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ORIGINAL ARTICLE

Simulation of urinary excretion of 1-hydroxypyrene in various scenarios of exposure to polycyclic aromatic hydrocarbons with a generic, cross-chemical predictive PBTK-model

Frans Jongeneelen · Wil ten Berge

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Abstract
Introduction A physiologically based toxicokinetic (PBTK) model can predict blood and urine concentrations, given a certain exposure scenario of inhalation, dermal and/or oral exposure. The recently developed PBTK-model IndusChemFate is a unified model that mimics the uptake, distribution, metabolism and elimination of a chemical in a reference human of 70 kg. Prediction of the uptake by inhalation is governed by pulmonary exchange to blood. Oral uptake is simulated as a bolus dose that is taken up at a first-order rate. Dermal uptake is estimated by the use of a novel dermal physiologically based module that considers dermal deposition rate and duration of deposition. Moreover, evaporation during skin contact is fully accounted for and related to the volatility of the substance. Partitioning of the chemical and metabolite(s) over blood and tissues is estimated by a Quantitative Structure–Property Relationship (QSPR) algorithm. The aim of this study was to test the generic PBTK-model by comparing measured urinary levels of 1-hydroxypyrene in various inhalation and dermal exposure scenarios with the result of model simulations.
Experimental In the last three decades, numerous biomonitoring studies of PAH-exposed humans were published that used the bioindicator 1-hydroxypyrene (1-OH-pyrene) in urine. Longitudinal studies that encompass both dosimetry and biomonitoring with repeated sampling in time were selected to test the accuracy of the PBTK-model by comparing the reported concentrations of 1-OHP in urine with the model-predicted values. Two controlled human volunteer studies and three field studies of workers exposed to polycyclic aromatic hydrocarbons (PAH) were included.
Results The urinary pyrene-metabolite levels of a controlled human inhalation study, a transdermal uptake study of bitumen fume, efficacy of respirator use in electrode paste workers, cokery workers in shale oil industry and a longitudinal study of five coke liquefaction workers were compared to the PBTK-predicted values. The simulations showed that the model-predicted concentrations of urinary pyrene and metabolites over time, as well as peak-concentrations and total excreted amount in different exposure scenarios of inhalation and transdermal exposure were in all comparisons within an order of magnitude. The model predicts that only a very small fraction is excreted in urine as parent pyrene and as free 1-OH-pyrene. The predominant urinary metabolite is 1-OH-pyrene-glucuronide. Enterohepatic circulation of 1-OH-pyrene-glucuronide seems the reason of the delayed release from the body.
Conclusions It appeared that urinary excretion of pyrene and pyrene-metabolites in humans is predictable with the PBTK-model. The model outcomes have a satisfying accuracy for early testing, in so-called *1st tier* simulations and in range finding. This newly developed generic PBTK-model IndusChemFate is a tool that can be used to do early explorations of the significance of uptake of pyrene in the human body following industrial or environmental exposure scenarios. And it can be used to optimize the sampling time and urine sampling frequency of a biomonitoring program.

Keywords PAH · Pyrene · PBTK · PBPK · Modeling · Urinary hydroxypyrene · Occupational exposure

F. Jongeneelen (✉)
IndusTox Consult, Universitair Bedrijven Centrum,
Toernooiveld 100, 6525, EC, Nijmegen, The Netherlands
e-mail: frans.jongeneelen@industox.nl

W. ten Berge
Santoxar, Westervoort, The Netherlands

Introduction

Physiologically based toxicokinetic (PBTK) models are mathematical descriptions of the flow of blood throughout the body, developed for the simulation of xenobiotic absorption, distribution and elimination. Thus, a PBTK-model can estimate the level of chemicals in blood and urine as equivalent to external exposure levels of inhalation, dermal load or oral intake.

Development of pharmaceuticals is the main area of application of these models. Ramsey and Andersen (1984) were pioneers in PBTK-modeling of occupational contaminants. A barrier in the application of PBTK-modeling is the need to know the values of all partitioning and kinetic parameters of a compound. This is particularly true for the blood-to-air and tissue-to-blood partition coefficients, which control the uptake and distribution over the body tissues. To solve this problem, QSPRs (Quantitative Structure–Property Relationships) algorithms have been developed that predict the substance-specific partition coefficients from common physical–chemical parameters. In fact this approach is a cross-chemical prediction approach. A generic PBTK-model with QSPRs for *1st tier* level modeling of environmental and occupational contaminants was recently published (Jongeneelen and ten Berge 2011). Also the PBTK-model contains newly developed algorithms that simulate the physiological process of dermal uptake. External verification of PBTK-model predictions is generally done by testing the match of experimental observations with model predictions. An interesting compound for external verification of the PBTK-model is pyrene. Pyrene [CAS nr.129-00-0] is a PAH (Polycyclic Aromatic Hydrocarbon) and is present in combustion exhaust and as an impurity in tar- and oil-derived products. Environmental exposure is widespread and occupational exposure is in several industries very significant. The proportion in the PAH-mixture may vary from 0.1 up to 5% of the total PAH.

Occupational exposure may occur through inhalation of vaporized or particulate pyrene and by dermal uptake. Dermal contamination of pyrene on the skin, due to occupational or environmental exposure, occurs in solid or fluid matrices. In the last three decades, numerous biomonitoring studies of PAH-exposed humans were published that used the bioindicator 1-hydroxypyrene (1-OHP) in urine. The concentration of metabolite 1-OHP can easily be determined in human urine, including the level of non-occupational exposed control populations (Levin 1995). Given the availability of a straightforward and sensitive method for 1-OHP determination in urine (Jongeneelen et al. 1987a), many researchers have resorted to this measurement for the biological monitoring of exposure to PAHs in the workplace and in the general environment (Jongeneelen et al. 1988; Buchet et al. 1992; Levin 1995; Dor et al. 1999; Jacob and Seidel 2002; Borak et al. 2002; Unwin et al. 2006; Hansen et al. 2008).

Longitudinal studies that encompass both dosimetry and biomonitoring of PAH with repeated sampling in time were searched for to test the accuracy of the PBTK-model prediction. This was done by comparing the pattern of excretion of 1-OHP in urine over time with the model-predicted values. Two controlled human volunteer studies and three field studies of workers exposed to polycyclic aromatic hydrocarbons (PAH) were included in the comparisons. Measured amounts and concentrations of hydroxylated pyrene metabolites in urine in various inhalation and dermal exposure scenarios were compared with the model-predicted amounts and levels. The aim of this study was to test the generic PBTK-model IndusChemFate by comparing published measured data of biomonitoring of the compound pyrene in various inhalation and dermal exposure scenarios with the results of the simulations PBTK-model simulations.

