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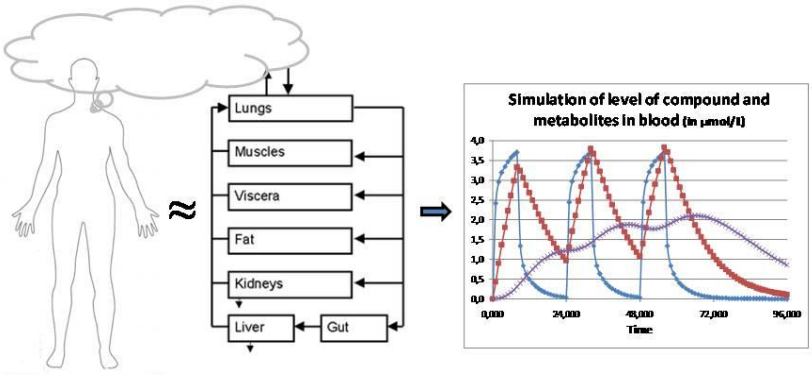

IndusChemFate

A multi-chemical PBTK-model in MS-Excel applicable

for workers, consumers and experimental animals

User manual, *version 2.00*

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- ✓ Occupational and Environmental Medicine
- ✓ Industrial Hygiene
- ✓ Toxicology
- ✓ Epidemiology
- ✓ Risk Assessment

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Summary

IndusChemFate is a Physiologically Based Toxicokinetic model (PBTK-model) to estimate blood and urine concentration of multiple chemicals, given a certain exposure scenario. It has been developed as a software application in MS-Excel.

Three uptake routes are considered (inhalation, dermal and/or oral) as well as two built-in exercise levels. The model holds the physiology of various humans, experimental rats and mice. The layout of the model is in line with most PBPK models. The model contains 11 body compartments (Lung, Heart, Brain, Skin, Adipose, Muscles, Bone, Bone marrow, Stomach & Intestines (lumped), Liver and Kidney).

The model holds the physiology of human subjects (adult males, adult females, children) and the experimental animals (rat and mouse). Standardized physiology parameters of humans with normal weight and obese are used to dimension organ and tissue volumes and blood flows through these tissues. These parameters are scaled relative to the total body weight. The level of exercise can be set on two levels of exercise (at rest and at light work).

The model contains published and in-house developed algoritmes (QSPRs = Quantitative Structure-Property Relationships) for blood:air partitioning, tissue:blood partitioning and renal excretion.

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.

Michaelis-Menten saturable metabolism is incorporated in the model. Metabolism can be modeled in any of 11 body compartments or in liver only. Tubular resorption is considered optionally either based on user input or based on a built-in QSPR, dependent on the octanol:water partition coefficient. Enterohepatic circulation is optional at a user-defined rate.

The model IndusChemFate is programmed in Visual Basic and runs in MS Excel. The data input proceeds via input in two worksheet of the Excel-file. Output is presented as numerical listing in time and in graphs and is presented in the same Excel-file. The program is provided as freeware (from CEFIC-LRI-website) and has a open source code.

References to this PBTK-model IndusChemFate are published in the scientific literature. Outcomes are aimed to have an accuracy within an order of magnitude. The model is regarded as a *first tier tool* or screening tool. The initial version 1.6 was published in August 2010. The extended version of the model is now published and available as IndusChemFate version 2.00.

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1 Introduction

Computational toxicology (or in-silico toxicology) is an emerging field between toxicology, chemistry, biostatistics and computer sciences. It may enhance the capacity to estimate risks of chemicals associated with exposure. PBPK modeling is one of the applications of this discipline.

Rise of PBPK modeling

Physiologically Based PharmacoKinetic or ToxicoKinetic (PBPK- or PBTK-, in this document the term PBTK is used) models describe the body as a composition of relevant compartments and physiological processes by means of mathematical equations. PBTK models were developed for the simulation of absorption, distribution, metabolism and excretion (ADME) of xenobiotics. The essential concepts were outlined roughly 70 years ago in a farsighted paper [1] that presented many of the mathematical relationships required to simulate blood flow and tissue distribution. Simulation modeling ideas were further developed to explain the effect of anesthetics [2], and early attempts to apply the approach to drugs were published in the 1960s [3]. Probably the most important contributions in that period were made by Bischoff and Dedrick [4], who demonstrated that PBPK models could be used for the a priori prediction of the pharmacokinetics of thiopental. During the following decades, developments were made by academics such as Rowland [5], Sugiyama [6] and Amidon [7], as well as scientists working in the environmental health field, in particular Anderson and Clewell [8, 9].

