg/l}$ ) <sup>d</sup>	$K_m$	48.4
Volumes (l)		
Volume of dead space	$V_{\text{ds}}$	0.15
Respiratory tract compartment volumes (l)		
Bronchioles <sup>e</sup>	$V_{\text{bro}}$	0.1
Mucosa <sup>e,f</sup>	$V_{\text{muc}}$	0.005
Alveolae <sup>e</sup>	$V_{\text{alv}}$	4.1

<sup>a</sup> Fiserova-Bergerova and Diaz (1986).

<sup>b</sup> Lindqvist (1977).

<sup>c</sup> Fiserova-Bergerova and Diaz (1986), Eq. (5).

<sup>d</sup> Kumagai and Matsunaga (1995).

<sup>e</sup> Johanson (1991).

<sup>f</sup> Schlesinger (1990).

and 2b, respectively, in the Wigaeus et al. (1981) study. In the Ernstgård et al. (1999) study the average body weight and height were 77 kg and 180 cm.

Average endogenous acetone concentrations were calculated based on measurements of acetone in arterial blood before the start of the exposures in the Wigaeus et al. (1981) and Ernstgård et al. (1999) studies (unpublished data). We assumed that the measurements were made at steady state. The average endogenous acetone concentrations were 1.4, 1.1 and 1.0  $\text{mg/l}$  for volunteers in series 1, 2a and 2b, respectively and 1.1  $\text{mg/l}$  for volunteers in the validation data set.

Pulmonary ventilation rates during rest and exercise were determined by experimental data (Table 2). The alveolar ventilation rate (Table 3) was calculated from the pulmonary ventilation rate by subtracting the product of breathing frequency (Table 2) and dead space (Table 1). Alveolar ventilation was in turn used to calculate the cardiac output by using the ventilation over perfusion ratio (Table 2). Finally, compartment blood flows were calculated as fractions of the cardiac output (Table 3). Blood flows at rest to the compartments for resting muscle and skin and working muscle were obtained by dividing the value representing the blood flow to muscles and skin (Åstrand, 1983) in two (Table 2). The blood flow to the resting muscle and skin compartment was considered independent of workload. In consequence, the increase in blood flow occurring during physical exercise was assigned solely to the working muscle compartment (Table 2).

Table 2  
Workload dependent PBTK-model parameters

Parameter	Abbreviation	Workload			
		0 W	50 W	100 W	150 W
Pulmonary ventilation (l/min) <sup>a</sup>	$Q_p$	9.1	23.8	35.3	50.1
Pulmonary ventilation (l/min) <sup>b</sup>	$Q_p$	14.8	23.1	35.3	50.1
Ventilation over perfusion ratio <sup>c</sup>	VPR	1.4	2.3	2.5	2.5
Respiratory frequency (min <sup>-1</sup> ) <sup>d</sup>	RF	15.4	17.1	19.9	24.0
Tissue blood flow (% of cardiac output)					
Fat <sup>c</sup>	$fQ_{fat}$	0.03	0.05	0.05	0.03
Richly perfused tissue <sup>c</sup>	$fQ_{rpt}$	0.44	0.29	0.21	0.19
Resting muscle and skin <sup>c</sup>	$fQ_{rm}$	0.105	0.05	0.04	0.03
Working muscle <sup>c</sup>	$fQ_{wm}$	0.105	0.45	0.61	0.70
Liver <sup>c</sup>	$fQ_{liv}$	0.32	0.16	0.09	0.05

<sup>a</sup> Wigaeus et al. (1981).

<sup>b</sup> Ernstgård et al. (1999).

<sup>c</sup> Åstrand (1983).

<sup>d</sup> Malmberg et al. (1987).

Metabolic parameters and tissue:air partition coefficients for acetone are listed in Table 1. The richly perfused tissue:air partition coefficient constitutes a weighted average of the kidney:air, white matter:air and grey matter:air partition coefficients. Liver:air partitioning was calculated from the fat:air partition coefficient. The mucosa:air partition coefficient was assumed to equal the water:air partition coefficient, as the mucous membrane consists mainly of water.