Methods and materials

The generic PBTK-model IndusChemFate

The generic PBTK (Physiologically Based ToxicoKinetic) model IndusChemFate holds physiological processes in mathematical representations. Partitioning over air, blood and tissue compartments is predicted by QSPRs (Quantitative Structure–Property Relationships) based on physical–chemical properties as octanol-to-water partition (log K_{ow}), molecular weight (MW), density, vapor pressure and water solubility. The model assumes a reference human of 70 kg and considers three uptake routes (inhalation, dermal and/or oral) for exposure as well as two built-in exercise levels (rest and light activities). Dermal uptake is estimated by the use of an advanced algorithm that considers intermittent dermal contact. Key feature of this module is that evaporation (from applied dermal dose and from stratum corneum) is fully accounted for. Also absorption of vapor over the skin is modeled. Oral intake of compounds is modeled as a bolus dose that is directly applied to the stomach and then transferred to the intestinal tissue at a first-order rate. Enterohepatic circulation is optional at a user-defined rate. The uptake in tissues is simulated by partition in an ideally mixed tissue compartment. Saturable metabolism following Michaelis–Menten kinetics is incorporated in the model. Metabolism is modeled in the liver compartment using human in vitro data of enzyme kinetics (maximum rate (V_{max}) and Michaelis–Menten constant (K_M). Exhalation and excretion via urine are excretion pathways. Physical–chemical properties of the chemical of interest provide most of the required model

input. The model is built in Visual Basic and runs in Microsoft Excel. Internal check of mass balance is done to demonstrate that the model is mathematically and computationally correctly implemented. A further description of the model has been published elsewhere (Jongeneelen and Ten Berge 2011). Version 1.6 of IndusChemFate has been used for the calculations.

Input data for PBTK-modeling of pyrene

Physical–chemical data

Physical–chemical input data of pyrene and its main metabolites are presented in Table 1.

Metabolism of pyrene in man and kinetic constants

1-Hydroxypyrene (1-OHP) is a major metabolite of pyrene (Boyland and Sims 1964; Jacob et al. 1982; Keimig et al. 1983). Bouchard et al. (1998); Bouchard and Viau (1998, 1999) concluded after thorough experimental rat studies that 1-OHP in urine is a reliable bioindicator due to linear dose excretion relationships, but concluded also that metabolites other than 1-OHP are present in significant proportions in urine and feces. However, they found that only 2,7% of an orally or intravenously administered dose is excreted as 1-OHP in urine (Bouchard and Viau 1998). That suggested that unaccounted metabolites of pyrene other than 1-OHP represent a large fraction of the excreted dose. Dermal application of [14C] pyrene showed that approximately 1% of the urinary [14C] was recovered as 1-OHP (Payan et al. 2008). This suggests also that other major metabolites in urine exist. Other biotransformation products of 1-OHP that have been identified are 1,6- and 1,8-dioxygenated metabolites of pyrene. Ruzgyte et al. (2005, 2006) showed significant excretion of pyrene-1,6- and pyrene-1,8-dioxygenated metabolites in urine of rats after i.v. injection of pyrene. The analysis of urine samples of human subjects exposed to pyrene confirmed the presence of these metabolites (Bouchard et al. 2009). Seidel et al. (2008) reported on levels of dihydroxypyrenes in urine of PAH-exposed workers. The levels of 1-OHP were in average 5- to 6-fold higher than the level of 1,6- and 1,8-dihydroxypyrenes. The minor metabolite pyrene-4,5-dihydrodiol originates from the formation of pyrene-4,5-epoxide (Grover et al. 1972). Results from an in vitro experiment conducted by Jacob et al. (1982) using liver microsomes from non-induced rats indicated that 1-OHP represented 57% of the total amount of metabolites, pyrene-4,5-dihydrodiol accounted for 21%, an unidentified diphenol for 6%, 1,6-dihydroxypyrene for 11% and an unidentified triol for 5%.

Boyland and Sims (1964) have identified several so-called phase II metabolism pathways. They identified in urine sulfo- and glucuronide conjugates of 1-OHP, of 1,6- and 1,8-dihydroxypyrene, and of pyrene-4,5-dihydrodiol, together with some *N*-acetyl -*S*- (4,5 -dihydro-4-hydroxy-5- pyrenyl)–Lcysteine. It was shown that after enzymatic hydrolysis of urine of PAH-exposed individuals, the level of free 1-OHP was 7–9 times increased (Jongeneelen et al. 1987a). The majority of urinary 1-OHP is conjugated to 1-OHP-glucuronide (=1-OHP-gluc) (Strickland et al. 1994). 1-OHP-gluc appeared to be more profound as metabolite in urine of exposed workers (0,3–0,9 μg/g creatinine) compared to the sulfate conjugate of 1-OHP (0,002–0,06 μg/g creatinine). The free 1-OHP was also low (0,07–0,2 μg/g creatinine) (Singh et al. 1995). Wang et al. (2005, 2006) studied the enzyme kinetics of glucuronidation (UGTs) and sulphation (SULTs) in several species including man. They showed that sulphation is a low

Table 1 Physical–chemical input data of pyrene, 1-OHP and 1-OHP-gluc

Property	Compound		
	Pyrene (source: EPI-suite)	1-OHP[a] (source: EPI-suite and chemspider)	1-OHP-gluc[b] (source: EPISuite and chemspider)
CAS	129-00-0	5315-79-7	154717-05-2
Density (mg/cm3 or grams/L)	1,270	1,000	1,000
Molecular weight	202.26	218.28	394
Vapor pressure (Pa)	0.0106	0.000022	3.2E−17
Log(K_{ow}) at skin pH 5.5	4.88	–	–
Log(K_{ow}) at blood pH 7.4	4.88	4.45	−2.12
Water solubility (mg/L)	0.135	4	40,000

[a] 1-OHP data based on US-EPA EPI-suite 4.0 Estimation Program Interface (EPI) Suite. US EPA (http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm) and/or Chemspider from RCS, ChemSpider Database (http://www.chemspider.com/)

[b] 1-OHP-gluc. Log Kow and water solubility is estimated by cross-reading of values of similar Glucuronides as N-Glucuronide of N-hydroxy-2-aminofluorene

capacity, high affinity clearance pathway (metabolic clearance in human liver preparations of SULTs is twofold lower than UGTs and V_{max} of SULTs is 5 times lower). This confirmed that sulphation of 1-OHP is a minor pathway. Mercapturic acids are formed after oxidation at the 4,5-position of pyrene (Boyland and Sims 1964) Fig. 1 gives an overview of the metabolism pathways of pyrene.