Principle of PBPK models

PBPK models generally contain various body compartments: lungs, liver, rapidly perfused tissues, slowly perfused tissues and fat. Depending on the route of administration, metabolism and excretion of a compound compartments may be added or lumped. As PBPK models take in account human physiology, physiological parameters as compartment volumes and blood flows are needed to describe the concentration in different compartments. Distribution is furthermore described by partitioning coefficients that control medium to medium transfer (for instance air to blood or blood to tissue).
Most PBPK models are substance specific and built for very specific purposes, for example the estimation of disposition of a certain drug prior to in vivo studies [7, 10] or cancer risk assessment for a specific industrial chemical [9, 11, 12] . The toxicokinetics of industrial chemicals are generally less extensively studied compared to medicines. For many medicines drug-specific PBPK models are (commercially) available [13]. Compound or substance specific models may zoom in on different compartments (depending on the critical endpoint) by alteration of the model layout. Compartments may be added or removed as well as circulations between compartments. As these models may get into great detail on multiple metabolites they typically require very detailed information that is obtained from experimental work. As a result this type of PBPK models is tailor-made to the compound of interest.

Generic PBTK models

The disadvantage of compound specific PBPK models is their need for compound specific input data and a relatively narrow application domain. From these perspectives several initiatives were developed to develop PBPK models that can be used for multiple compounds. In the environmental and occupational risk assessment methodology PBPK modeling was introduced. In this context the term 'generic PBTK' (Physiologically Based ToxicoKinetic) modeling is often used, meaning that these models can be applied to multiple compounds [14-17].

Several generic PBTK models for multiple compounds have been developed. Cahill, Mackay et al [18] published a generic PBPK-model for multiple environmental contaminants in an easy accessible software program in MS Excel. The model relies on available physical-chemical partitioning and reactivity data, but experimental partitioning and absorption efficiency data can also be used to refine the parameters. Tissue concentrations for each of the chemicals and metabolites are simulated for acute, occupational and environmental exposure regimes. The same model may be used for all chemicals and exposure regimes with only the physical-chemical properties, reaction rates, and exposure conditions being variables. Luecke et al. [19] reported on a generalized PBPK model (called PostNatal) that could be used by supplying appropriate pharmacokinetic parameter estimates for the chemicals of interest. The Windows based program consists of four PBPK models in one with each PBPK model acting independently or totally integrated with the others through metabolism by first order or Michaelis–Menten kinetics. Dosing may occur by ingestion, dermal, inhalation, or more. Elimination can be modeled through the feces, urine, and/or hair.

Beliveau and Krishnan [20] developed a PBPK spreadsheet program in MS Excel for the inhalation of volatile organic compound (VOC). It is driven by a QSPR (Quantitative Structure-Property Relationship) that they derived from experimental rat data, based on structural fragments [21]. US-EPA also developed a very detailed generic PBPK model, the so-called Exposure Related Dose Estimating Model (ERDEM), focusing on risk assessment for environmental agents [22]. It allows the user to input data up to a very high level of detail. Disadvantage of the model is that detailed compounds-specific data are needed. Other generalized PBPK models that were published are among others PKQuest by Levitt [23] (based on experimental data, bridging the gap between animal experiments and human values), PKSim by Willman [24] (focusing merely on drugs) and a generic PBPK model by Brightman [25] (predicting plasma concentrations based on extrapolation of animal data).

Table 1. Overview of generic PBPK models.

Authors	Model Name	Reference
Cahill et al	No name	[18]
Luecke et al	PostNatal	[19]
Beliveau & Krishnan	No name	[20]
EPA	ERDEM	[22]
Levitt et al	PKQuest	[23, 26-28]
Willman et al	PKSim	[24]
Brightman et al	No name	[25, 29]

A drawback of some generic models is that they are not built in easily accessible software, although Cahill et al and Beliveau & Krishnan present their models in MS Excel. The applicability of QSPRs (Quantitative Structure-Property Relationships) in the model is an advantage, especially for generic models.
Generic models are screening (or *first tier*) tools for the evaluation of chemical risk assessment as it supports the assessment of internal exposure especially when little is known of the toxicokinetics of a compound.