## 2.5. Model equations

The two PBTK models are described by sets of differential equations which quantify the rate of change of the amount of acetone in each model compartment. The parameters  $P$ ,  $Q$  and  $V$  denote partition coefficients, flows and volumes. The variable  $C$  marks concentrations. The subscripts inh, art, liv, bro, muc and alv represent inhaled air, arterial blood, liver, bronchioles, mucosa and alveolae, respectively. Further, the subscripts b, w

Table 3  
Scaling of the physiological parameters of the PBTK models

Parameter	Scaling	Reference
Total body water (males) (cm, kg)	$TBW = -12.86 + 0.1757ht + 0.331bw$	Watson et al. (1980)
Fat free mass (kg)	$FFM = TBW/0.72$	Widdowson (1965)
Lean body volume (l)	$LBV = FFM/1.1$	Behnke et al. (1953)
Compartment volumes (l)		
Fat	$V_{fat} = (bw - FFM)/0.92$	Fidanza et al. (1953)
Arterial blood <sup>a</sup>	$V_{art} = (0.01933 + 0.00907)LBV$	Cowles et al. (1971)
Arterial blood <sup>b</sup>	$V_{art} = (0.0256 + 0.01933 + 0.00907)LBV$	Cowles et al. (1971)
Richly perfused tissue	$V_{rpt} = (0.00532 + 0.0103)LBV$	Cowles et al. (1971)
Resting muscle and skin	$V_{rm} = 0.344LBV$	Cowles et al. (1971)
Working muscle	$V_{wm} = 0.344LBV$	Cowles et al. (1971)
Liver	$V_{liv} = 0.0285LBV$	Cowles et al. (1971)
Ventilation and flows (l/min)		
Alveolar	$Q_{alv} = Q_p - (V_{ds}RF)$	
Cardiac output	$Q_c = Q_{alv}/VPR$	
Fat	$Q_{fat} = Q_c fQ_{fat}$	
Richly perfused tissue	$Q_{rpt} = Q_c fQ_{rpt}$	
Resting muscle and skin	$Q_{rm} = Q_c fQ_{rm}$	
Working muscle	$Q_{wm} = Q_c fQ_{wm}$	
Liver	$Q_{liv} = Q_c fQ_{liv}$	

<sup>a</sup> Washin–washout model.

<sup>b</sup> Inert-tube model.

and  $a$  denote blood, water and air. Subscript  $c$  represents the cardiac output.  $V_{\max}$  is the maximal rate of metabolism and  $K_m$  denotes the Michaelis–Menten constant.

The non-metabolizing compartments for acetone ( $i$  indicates fat, richly perfused tissue, resting muscle and skin and working muscle compartments) are described identically in both models:

$$\frac{d(C_i)}{dt} V_i = Q_i \left( C_{\text{art}} - \frac{C_i P_{b:a}}{P_{i:a}} \right) \quad (1)$$

as is the metabolizing liver compartment, which also includes production of endogenous acetone:

$$\begin{aligned} \frac{d(C_{\text{liv}})}{dt} V_{\text{liv}} = & Q_{\text{liv}} \left( C_{\text{art}} - \frac{C_{\text{liv}} P_{b:a}}{P_{\text{liv:a}}} \right) + \text{PR} \\ & - \left( \frac{V_{\max} C_{\text{liv}} P_{b:a} / P_{\text{liv:a}}}{K_m + C_{\text{liv}} P_{b:a} / P_{\text{liv:a}}} \right) \end{aligned} \quad (2)$$

The rate of acetone production (PR) is calculated for rest conditions from the average endogenous concentration of acetone in blood ( $C_{\text{endo}}$ ) assuming steady-state conditions. At steady state, the production rate equals the elimination rate via exhalation and metabolism. The production rate in the washin–washout model is calculated as:

$$\begin{aligned} \text{PR} = & Q_{\text{alv}} \frac{C_{\text{endo}}}{P_{b:a}} + \frac{V_{\max} C_{\text{liv}}^{\text{ss}} P_{b:a} / P_{\text{liv:a}}}{K_m + C_{\text{liv}}^{\text{ss}} P_{b:a} / P_{\text{liv:a}}} \\ & + Q_c \left( \frac{C_{\text{endo}} P_{b:a}}{P_{w:a}} - C_{\text{muc}}^{\text{ss}} \right) - Q_{\text{alv}} C_{\text{alv}}^{\text{ss}} \end{aligned} \quad (3)$$

The steady-state concentrations in liver ( $C_{\text{liv}}^{\text{ss}}$ ), mucosa ( $C_{\text{muc}}^{\text{ss}}$ ) and alveolar air ( $C_{\text{alv}}^{\text{ss}}$ ) are obtained from the steady-state solution of Eqs. (2), (5) and (6), respectively. To calculate the acetone production rate in the liver compartment of the inert-tube model, the two last terms in Eq. (3) were removed.