In summarizing the available information on the pyrene metabolism in man, it seems clear that the main pathway is hydroxylation to 1-hydroxypyrene by cytochrome P450 iso-enzymes. 1-OHP is conjugated in a second step and is excreted in urine as a glucuronide-conjugate (=1-OHP-gluc). The Michaelis–Menten constants of the enzyme kinetics of pyrene to 1-OHP and of 1-OHP to 1-OHP-gluc have been determined in in vitro studies using human microsomal liver tissue preparations (Jongeneelen et al. 1987b; Luukkanen et al. 2001). Table 2 shows the results. For the metabolic elimination of pyrene, it was assumed that the stoichiometric yield of pyrene to 1-OHP was 0.5, thus half of the absorbed amount of pyrene is metabolized to 1-OHP. It was further assumed that all 1-OHP was conjugated to 1-OHP-glucuronide. The other half of the absorbed amount of pyrene is assumed to convert to unidentified metabolites. This has been reached by setting the metabolic elimination of pyrene in the liver is twice as high as the production of 1-OHP. The total metabolic clearance of pyrene was set at twice as high as assumed from the formation of 1-OHP (360/4.5 vs. 180/4.5), thus leaving room for the formation of other unidentified primary metabolites. It is further assumed that all 1-OHP is metabolized to 1-OHP-gluc, thus that removal of 1-OHP is in balance with the production of 1-OHP-gluc. This latter metabolite is the end-metabolite and is not further metabolized. Table 3 shows the Michaelis–Menten kinetic constants of the metabolism as used in the PBTK-model.

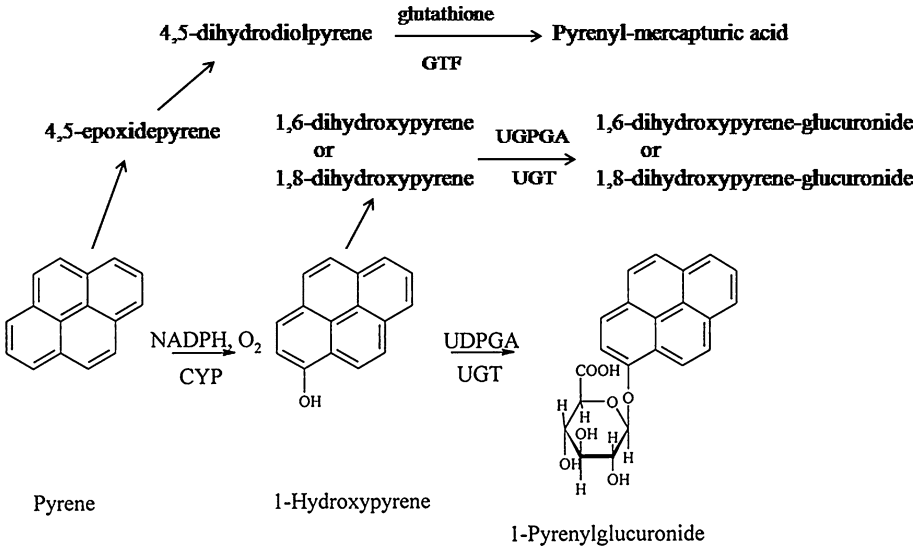

Fig. 1 Metabolism pathways of pyrene

Table 2 Experimental data of metabolism enzyme kinetics of pyrene in man

Metabolism step, tissue, metric and value	Value converted to the value used in the PBTK-model	Reference
Hydroxylation of pyrene to 1-OHP in human hepatic 9000*g supernatant samples of 12 individuals V_{max}: mean = 0.18; range = 0.03–4.5 nmol/h/mg wet liver Apparent K_M = 0.4–170, mean = 4.4 μM	V^a_{max} = 180 μmol/h/kg liver tissue Apparent K_M = 4.4 μM	Jongeneelen et al. (1987b)
Glucuronidation of 1-OHP in human liver microsomes samples of 3 individuals V_{max} = 4.9–6.6 nmol/min/mg microsomal protein Apparent K_M = 6.9–8.6 μM	V^b_{max} = 6,900 μmol/h/kg liver tissue Apparent K_M = 7.7 μM	Luukkanen et al. (2001)

[a] V_{max} 1-OHP = 0.18*1,000,000 = 180,000 nmol/h/kg liver tissue

[b] It is assumed that the hepatic 9000*g supernatant fraction (=S9) corresponds to a protein content of 20 mg protein per mL liver (Chalbot and Morfin 2005) which is assumed to correspond roughly with 20 mg/g Liver. V_{max} 1OHP-gluc = 5.8 × 20,000 (mg protein per kg liver) × 60 (min) = 6,900,000 nmol/h/kg liver tissue

Table 3 Metabolic enzyme kinetics of pyrene and 1-OHP as used in the model

Property	Compound		
	Pyrene	1-OHP	1-OHP-gluc
V_{max} liver of elimination of parent compound (μmol/(kg tissue * h)	360 (removal of pyrene, estimate)	6,900 (removal of 1-OHP; estimate)	–
k_M liver of elimination of parent compound (μmol/L)	4.5 (removal of pyrene, estimate)	7.7 (removal of 1-OHP; estimate)	–
V_{max} liver of metabolite formation (μmol/(kg tissue * h)	180 (formation 1-OHP; Jongeneelen et al. 1987b)	6,900 (formation 1-OHP-gluc; Luukkanen et al. 2001)	–
k_M liver of metabolite formation (μmol/L)	4.5 (formation 1-OHP; Jongeneelen et al. 1987b)	7.7 (formation 1-OHP-gluc; Luukkanen et al. 2001)	–

In a study with rat post-mitochondrial fractions of liver and lung, it was found that metabolic activity of lungs toward pyrene was negligible (Haddad et al. 1997). That is why only metabolism in the liver is taken into account in the pyrene PBTK-model.

It was assumed that induction of the P450 iso-enzymes relevant for the pyrene metabolism does not occur, since the percentage of the pyrene dose excreted as 1-OHP at different time points was not significantly different between doses of pyrene and doses of PAH-mixture reference material over a large range (Bouchard et al. 2002). The excretion rate of 1-OHP remained proportional to the pyrene exposure dose over the PAH dose range.

Toxicokinetic parameters

The toxicokinetic parameters needed for PBTK-modeling of pyrene are presented in Table 4. The oral absorption rate of pyrene has been estimated by Buckley and Lioy (1992) in an experiment with human dietary exposure to PAH. The best fit with a biexponential model assuming first-order appearance and first-order elimination showed an median oral absorption rate of 0.18 h^{-1} (range: 0.13–0.65 h^{-1}, $n = 5$).

Tubular resorption was expected for pyrene and 1-OHP, but not for 1-OHP-gluc given the high water solubility. Enterohepatic recirculation is true for 1-OHP-gluc. This recirculation is driven by reabsorption of 1-OHP in the intestines after bacterial hydrolysis of the biliary excreted 1-OHP-gluc. In our model, enterohepatic circulation was incorporated by defining the ratio of excretion to bile relative to excretion to the blood. This ratio is defined as the fraction of the amount of a metabolite in liver tissue that is excreted to the intestinal lumen via bile (expressed as ratio of the amount drained off to bile relative to the amount removed to liver venous blood). If, for example, the removal ratio of a non-conjugated metabolite from the liver by enterohepatic recirculation is set 0, 100% of the total amount that leaves the liver per unit of time is transferred to blood and there is no enterohepatic recirculation. If in the case of a conjugated metabolite, the removal ratio is set to 1, 50% of the total amount that leaves the liver per unit of time is transferred to blood and 50% is transferred to the intestinal lumen via bile, available for reabsorption with a fixed rate of 0.3/h.