Why another new generic PBTK model?
In silico tools for internal and external exposure modeling receive increasing attention in the chemical industry, driven by the need to limit the use of experimental animals and by the demand for data in the context of the European REACH legislation for chemicals. The latter regulation require to derive so called DNELs (Derived No Effect Level) as limit values for the protection of health of workers and for the general population. The DNELs are specified for the route of entry, by inhalation, ingestion or direct skin contact. The DNELs are mostly obtained from NOAELs (No Observed Adverse Effect Levels) in experimental animal studies after applying assessment factors. Unfortunately, the available experimental animal studies often deal with only one route of entry, so the NOAELs via other routes of entry have to be estimated by applying fixed regulatory adjustment factors. These adjustment factors tend to be conservative. A generic PBTK model can be used to improve route-to-route extrapolation of DNELs.
Furthermore it can help to improve (bio)monitoring strategies by the estimation of an internal dose-metric (*biological equivalent guidance value* or BEGV) based on external exposure limits. Also sampling could be optimized, for instance by defining an appropriate sampling medium and sampling time.
The aim was to develop a *(first tier)* screening tool for data-poor substances that should be able to estimate blood- and urine concentrations of various chemicals and its metabolites, given a certain exposure scenario. The resulting model is called IndusChemFate. It is a generic, cross-chemical predictive PBTK Model that is available as freeware with a open source code. The scientific background and rationale, together with examples of simulations of experiments with dosimetry and body fluid concentrations of various compounds are published in two papers (table 2):

Table 2. Scientific references of the PBTK-model IndusChemFate.

No	*Reference of paper*
1	Frans J. Jongeneelen; Wil F. Ten Berge. A Generic, Cross-Chemical Predictive PBTK Model with Multiple Entry Routes Running as Application in MS Excel; Design of the Model and Comparison of Predictions with Experimental Results. Annals of Occupational Hygiene 2011 55: 841-864. Including two supplements to this paper.
2	Frans J. Jongeneelen; Wil F. Ten Berge. Simulation of urinary excretion of 1-hydroxypyrene in various scenarios of exposure to PAH with a generic, cross-chemical predictive PBTK-model. International Archives of Occupational and Environmental Health 2011 (first on line, November 2011).

2 The PBTK model IndusChemFate

2.1 Program features and limitations

The PBTK-model IndusChemFate2.00 is available as a software tool in MS-Excel to estimate blood and urine concentration of multiple chemicals, given a certain exposure scenario. Three uptake routes are considered (inhalation, dermal and/or oral) as well as two built-in exercise levels. The model accounts for various human subjects (a reference male human , a female and a child, normal or obese) or experimental animals (rat or mouse). The layout of the model is shown in figure 1.