The kinetics of acetone in the respiratory tract of the washin–washout model is described by the following four equations:

$$\begin{aligned} \frac{d(C_{\text{bro}})}{dt} V_{\text{bro}} = & Q_{\text{alv}} (C_{\text{inh}} + C_{\text{alv}} - 2C_{\text{bro}}) \\ & - Q_{\text{alv}} \left( C_{\text{bro}} - \frac{C_{\text{muc}}}{P_{w:a}} \right) \end{aligned} \quad (4)$$

$$\begin{aligned} \frac{d(C_{\text{muc}})}{dt} V_{\text{muc}} = & Q_{\text{alv}} \left( C_{\text{bro}} - \frac{C_{\text{muc}}}{P_{w:a}} \right) \\ & + Q_c \left( \frac{C_{\text{art}} P_{w:a}}{P_{b:a}} - C_{\text{muc}} \right) \end{aligned} \quad (5)$$

$$\frac{d(C_{\text{alv}})}{dt} V_{\text{alv}} = Q_{\text{alv}} \left( C_{\text{bro}} + \frac{C_{\text{art}}}{P_{b:a}} - 2C_{\text{alv}} \right) \quad (6)$$

$$\frac{d(C_{\text{art}})}{dt} V_{\text{art}} = Q_{\text{alv}} \left( C_{\text{alv}} - \frac{C_{\text{art}}}{P_{b:a}} \right) + Q_c \left( C_{\text{muc}} - \frac{C_{\text{art}} P_{w:a}}{P_{b:a}} \right) - Q_c C_{\text{art}} + \sum Q_i \frac{C_i P_{b:a}}{P_{i:a}} \quad (7)$$

whereas the kinetics in the respiratory tract of the inert-tube model is represented by:

$$\frac{d(C_{\text{art}})}{dt} V_{\text{art}} = Q_{\text{alv}} \left( C_{\text{inh}} - \frac{C_{\text{art}}}{P_{b:a}} \right) - Q_c C_{\text{art}} + \sum Q_i \frac{C_i P_{b:a}}{P_{i:a}} \quad (8)$$

## 2.6. Relative uptake

To allow comparisons with experimental data during rest and physical exercise, relative uptake was calculated independently for each 30 min time period in series 2b of the Wigaeus et al. (1981) study. Simulated relative uptake ( $R_{\text{up}}$ ) was calculated as follows:

$$R_{\text{up}} = \frac{Q_{\text{alv}}}{Q_p} \left( 1 - \frac{X}{C_{\text{inh}}} \right) 100 \quad (9)$$

where  $C_{\text{inh}}$  is the concentration in inhaled air.  $Q_p$  and  $Q_{\text{alv}}$  denote pulmonary- and alveolar-ventilation, respectively.  $X$  equals the concentration in bronchiolar air (washin–washout model) or the concentration in arterial blood divided by the blood:air partition coefficient (inert-tube model).

## 2.7. Sensitivity analyses

In order to identify the parameters with the greatest impact on acetone levels in all three end-points (arterial blood, end-exhaled air and mixed exhaled air) during resting and working (100 W) conditions, sensitivity analyses were performed. The washin–washout model was first run using the original model parameters and then repeatedly rerun using a 1% increase in each parameter value to determine the changes in predicted acetone concentration. In the model, compartment blood flows are obtained as fixed fractions of the cardiac output. However, in the sensitivity analyses of the individual compartment blood flow parameters, the calculation had to be made in the opposite way in order to preserve mass balance. Thus, cardiac output was calculated as the sum of the individual blood flows. The exposure levels were set at 1309 mg/m<sup>3</sup> during rest and 737 mg/m<sup>3</sup> during physical exercise with duration of 120 min. The sensitivity coefficients ( $S_c$ ) were calculated using the following equation:

$$S_c = 100 \left( \frac{C_{1.01}}{C} - 1 \right) \quad (10)$$

where  $C$  is the output (concentration in arterial blood, end-exhaled air or mixed exhaled air) using the original parameter value and  $C_{1.01}$  is the output with an 1% increase in parameter value. Model parameters were considered sensitive if their absolute sensitivity coefficient was 0.5 or greater for more than 10 min.