Viau et al. (1999) reported on the biliary to urinary 1-OHP ratio in rats with biliary cannulae and the fecal to urinary ratio in non-cannulated rats. They found ratios of 3 versus 0.6, respectively. That suggests that 1-OHP-gluc undergoes major hepatic recycling.

Based on the elimination curves of 1-OHP in urine with a half-life of 20 h, the human enterohepatic removal ratio of 1-OHP-gluc, set to a value of 0.3, was found to provide the best fit. With this enterohepatic removal ratio, 77% of the total amount that leaves the liver per unit of time is transferred to blood and 23% is transferred to the intestinal lumen via bile (23%/77% = 0.3).

Table 4 Toxicokinetic input data of pyrene, 1-OHP and 1-OHP-gluc for modeling

Property	Compound		
	Pyrene	1-OHP	1-OHP-gluc
Oral absorption rate (h^{-1})	0.18 (Buckley and Lioy 1992)	–	–
Resorption tubuli (y/n/?)[a]	Yes	Yes	No
Enterohepatic removal ratio (relative to liver venous blood)	0	0	0.3

[a] It is assumed that pyrene and 1-OHP are easily resorbed in the kidney and that 1-OHP-gluc is not

Selected studies with biomonitoring results of measured occupational pyrene exposure

A literature search was performed to identify studies with various exposure scenarios of inhalation and/or dermal pyrene exposure combined with excreted amounts or spot samples concentration of its metabolite 1-OHP in urine. These studies were used to test the accuracy of the PBTK-model predictions. The identified studies are listed in Table 5.

Conjugated urinary 1-OH-Pyrene

The analytical method for 1-OHP is urine may utilize an enzymatic hydrolysis step with glucuronidase or a mix of glucuronidase and sulphatase. Other studies apply acidic hydrolysis to liberate the conjugated metabolites. In all selected studies, hydrolysis of the conjugated 1-OHP was applied. That means that the reported concentration of 1-OHP in urine in the selected studies is the sum of the free fraction and the conjugated fraction of 1-OHP = total 1-OHP.

Different units are used to express the level of 1-OHP in urine. Spot urine samples are often corrected for dilution using creatinine. The kinetics of creatinine excretion parallels that of 1-OHP and a creatinine excretion rate—normalized excretion rate of 1-OHP appears to allow for a better metric of 1-OHP urinary excretion (Viau et al. 2004). Such units (in μg/g creatinine or μmol/mol creatinine) and excretion rates (in μg/h or μmol/h) need to be converted to the predicted units of the PBTK-model, that is amounts in μmol and concentrations in μmol/L. Units that have been used for the conversion of urine levels are presented in the "Appendix".

Results

Partition coefficients of pyrene

Absorbed pyrene is distributed in the body over fluids and tissues. The tissue-to-blood partition coefficients and the tissue-specific (arterial) blood flows control the supply and discharge of the substance over the body compartments and therefore also the concentration in the various model compartments.

In the rat PBTK-model for oral and i.v. pharmacokinetics of pyrene of Haddad et al. (1998), tissue-to-blood

Table 5 Studies on absorption, metabolism and urinary excretion of pyrene and metabolites in workers and/or volunteers

Nr	Study item and type of study	Subjects and exposure	Main results	References
1	Measure of tissue-to-blood partition coefficients of pyrene Indirect measurement	Blood of rat	Pyrene partitioning tissue-to-blood of 7 tissues	Haddad et al. (1998)
2	Ration of parent pyrene and metabolite 1-OHP in urine	Coke oven workers	Average level 1-OHP = 15.4 μg/L, Average level of unmetabolized pyrene 0.020 μg/L. Ratio is 800	Rossella et al. (2009)
3	Experimental study after inhalation (and retention) of PAH and excretion of urinary 1-OHP in aluminum plant workers + contribution of dermal route to total absorbed dose	5 volunteers, 6 h inhalation exposure (2 times) 3 volunteers, 8 h dermal exposure from air concentration	About 60% of inhaled pyrene was retained. The biological half-life of 1-OHP was determined at 9.8 h. Dermal exposure may account for up to 20% of the total absorbed pyrene dose	Brzeznicki et al. (1997)
4	Exposure chamber study after dermal and inhalatory exposure of bitumen fumes and urinary excretion of 1-OHP	10 volunteers with respirators. 8 h dermal exposure via vapor	About 55% of bitumen vapor was absorbed by the skin	Walter and Knecht (2007)
5	Observational study in electrode paste plant workers. Protective effect of dust respirator mask on urinary 1-hydroxypyrene concentration	18 workers, two weeks of 5 × 8 h exposure to PAH + 2 * 5 days urine sampling	Significant reduction of 1-OHP when workers are more intensive using respirators	Bentsen et al. (1998)
7	Observational study of the exposure to PAH in oil shale industry	49 workers and 10 controls. Personal air sampling + skin wipe samples + post-shift urine samples	High exposure to PAH. Poor correlation airborne pyrene with urinary 1-OHP	Kuljukka et al. (1996)
8	Observational study of the exposure to PAH in coal liquefaction	5 workers were followed for 4 shifts and 4 days off work. PAS sampling and pre-, peri- and post-shift urine samples	Increase and decrease of urinary 1-OHP over a nine-day period	Quinlan et al. (1995)

partition coefficients have been used that were measured experimentally by equilibrium dialysis. Tissue homogenate and blood were dialyzed in 0.1 M Tris–HCl buffer of pH 7.4 to the same buffer in a metabolic shaking bath. The tissue-to-buffer partition coefficient was divided by the plasma-to-buffer partition coefficient to get the tissue-to-plasma partition coefficient. Mean ± SD of the values obtained for five incubation concentrations (0.05, 0.1, 0.2, 0.4 and 0.6 mmol/L) for each tissue. Table 6 shows the results.

In the PBTK-model, the partition coefficients of tissues to blood for pyrene are estimated by a QSPR (Quantitative Structure–Property Relationship). The algorithm of the partition coefficient is a function of the octanol-to-water partition coefficient (K_{ow}) (Jongeneelen and Ten Berge 2011). The predicted partition coefficients of pyrene are listed in Table 6. The table shows that the estimated values are at maximum one order of magnitude higher than measured, leading to a higher tissue preference for pyrene.

It should be noted that the comparison of measured and modeled tissue-to-blood partition coefficients might be biased, since rat and human blood differ in composition. Also partitioning to plasma of the rat was compared to the predicted values in human blood. Moreover, experimental conditions in measuring partition coefficients are not standardized, and this may hamper the comparison.

Ratio of parent pyrene, 1-OH-pyrene and 1-OH-pyrene-gluc in urine

A recent study of coke oven workers reported on concentrations of both parent PAH and hydroxylated PAH metabolites in urine of coke oven workers. In a series of urine samples, taken at the end of a 3-day working period, a median level total 1-OH-pyrene of 15.4 μg/L was found, whereas in the same samples, a median level of 0.020 μg/L unmetabolized pyrene was found (Rossella et al. 2009).