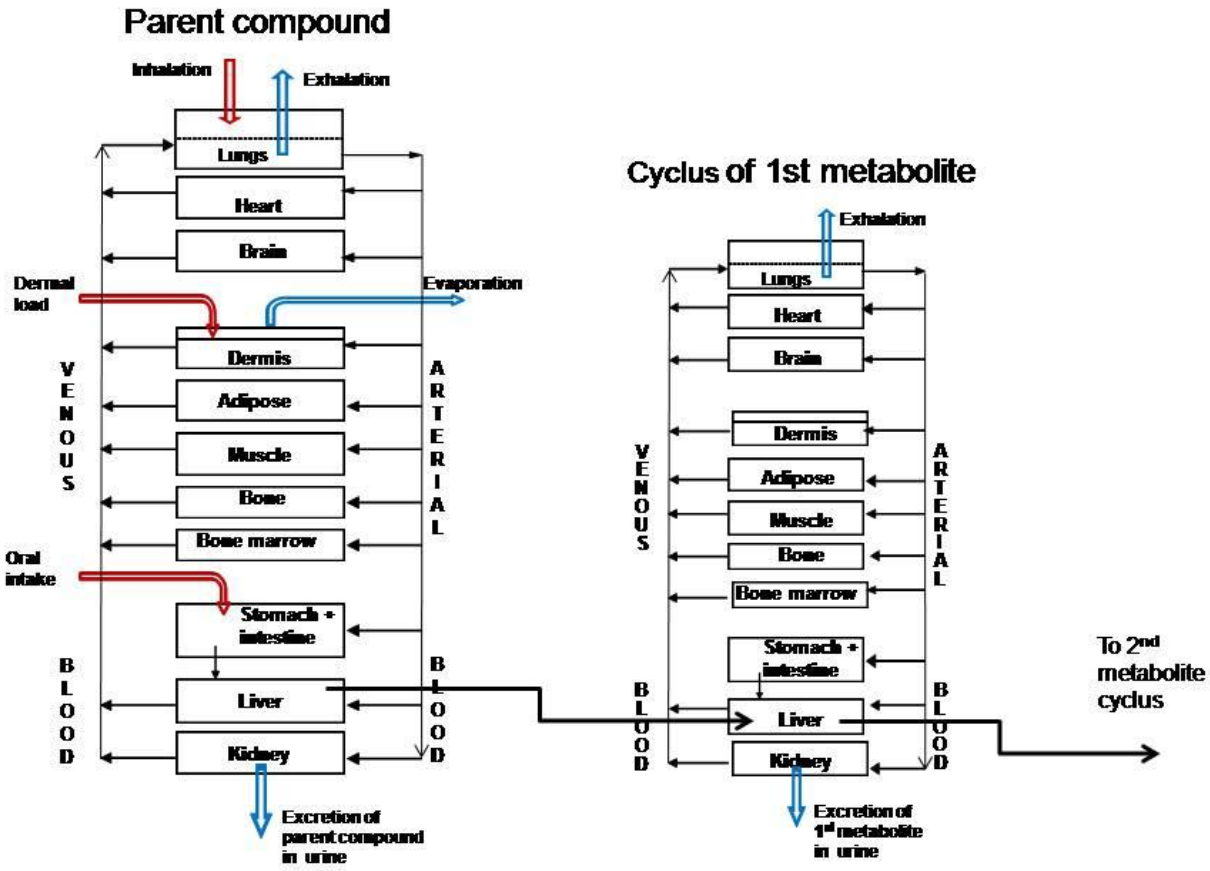


Figure 1. The scheme of the PBPK-model IndusChemFate

The model contains 11 body compartments (Lung, Heart, Brain, Skin, Adipose, Muscles, Bone, Bone marrow, Stomach & Intestines (lumped), Liver and Kidney). All physiological parameters such as blood flows, tissue dimensions, cardiac output and alveolar ventilation

are adopted from reference documents such as the EU Technical Guidance Documents and EU REACH documentation [30-32]. The impact of exercise on cardiac output and alveolar ventilation that may influence uptake, distribution, metabolism and excretion is also modeled by definition of two levels of exercise (at rest and at light work).

The PBTK-model IndusChemFate holds QSPR-algorithms (Quantitative Structure-Property Relationships) to minimize the number of necessary input parameters. The Input variables are limited to some physico-chemical properties (e.g. molecular weight, density, vapor pressure, water solubility) and metabolic kinetic parameters (e.g. maximum velocity of metabolism (= V_{max}) and the Michaelis-Menten constant (= K_m)). The physico-chemical properties required to run the model can be obtained from freely available (internet) resources [33-35].

The adopted algorithms or QSPRs (Quantitative Structure-Property Relationships) for partitioning are either published in scientific literature or in-house developed for this model. Alveolar uptake is determined by the blood:air partition coefficient of the compound. The blood:air partitioning is estimated by means of a newly developed QSPR derived from experimental substance-specific physico-chemical parameters [36]. Distribution of xenobiotics over the body is largely determined by tissue:blood partition coefficients that are obtained from a published QSPR [37]. That means that this model IndusChemFate does not require compound-specific blood:air and/or tissue:blood partitioning information.

Dermal uptake is estimated by the use of a novel dermal physiologically based module that considers dermal deposition rate and duration of deposition [38, 39]. This module predicts the fraction of the substance on the skin surface, in the skin and finally absorbed in the blood dependent on the time after first contact. Moreover, evaporation during skin contact is fully accounted for and related to the volatility of the substance.

Oral intake of compounds is considered as a bolus dose that is directly applied to the stomach and then transferred to the intestinal tissue at a first order rate.

Saturable Michaelis-Menten metabolism kinetics is incorporated in the model, based on the principle of removal of the parent compound at a rate determined by tissue specific V_{max} and K_m. Also the subsequent production of one or more metabolites is determined by specific V_{max} and K_m values for production. The metabolic parameters can be used independently (i.e. removal of parent compound or initial metabolite and production of secondary metabolite are not necessarily equal). Metabolism can be modeled in all organs. The simulation of metabolism requires experimental input tissue-specific values (V_{max} and K_m). The model supports simulation up to 4 subsequent metabolites.