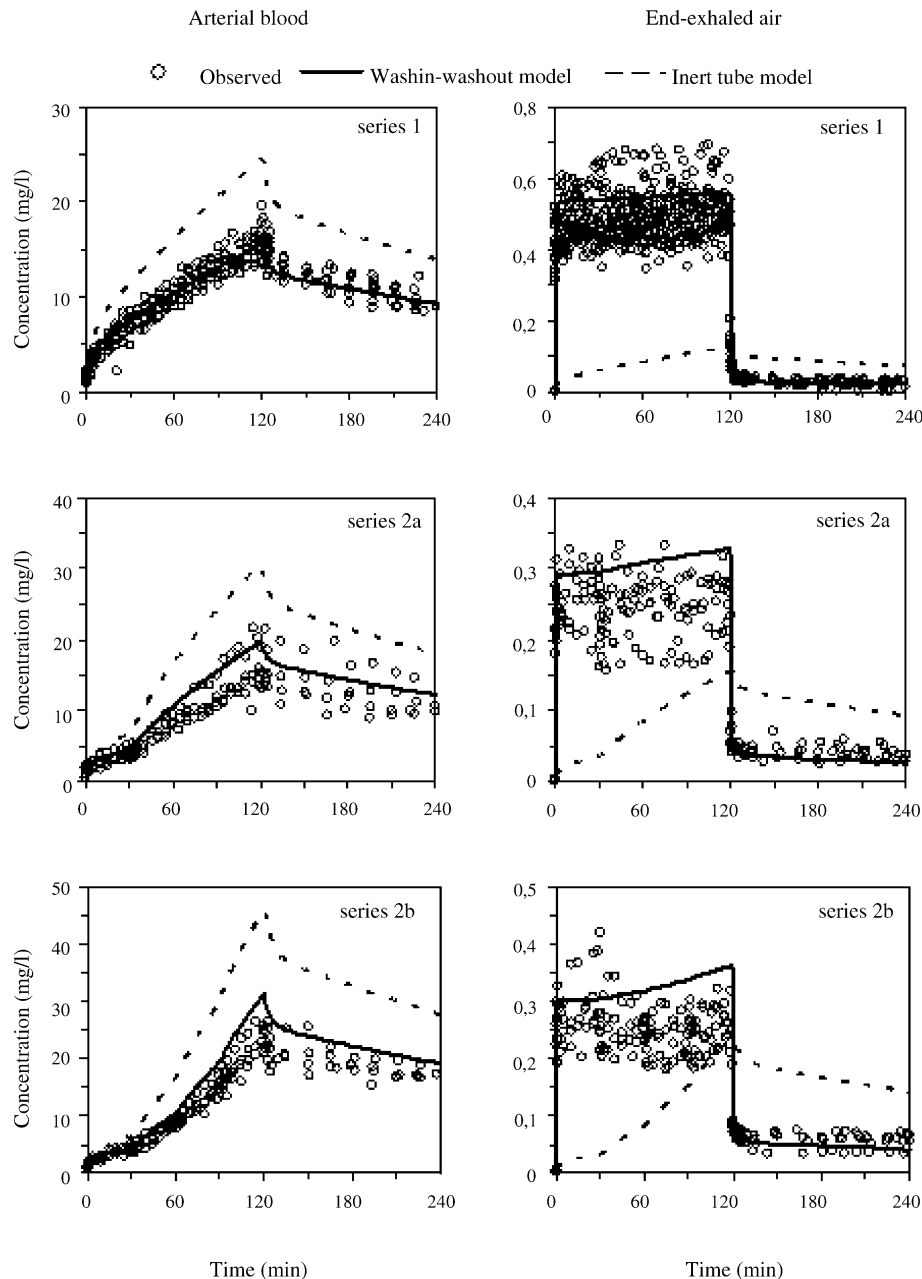


Fig. 2. Washin–washout and inert-tube model predictions are compared to experimental acetone concentrations (Wigaeus et al., 1981) in arterial blood and end-exhaled air. Series 1: exposure to 1309 mg/m<sup>3</sup> for 120 min during resting conditions. Series 2a: exposure to 712 mg/m<sup>3</sup> for 30 min at rest followed by 90 min of physical exercise at 50 W workload. Series 2b: exposure to 737 mg/m<sup>3</sup> for 30 min at rest followed by a stepwise increase in workload (50, 100 and 150 W) for 30 min periods.

Numerical calculations were carried out with Berkeley Madonna version 8.0.

### 3. Results

In order to assess the performance of the washin–washout PBTK-model and to elucidate the impact

of the washin–washout effect on acetone inhalation kinetics, simulations were performed with both the washin–washout and the inert-tube PBTK models. The simulations were based on the exposure conditions and the average physiological parameters (pulmonary ventilation, body weight, height and endogenous acetone blood concentrations) reported in

series 1, 2a and 2b, respectively (Wigaeus et al., 1981).

The inert-tube model simulations of acetone levels in arterial blood were consistently higher than the experimentally observed concentrations in all three series (Fig. 2, dotted lines). In contrast, the predictions for acetone in end-exhaled air were much lower than expected from experimental data. Moreover, the shape of the end-exhaled air concentration–time curve was not comparable with the experimental curve (Fig. 2, dotted lines). In effect, the simulated average relative uptake of acetone was significantly higher than experimentally measured (Fig. 3).