The simulation in the PBTK-model of the level of parent pyrene and the two hydroxylated pyrene-metabolites in urine was done using an typical exposure scenario of coke oven workers (3.0 μg/m^3 pyrene in the breathing zone and dermal exposure rate of 1.0 ng * cm^{-2} * h^{-1} pyrene at a skin surface of 5,000 cm^2 repeated over 3 consecutive shifts of 8 h, based on exposure data of VanRooij et al. 1993). The model-predicted concentrations are presented in Table 7. The simulation predicts that 1-OHP-glucuronide is the very dominant urinary metabolite. Free 1-OHP and unmetabolized parent pyrene are present in urine as a trace. The ratio of total 1-OHP versus the parent pyrene as predicted by the PBTK-model is 240.000. This is much higher than the ratio of 800, as measured by Rossella et al. (2009). This seems a large omission in the model, but it is obvious that only a trace of parent pyrene is excreted in urine. It is known that the assays for the determination of traces of unmetabolized PAH in urine at the ng/L level are with large errors. The analytical uncertainty obscures solid conclusions. However, it is clear that the model seems to favor the pathway of urinary excretion of 1-OHP-gluc compared to the real-life situation.

Table 6 Rat tissue-to-plasma partition coefficients for pyrene determined by equilibrium dialysis (Haddad et al. 1998) and QSPR predicted tissue-to-human blood partition coefficient in the PBTK-model

Tissues	Measured value (Haddad et al. 1998) Tissue-to-rat plasma partitioning coefficient	QSPR-estimate Tissue-to-human blood partition coefficient
Liver	2.37 ± 0.16	8.27
Kidney	2.38 ± 0.10	7.6
Brain	1.71 ± 2.25	14.4
Lung	2.25 ± 0.12	5.24
Heart	1.85 ± 0.06	5.24
Muscle	1.58 ± 0.09	5.24
Small intestine	1.54 ± 0.05	8.27
Testes	1.67 ± 0.09	–
Fat	11.84 ± 0.54	142

Comparisons of observations with model predictions

Comparison 1: controlled human volunteer inhalation study (Brzeznicki et al. 1997)

An experimental study was carried out with 5 volunteers to study the elimination of 1-OHP after exposure to pyrene (Brzeznicki et al. 1997). The volunteers were exposed during 6 h to pyrene at an aluminum plant. The individual 6 h-TWA concentrations of pyrene were between 4.2 and 23.5 μg/m^3. The volunteers were breathing at rest. The experiment was carried out twice. The mean 6 h-TWA inhaled concentration of the 10 observations was 11.8 μg/m^3.

Urine samples for 1-OHP determinations were collected before exposure and during the second, fourth, sixth, eighth, tenth, 12th, 14th, 16th, 23rd, 25th, 27th, 29th, 31st, 35th, 39th, 47th, 51st, 55th, 59th, 63rd and 71st hours since the beginning of the experiment. Urine concentrations of 1-OHP were determined after enzymatic hydrolysis, thus the sum of free 1-OHP + 1-OHP-gluc was determined. The excreted amount 1-OHP in urine over 71 h ranged from 3.2 to 14.9 μg that is equal to a range of 16–74 nmol. The average excreted amount in urine was 40 nmol. The predicted amount was calculated in the PBTK-model using two different absorption scenarios after inhalation:

Absorption scenario 1: only pulmonary uptake The average concentration of 11.8 μg/m^3 pyrene, inhaled over

Table 7 Ratio of total 1-OHP versus unmetabolized pyrene in urine samples of PAH-exposed coke oven workers

Compound	Measured (Rossella et al. 2009) Concentration in end-of-shift urine after three shifts	Predicted with PBTK-model Concentration in end-of-shift urine after three shifts (based on a typical exposure scenario of coke oven workers)
Pyrene (μg/L)	AM: 0.020 Range: 0.005–0.742 $N = 54$	0.000134
Free 1-OHP (μg/L)	–	0.000001
1-OHP-gluc (μg/L)	–	48.8
Total 1-OHP (=free 1-OHP + 1-OHP-gluc) (μg/L)	AM: 15.4 Range: 1.1–147.1 $N = 54$	48.8
Ratio total 1-OHP versus pyrene in urine	775	240.000

6 h, was used as input for the PBTK-model prediction. The model predicted an amount of 119 nmol 1-OHP excreted in urine in 71 h. That is 2.5-fold higher than the measured average and above the highest individual value.

Absorption scenario 2: 75% pulmonary uptake +25% secondary ingestion The second scenario of absorption is the scenario with in part secondary ingestion of the particulates. It was assumed that 25% in the inhaled dose was secondary ingested as coarse particles. The secondary ingested dose was modeled as oral bolus dose. The scenario 2 was: 6 h of 8.9 μg/m^3 inhalation and 14.7 μg as oral dose (based on inhalation of 10 m^3 in 6 h with 2.95 μg/m^3 as course particles and 50% retention). The oral dose is equal to 14.7/70 = 0.21 μg/kg body weight.

The PBTK-model predicted for this scenario an amount of 124 nmol 1-OHP excreted in urine in 71 h. It seems that secondary ingestion does lead to the same excreted amount.

The observed and predicted data are summarized in Table 8. The model-predicted uptake after inhalation of pyrene is approximately a factor 2–3 overestimated.

Comparison 2: controlled transdermal exposure study of bitumen fume (Walter and Knecht 2007)

Walter and Knecht (2007) conducted an experimental study in which volunteers were dermally exposed in an exposure chamber to bitumen fume. Ten male non-smokers were exposed for 8 h to bitumen fume. They wore only short pants and shoes during the experiment. This results in an exposed skin surface of approximately 15,000 cm^2. To prevent inhalatory intake, the volunteers used a powered air purifying respirator. The efficacy of protection by the respirator was confirmed. Concentrations of PAH were controlled by a constant generation of bitumen fume. Both particles and vapor were sampled and analyzed for PAH's. The bitumen particulate concentration was 2.5 mg/m^3 and the bitumen vapor concentration was 17.9 mg/m^3. The concentration pyrene in aerosol and vapor was reported as 0.55 and 0.10 μg/m^3, respectively. Samples of urine were collected before starting, during the experiment and after the end of exposure over a period of 24 h. Urine samples were analyzed for hydroxylated PAH metabolites, among them 1-OHP after acidic hydrolysis. Figure 2 shows the

Table 8 Measured excreted mass of total 1-OHP after inhalation of pyrene (Brzeznicki et al. 1997) and PBTK-model predicted

Exposure conditions	Measured excreted amount of total 1-OHP in urine of 5 volunteers (in μmol over 71 h)	Predicted amount as sum of 1-OHP + 1-OHP-gluc in urine (in μmol over 71 h)
Absorption scenario 1: Only pulmonary uptake. Six h inhalation of an average concentration pyrene of 11.8 μg/m^3. No secondary ingestion was assumed	0.040 (0.016–0.074)	0.119
Absorption scenario 2: Ninety percentage pulmonary uptake + remainder secondary ingested. It was assumed that 10% was secondary ingested as course particles, taken up as oral bolus dose (scenario = 6 h of 8,9 μg/m^3 inhalation and 6 μg oral dose, based on inhalation of 10 m^3 in 6 h and 50% absorption)	0.040 (0.016–0.074)	0.124