The residence time of the parent compound or one of its metabolites in the human body is determined by the rate of circulation, storage in tissues and the rate of excretion. Enterohepatic circulation (of phase II metabolites) is adopted in the model by means of a

bypass from the liver to the intestines (by bilary excretion) at a user-defined rate. Furthermore regulation of renal clearance is an additional option. It can be determined actively by the user or passively by the model using a built-in cut-off value based the (log) octanol:water partition coefficient (at a pH of 7.4, the pH of the blood). The other excretion route is via exhalation, which is based on the blood:air partition coefficient (also at pH 7.4).

The IndusChemFate PBTK-model is available free of charge from the CEFIC-LRI website as a Visual Basic application in Microsoft Excel (available at http://www.cefic-lri.org/lri-toolbox/induschemfate).

2.2 New items in version 2.00 of IndusChemFate

2.2.1 Various human subjects

The model considers various human subjects (a reference male human, a female and a child, normal or obese). The model holds 12 preselected subjects: male, female, child; normal or obese; in rest or light activity (see appendix 1).

2.2.2 Experimental animals

The model holds 2 preselected experimental animals: rat or mouse in rest. New QSPR algorithms are derived for blood:air and tissue:blood partitioning in experimental animals (see appendix 1).

2.2.3 Altered decision rules for renal excretion

The urinary excretion of compounds and metabolites is estimated with QSPR-algoritmes that is based on the log Kow of the compound/metabolite. In version 2.00 the earlier estimate of versions 1.x using a fixed point decision is changed to an sigmoide excretion model with certain boundaries (see paragraph 4.3.2.).

2.2.4 Improved entry of exposure

The entry of periods with exposure is made more flexible. Three items govern the exposure time and period:

- Duration per day in number of hours/day. Duration of inhalation exposure and and dermal exposure can be set independent. Oral intake is modeled as bolus-dose;
- Length of exposure period in number of days;
- Number of shifts/periods with exposure per week (from 1 to 7).

This gives the flexibility to enter exposure periods for workers, consumer and experimental animals.

3 Running the model

3.1 Instructions in short

The PBTK-model IndusChemFate runs as an application in MS Excel. The Excel-file has 4 sheets:

1. Tutorial;
2. Exposure Conditions+Calculation;
3. Database and;
4. Graphical Output.

Table 3 summarises the instructions for running the model in short.

Table 3. Short instructions for running the application IndusChemFate.

Step	Action
01	**First step** after opening is to activate the macro's of the MS Excel-file.
02	**Second step** is to save the opened MS Excel-file under a new name.
03	**Enter data of chemical:** Open sheet 'Database'. Either data of the chemical under study are already available in a column of the sheet 'Database' or the chemical is not yet available and the data should be entered. Data to be entered are phys-chem data and toxicokinetic data. Metabolism can be modeled in all tissues or in liver only. Start initially with only metabolism in liver by pushing the button *"Metabolism only in liver".*
04a	**Select a subject** out of 12 human subjects or select an experimental animal (code for mouse =13, for rat = 14).
04b	**Define exposure period(s)**. Enter the duration of inhalation per day (in hours/day) and duration of dermal exposure per day (in hours/day). Then, enter the period of exposure (in days) and the number of successive shifts with exposure per week (from 1 to 7) (Example: If the period of exposure is 14 days and the number of successive shifts per week is 5, the total number of days with exposure is 10 in the period of 14 days).
05	**Copy data of chemical:** Open sheet 'Database' and click the name of the parent compound in Row 3. All specific data of the selected chemical will be copied from the database into the cells B31 through B305 in the worksheet 'Exposure Conditions+ Calculation' and activates the latter worksheet.
06	**Enter exposure level**: Open sheet 'Exposure Conditions+ Calculation' and enter your exposure conditions in cells B2 – B18.
07	**Run the model:** After entry of all exposure data, press the button *"Calculate*" in sheet 'Exposure Conditions+ Calculation'. The calculated concentrations and amounts in body fluids and in body compartments over time will appear the active sheet. After running, the results are presented in graphs in sheet 'Graphical Output'.
08	**Tabular listing of output:** The calculated concentrations (indicated as Cn with 0=parent compound, 1 = 1st metabolite, 2 = 2nd metabolite) and amounts (indicated as Mn) in body fluids, exhaled air and in 11 body compartments in the observation periode will be listed in the active sheet 'Exposure Conditions+ Calculation'.