In comparison, using the washin–washout PBTK-model, good agreements between simulated and observed values in arterial blood and end-exhaled air were obtained (Fig. 2, solid lines). The simulated relative uptake corresponds well with the experimentally observed during rest as well as during physical exercise (Fig. 3).

The sensitivity analyses revealed that, as expected, alveolar ventilation, the blood:air partition coefficient, the water:air partition coefficient, the muscle:air partition coefficient and pulmonary ventilation were the most sensitive parameters at rest (Fig. 4) and during exercise (not shown). All other model parameters had only marginal effect on the output.

The washin–washout PBTK-model was validated by simulating concentrations of acetone in arterial blood and mixed exhaled air in a second, independent data set (Ernstgård et al., 1999). Experiment specific exposure

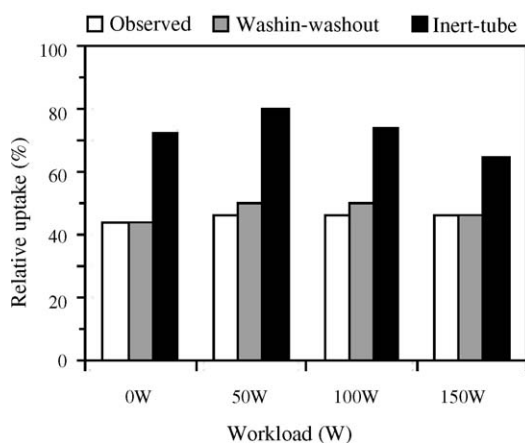


Fig. 3. Washin–washout and inert-tube PBTK-model simulations of the relative uptake of acetone during rest and physical exercise compared to experimentally determined values. The exposure level was 737 mg/m<sup>3</sup>. The exposure was carried out during rest for 30 min followed by a stepwise increase in workload (50, 100 and 150 W) for 30 min periods (Wigaeus et al., 1981, series 2b).