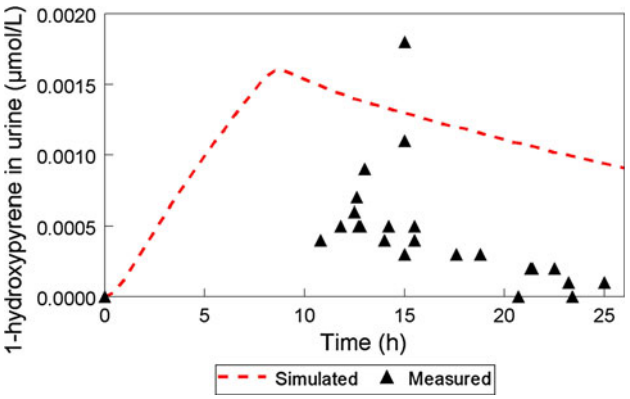

Fig. 2 Concentrations of 1-hydroxypyrene in urine of naked volunteers with respirators who were dermally exposed to bitumen fume (Walter and Knecht 2007). The data are corrected for background excretion

measured concentrations of 1-OHP in urine of the 10 volunteers after the end of exposure until 24 h. The concentrations were taken from Fig. 5 of the original paper and are expressed as net value (=pre-exposure background of 65 ng/g creatinine was subtracted from measured value). Figure 2 shows that the interindividual variation of 1-OHP in urine in this experiment is at maximum fourfold.

This exposure scenario of the experiment was entered in the PBTK-model to predict the concentration of 1-OHP-gluc in urine:

- Airborne pyrene concentration: 0.65 µg/m^3 during 8 h;
- Respiratory protection factor: 100,000,000;
- Level of activity: at rest.

The dermal uptake was simulated in the model only as vapor, since actual dermal dose rates in this experiment have not been reported in the paper of Walter and Knecht (2007). The predicted level of 1-OHP-gluc in urine is within the boundaries of the measured level of total 1-OHP in the experiment. However, the exposure level to the skin is very low. At a trace level of exposure, one can expect a growing similarity between measured and model simulated levels when the exposure level come close to the zero level. One should realize that the observed similarity in this experiment might partly be caused by the very low exposure level of the skin.

The mass balance of the simulation showed that in this experiment, 11 * 10^{-5} µmol/kg BW of pyrene has been absorbed from transdermal uptake of pyrene vapor through the skin. The simulation showed that a quarter of this amount (2.5 * 10^{-5} µmol/kg BW) is excreted in urine over 26 h as 1-OHP-gluc. The remainder of the parent pyrene and metabolites is still in tissues (2.1 * 10^{-5} µmol/kg BW), in enterohepatic circulation tissues (1.5 * 10^{-} µmol/kg BW) or lost in other metabolites (4.6 * 10^{-5} µmol/kg BW).

Comparison 3: reduced inhalation of pyrene in electrode paste workers (Bentsen et al. 1998)

The protective effect of dust respirator masks was studied by measuring urinary 1-hydroxypyrene in PAH-exposed paste workers. Eighteen workers in 4 job categories were involved. Personal air sampling of particulate and vapor pyrene was performed every work shift for two consecutive weeks. Urine samples were collected as pre- and post-shift samples every work shift for two consecutive weeks. In the second week of the study, an intervention was introduced: the workers were encouraged to wear FFP3SL respirator masks persistently. In this respirator intervention week, a significant reduction in urinary 1-hydroxypyrene in end-of-shift samples was found. Table 9 shows the exposure level. The urinary concentrations of 1-OHP are converted to µmol/L.

The time course of the average 1-OHP excretion of the 18 workers in the normal and the intervention week is presented in Fig. 3. The figure shows that the reduction due to the use of the respirator is of borderline significance.

The level and increase over the working week of the concentration 1-OHP in urine of the workers was predicted by the PBTK-model using the week exposure scenario of 5 days * 8 h working in a working atmosphere with an average concentration of pyrene in vapor and particulates of 2.75 µg/m^3. The predicted pattern of total 1-OHP in urine is shown in Fig. 3.

Table 9 Pyrene exposure metrics of 4 job categories of an electrode paste plant (Bentsen et al. 1998)

Group	*N*	Name	Pyrene in breathing air		Urinary 1-OH-Pyrene	
			Vapor (µg/m^3)	Particulate (µg/m^3)	Normal week increase over shift (µmol/L)	Respirator intervention week increase over shift (µmol/L)
1	3	Mixing	2.4	1.04	0.061	0.051
2	8	Truck driving	1.8	0.20	0.082	0.088
3	4	Mold filling	4.9	0.60	0.155	0.098
4	3	Miscellaneous	2.2	0.34	0.093	0.053
All	18	All	2.4	0.35	0.090	0.084

Median levels are reported

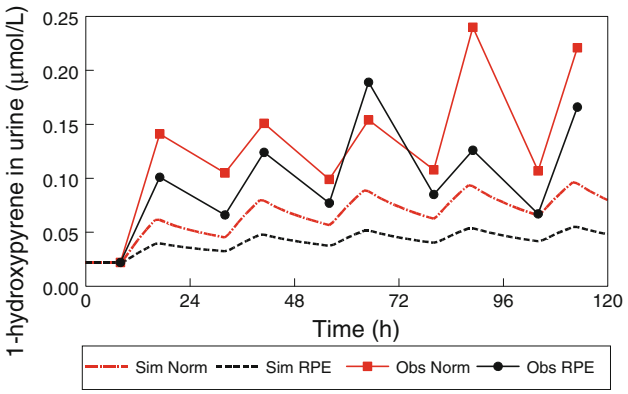

Fig. 3 Measured concentrations of 1-OHP in urine of exposed workers in a normal week and in a week with increased use of respirators (Bentsen et al. 1998). Background excretion was added to the simulated level

True respirator protection depends on the quality of the respirator and the efficacy of use by the workers. The assigned protection factor of a FFP3SL respirator is 5–10, but in practice, the average protection factor is lower due to inappropriate use. For the prediction of total 1-OHP in urine in the respirator intervention week, we applied a protection factor of 5 for the respirators. The result is shown in Fig. 3. Comparison of the simulations with the measured data showed that the model-predicted effect of the respirator is far greater than the observed effect. Given the approximately threefold higher measured concentrations than modeled concentrations with the scenario of unprotected inhalation of pyrene, it seems that there is another significant route of uptake in the electrode paste working environment. It should be kept in mind that inhalation and skin uptake are both important routes for PAH exposure in this work environment. The dermal exposure dosimetry could not be taken up in the model, since the dermal exposure levels were not reported.