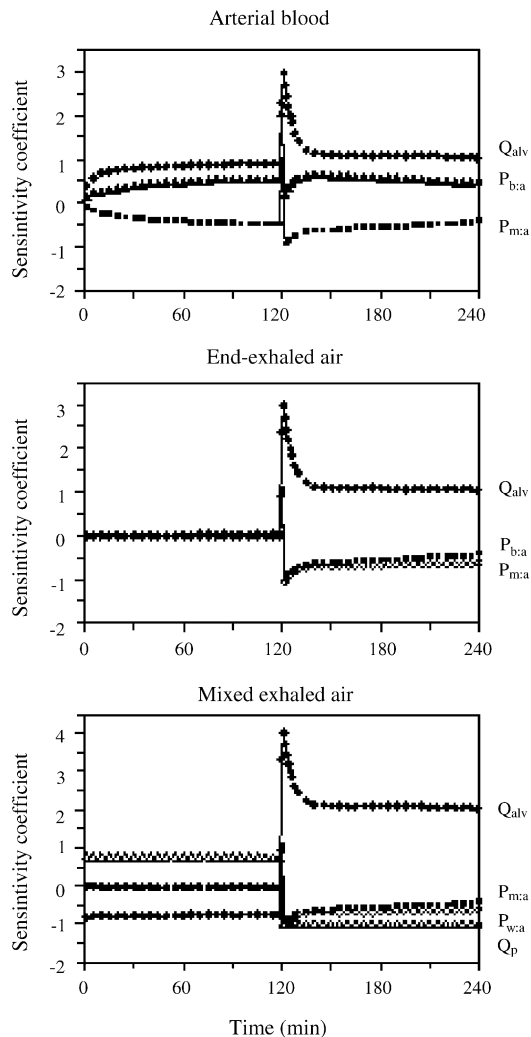


Fig. 4. Sensitivity analyses of the washin–washout acetone PBTK-model. Only parameters with absolute sensitivity coefficients of 0.5 or greater are displayed in arterial blood, end-exhaled air and mixed exhaled air. Sensitive parameters were alveolar ventilation ( $Q_{alv}$ ), pulmonary ventilation ( $Q_p$ ) and the partition coefficients of blood:air ( $P_{b:a}$ ), muscle:air ( $P_{m:a}$ ) and water:air ( $P_{w:a}$ ).

conditions and average physiological parameters were used as input for the model. Simulated and observed arterial blood and mixed exhaled air levels agreed well (Fig. 5). The experimental average relative uptake was 43% compared to 47% obtained in the simulation of the experiment.

#### 4. Discussion

The washin–washout acetone PBTK-model developed herein explains levels of acetone in breath and blood in two independent experimental data sets during

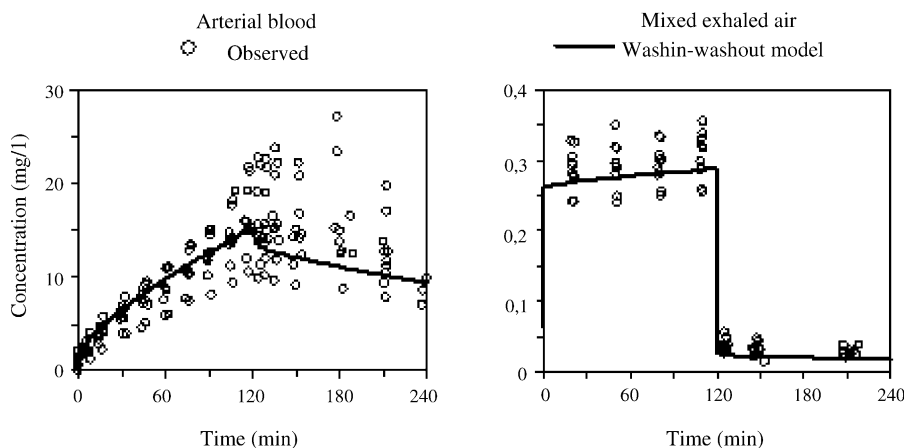


Fig. 5. The washin–washout model predictions are compared to experimentally observed acetone concentrations (Ernstgård et al., 1999) in arterial blood and mixed exhaled air. The exposure level was 554 mg/m<sup>3</sup> for 120 min and the exposure was performed during physical exercise (50 W).

exposure at several different levels of physical exercise. In contrast, the more common inert-tube model is unable to adequately describe the experimental data.

The inert-tube model overestimates the relative uptake of acetone during physical exercise, resulting in over predictions of acetone levels in blood and vice versa for breath. The behavior of the inert-tube model stems from the assumption of a workload independent dead-space volume. As a result, at increasing workloads, the relative contribution of alveolar ventilation decreases and, hence, the relative uptake increases. This effect is partly counteracted at higher workloads (100 and 150 W in Fig. 3) as acetone builds up in blood. Overall, our exercises clearly illustrates that the inert-tube model cannot explain the kinetic behavior of a polar volatile substance such as acetone.

Previously published human PBTK models for acetone used similar descriptions of the washin–washout effect (Clewett et al., 2001; Kumagai and Matsunaga, 1995). The breathing was treated as a continuous parallel process and only the outermost part of the mucous membrane was involved in the absorption and desorption of acetone in the airways. Both model simulations agreed well with experimentally observed acetone levels in arterial blood and end-exhaled air at rest. Only Kumagai and Matsunaga (1995) evaluated the model on toxicokinetic data from exposures to acetone during physical exercise (50 W). However, to fit experimentally observed arterial blood and end-exhaled air concentrations, the volume of the mucosa had to be increased about three times. This probably indicates that the transfer rate of solvent to the mucosa is higher during physical exercise than during rest.

Fifteen years later, Kumagai and Matsunaga (2000) used a slightly different approach to model the washin–washout of seven polar solvents including acetone. This model included cyclic breathing and systemic uptake via the mucosa. Further, it utilized several compartments to represent the respiratory system but only a single compartment to represent the body. The authors showed a good fit to experimental data of relative uptake and acetone levels in end-exhaled air. However, simulations were only performed during rest and for the first 10 min of exposure.

In contrast to the other PBTK models for acetone, our washin–washout model involves the deeper part of the mucous membrane in the absorption and desorption of acetone vapor. It also includes separate compartments for working and resting muscles. Similar to Kumagai and Matsunaga (2000), we included transfer of solvent between the mucosa and arterial blood. Further, as the washin–washout effect appears to increase during physical exercise, we scaled the rate of the transfer to ventilation and blood flow. Since the alveolar ventilation rate governs the transport of solvent vapor through the respiratory tree, it was assumed to be the major determinant of the rate of solvent transfer between the air in the bronchioles and the mucosa. As the major part of the systemic blood flow passes through the blood vessels that run in parallel with the conducting airways, we assumed that the exchange rate between the deeper portion of the mucosa and arterial blood was best represented by the cardiac output.

The values of the metabolic parameters ( $V_{\max}$  and  $K_m$ ) for acetone provided by Kumagai and Matsunaga (1995) are different from that of Clewett et al. (2001). The values from the latter study were derived by adjusting rat

acetone metabolic parameters to fit human toxicokinetic acetone data, whereas Kumagai and Matsunaga scaled the rat acetone metabolic parameters to humans. When using the metabolic parameters values from the Clewell (2001) study, our model predictions did not fit the experimental data used in this study. In contrast, when adopting the  $V_{\max}$  and  $K_m$  values from Kumagai and Matsunaga, our model predictions agreed well with the data. According to the sensitivity analyses, our model is not very sensitive to the metabolic parameters (sensitivity coefficients below 0.1, except for the first few minutes after the exposure ended for  $V_{\max}$ , and below 0.4 for  $K_m$ ), suggesting that estimates of these parameters obtained by fitting to concentration–time curves have low reliability.

The washin–washout PBTK-model developed in this study have a slight tendency to overestimate the acetone levels in blood and breath at higher workloads. Consequently, the simulated relative uptake during physical exercise is somewhat higher than reported in corresponding experiments. Possible explanations to the small deviations are that the model simplifies the breathing process (continuous rather than cyclic flows) and/or the gas exchange process. For example, the ventilation over perfusion ratio varies in different regions of the lung and the ventilation/perfusion inequality in the lungs has been shown to increase during heavy exercise (Hammond et al., 1986). The inclusion of a more elaborate description of the breathing and gas exchange processes might improve the predictions of acetone kinetics during physical exercise, but quantitative data are lacking at present.

Our efforts have resulted in a PBTK-model able to describe experimental levels of acetone in blood and breath during various levels of physical exercise. They also illustrate the importance of including washin–washout in models describing the inhalation of polar volatile substances. The new model may be of use to obtain more accurate estimates of internal dose or when using exhaled air as a biomarker for exposure. Further, it may help to explain the absorption and desorption processes of polar solvents in the airways. However, in that respect, studies of additional polar volatiles are needed in order to strengthen the model.

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