Comparison 4: exposure to pyrene in cokery workers of shale oil industry (Kuljukka et al. 1996)

The exposure of 49 Estonian cokery workers to PAH at an oil shale processing plant was assessed by occupational hygiene and biomonitoring measurements. To assess the external dose of exposure, the concentration of pyrene was measured in the breathing zone of workers during an 8 h work shift. Skin contamination with pyrene was assessed by skin wipe sampling. As a biomarker of exposure to polynuclear aromatic hydrocarbons and as an integral of all possible absorption routes of pyrene, 1-hydroxypyrene concentration was measured in post-shift urine samples. Mean airborne concentration of pyrene was 8.1 (range: 0.01–69.6) µg/m^3. Based on skin wipe sample analyses, the skin contamination was also obvious. The mean value of pyrene on the samples collected after the 8 h shift was 1.6 (range 0.1–9.0) ng/cm^2. The mean value of urinary 1-hydroxypyrene concentration was 6.0 (range 0.2–69.5) nmol/mmol creatinine for the exposed workers and 0.5 nmol/mmol creatinine for the 10 controls. The urinary concentrations of 1-OHP are presented in Table 10. The urinary concentrations were converted to µmol/L.

The average exposure data (8.1 µg/m^3 pyrene in the breathing zone and dermal exposure rate of 0.2 ng * cm^{-2} * h^{-1} pyrene on the hands and upper arms with an estimated skin surface of 2,000 cm^2) were used to predict the concentration of total 1-OHP in urine of the exposed cokery workers with the PBTK-model. The predicted concentrations at the end of the 8 h shift are presented in Table 10. The predicted level of 0.131 µmol/L is nearly 3 times higher compared to the average measured level, but lies within the large range of the observed level. Additional simulations showed that an exposure scenario of inhalatory exposure only resulted in a post-shift concentration of 0.129 µmol/L. Dermal exposure only resulted in a post-shift concentration of 0.002 µmol/L. Inhalation of pyrene seems to be the major route of exposure.

In this case, the range of exposure to airborne pyrene was very wide, and the concentration ranged from 0.01 to 69.6 µg/m^3. That resulted in a wide range of 1-OHP in urine: 0.002–6.19 µmol/L. The large range of individual exposure levels of the workers obscures a detailed comparison of the measured level of 1-OHP with the modeled level of 1-OHP, only average levels (with ranges) were reported by Kuljukka et al. (1996).

Comparison 5: exposure to pyrene in coal liquefaction workers (Quinlan et al. 1995)

A study in five coal liquefaction workers (E1, E2, E3, E6 and E7) was conducted to examine relationships between exposure to polycyclic aromatic hydrocarbons (PAHs) and

Table 10 Measured levels of total 1-OHP in urine of cokery workers of shale oil industry and PBTK-model predicted urine levels

Group	*N*	Measured post-shift total 1-OHP (Kuljukka et al. 1996)		PBTK-model predicted post-shift concentration total 1-OHP (In µmol/L)
		(In nmol/mmol)	(Converted to µmol/L)	
Cokery workers	49	6.0 (0.2–69.5)	0.054 (0.002–6.19)	0.13
Controls	10	0.5	0.005	<0.000004

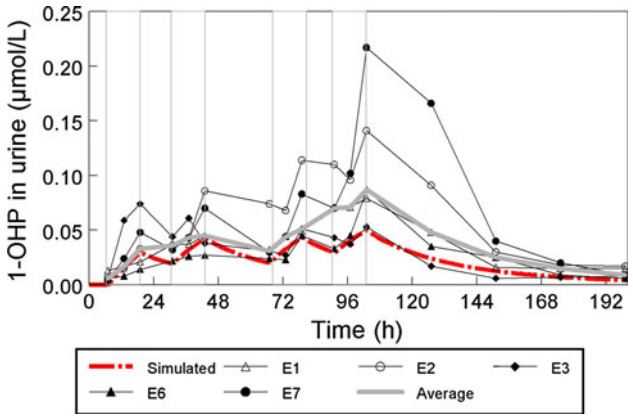

Fig. 4 1-OHP in urine of five coal liquefaction workers in a 9-day period (Quinlan et al. 1995). The data are corrected for background excretion

excretion of urinary 1-hydroxypyrene (1-OHP). During a study period of 8 days, workers were followed in the course of four 12 h shifts and 4 consecutive days off work. At each shift, a urine spot sample was collected at the start. All urine voided during the 12 h shift was collected, a representative aliquot was taken as perishift sample and a further spot sample was taken at the end of the shift. This was repeated for all four shifts. On the days off work, morning urine was collected. The individual concentration of 1-OHP in the course of 8 days is shown in Fig. 4. Also the average level is indicated. The 1-OHP levels are corrected for the background, as determined at the start of the study. Individual exposure levels varied largely, the variation between workers was larger than the between-day variation.

Personal air monitoring of particulate and vapor pyrene concentrations was performed as average values over 2 shifts for each worker. The median airborne concentration of particulate pyrene was 0.8 µg/m^3, whereas the vapor phase concentration was 0.5 µg/m^3. This makes the total airborne pyrene concentration 1.3 µg/m^3.

The concentration of total 1-OHP in urine was predicted with the PBTK-model using the exposure scenario of 4 * 12 h inhalation of 1.3 µg/m^3 pyrene, with an extra 12 h off after the 2nd shift, due to changed day/night service. The predicted concentration in the course of the 9-day period is very close to the average of the measured level (Fig. 4). The median excreted amount of total 1-OHP as calculated from the area under curve of 1-OHP excretion rate from day 1 to day 8 was 0.31 µmol (Quinlan et al. 1995). The model-predicted amount of total 1-OHP in urine over 192 h was 0.28 µmol. That suggests that in the second exposure route—dermal exposure—was not very dominant. However, knowing the large variation of exposure between workers and between shifts, occasionally high dermal exposure on certain days cannot be excluded.

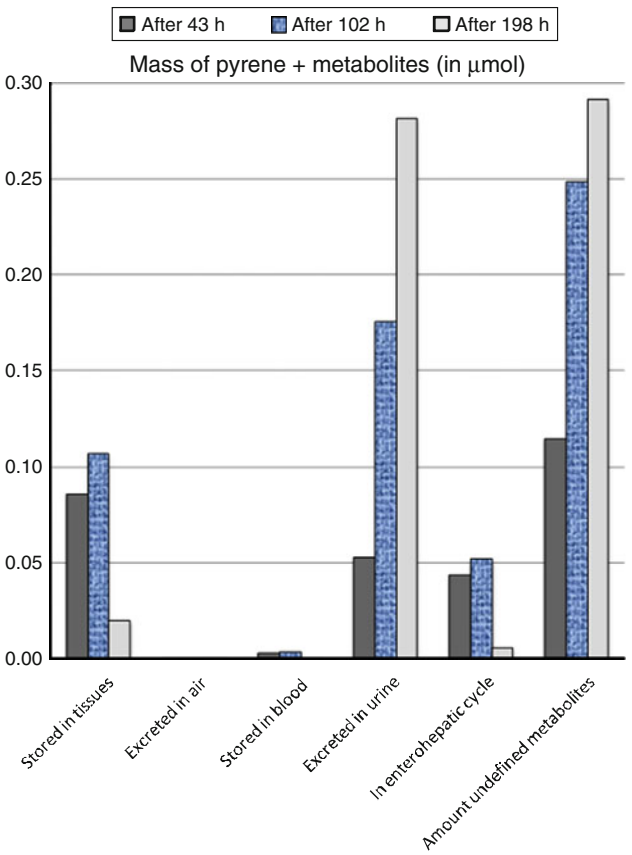

Fig. 5 The predicted mass of pyrene and metabolites in the different compartments and fluids of coal liquefaction workers at three time points in the course of a period with 4 consecutive shifts on work followed by 4 shifts off work (as indicated in Fig. 4)

The predicted mass of pyrene and metabolites in the different compartments and body fluids at three time points (after the first 2 shifts, after 4 shifts and after 8 days) is shown in Fig. 5. The figure shows that the amount in tissues is only a little increasing after the first two shifts. The amount in the enterohepatic circuit is about the half of the amount in tissues. The quantity excreted in urine is as large as the quantity of lost undefined metabolites. Figure 5 illustrates the fate of pyrene in a longitudinal 8-day period with 4 shifts with exposure and the last 4 shifts off work. The amount in the enterohepatic circuit is significant. It is the metabolite 1-OHP-gluc that is biliary excreted, hydrolyzed in the intestine, and the free 1-OHP is reabsorbed from the gut.

Discussion

In this study, published studies with human biomonitoring data of pyrene were selected to test/validate the generic PBTK-model IndusChemFate. Pyrene is an interesting compound because many human biomonitoring data are available. And uptake may occur through inhalation and by

transdermal absorption. The main metabolism of pyrene is the pathway: pyrene ==> 1-OHP ==> 1-OH-P-gluc. As the PBTK-model allows a metabolism setting in which the removal of the parent compound may differ from the formation of metabolite, loss of parent compound and production of metabolite can be modeled separately. This makes that within a complex metabolism scheme a certain pathway can be modeled. This option was used in the PBTK-model entries for pyrene to account for the production of unknown pyrene-metabolites. The PBTK-model also allows additional metabolism in other tissues as lungs and skin. However, no data of metabolism kinetics of pyrene in these tissues of human origin were available. Thus, metabolism was assumed to occur in the liver only.

Absorption of pyrene was simulated in scenarios with inhalation, dermal uptake and secondary oral uptake. The simulations show that time course, concentrations and total excreted amount of pyrene and metabolites are roughly predictable, within an order of magnitude. The fate of pyrene in the body was also tested. The accumulation of 1-OHP-gluc in urine might either be caused by the storage of the parent compound pyrene in the adipose tissue, by delayed dermal uptake, due to a dermal depot or reservoir effect of pyrene in skin or by severe enterohepatic circulation of the metabolite 1-OHP-gluc. Our results underline the suggestion of Viau et al. (1999) that enterohepatic circulation of the 1-OHP-gluc was the strongest determinant that delays the excretion and thus is the reason of the delay in excretion of the hydroxylated metabolites in urine. The predicted amount of storage in different tissues including adipose tissues compared to storage in the intestinal lumen storage confirms this hypothesis.

Dermal contamination of pyrene on the skin, due to occupational or environmental exposure, occurs never as a pure compound. In contrary, pyrene is always present in a complex matrix with many different compounds, mostly unidentified. In most cases a mix of heavy MW PAH and lighter MW aromatic compounds are present. This might results in unknown interactions in the dermal absorption process.

Transdermal uptake of vapor of pyrene seems to be relevant. Transdermal uptake of vapor has also shown for other low-volatile compounds such as NMP (Bader et al. 2008) and glycol ethers (Corley et al. 1997; Johanson and Boman 1991).

The widely accepted bioindicator of exposure to PAH is urinary 1-OHP after enzymatic hydrolysis (=Σ1-OHP + 1-OHP-gluc). The level of unmetabolized pyrene in urine is 3 orders of magnitude lower than the hydroxylated metabolites. Biomonitoring of unmetabolized PAH does not seem an attractive alternative. Another disadvantage of unmetabolized PAH as bioindicator is possible contamination of urine samples.

Fecal excretion is not taken up in the model. Rat studies show that this excretion pathway is significant. However, human data are not available. We assumed that biliary and fecal excretion in humans is not very dominant.

One should be aware of a background-level of 1-OHP in urine, due to smoking and dietary intake of PAH. That will be important to account for, especially when the levels of exposure are only just above the background exposure, such as in cases of environmental exposure.

Possible bias in model predictions is caused by the following:

- Inaccurate metabolic parameters;
- Matrix effects in the dermal uptake process.

When the tables and plots of the model predictions are inspected with the experimental values, the correspondence of predicted shape curves and levels of total 1-OHP in urine suggest confidence in the model structure and parameters. The PBTK-model mimics real-life observations of hydroxylated metabolites of pyrene in urine within one order of magnitude. Sensitivity testing was not yet done; however, some initial tests showed that enzyme kinetics and enterohepatic circulation are the strongest parameters that have a substantially impact on the pyrene model output results.

It appeared that urinary excretion of pyrene and pyrene-metabolites in humans is predictable with the PBTK-model. The model outcomes have a satisfying accuracy for early testing, in so-called *1st tier* simulations and in range finding. This newly developed generic PBTK-model IndusChemFate is a tool that can be used to do early explorations of the significance of uptake of pyrene in the human body following industrial or environmental exposure scenarios. And it can be used to optimize the sampling time and urine sampling frequency of a biomonitoring program.

In conclusion,

- Time course, concentration and total excreted amount are predictable under different exposure scenarios. PBTK-modeling of excretion of the main urinary metabolites of pyrene with the newly developed generic model seems accurate;
- This pyrene PBTK-model is sensitive to enzyme kinetics and hepatic circulation rate;
- Enterohepatic circulation, but not storage in adipose tissue, seems the reason of accumulation;
- The pyrene PBTK-model allow to estimate real-time concentrations in blood and urine, as well as peak-concentrations and total excreted amounts of primary metabolites after occupational and or environmental exposure to PAH, with an accuracy of an order of magnitude.

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Conflict of interest The authors declare that they have no conflict of interest.

Appendix

See Table 11.

Table 11 The excreted amount or concentration is given in different units

1-OHP		
Mol weight	218.26	
1 μg/L	0.71 μg/g creatinine	0.37 μmol/mol creatinine
1 μmol/mol creatinine	1.93 μg/g creatinine	2.70 μg/L
1 μmol/mol creatinine	0.0124 μmol/L	12.4 nmol/L
1 μg/g creatinine	6.41 nmol/L	
1 μg/h	16 μg/L	0.073 μmol/L
Pyrene		
Mol weight	202.25	
Creatinine		
Mol weight	113.12	

Conversion to equal units can be done on the assumption that a 75 kg person void 1.5 L urine in 24 h and that 1 L urine contain 1.4 g creatinine

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