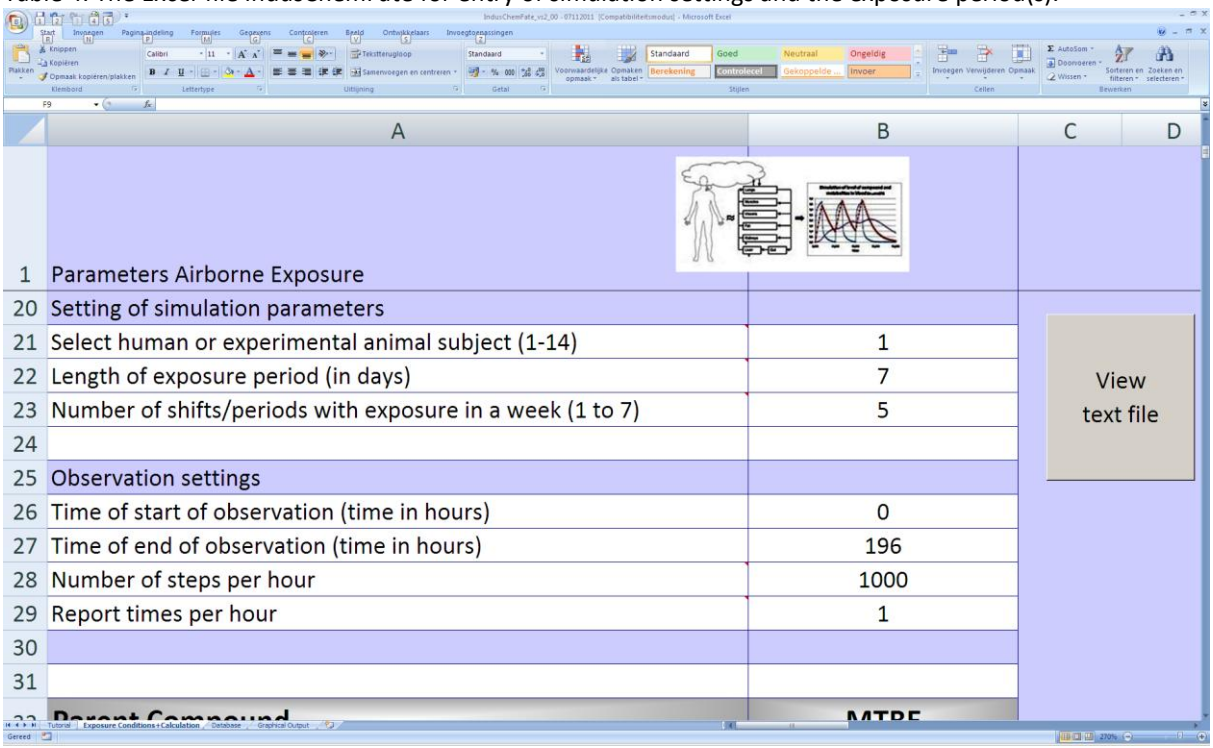
Step	Action
09	**Graphical output:** The concentrations in blood, urine and exhaled are presented in graphics in the sheet 'Graphical Output'.
10	**Other output of the model**: The QSPR-estimated partition coefficients are presented in sheet 'Exposure Conditions+ Calculation' , just below the data. Below these data, the QSPR-estimated fraction of tubular resorption is reported.
11	**Internal check of the model**: The mass-balance is presented in sheet 'Exposure Conditions+ Calculation' , just below the data.
12	**Save results:** To save the input data and output tables, press button *"Save calculations*" in sheet 'Exposure Conditions+ Calculation'. The data are written to a simple notepad textfile that will appear in the active directory with the filename of the substance (from cell B31 of sheet 'Exposure conditions + Calculation'). In the header time and date of saving are printed.
13	**View output as text file:** To view the saved data, press button "*View text file*" in sheet 'Exposure Conditions+ Calculation'.

3.2 Extended instructions

3.2.1 Initial settings

The initial settings of the model simulation can be determined by the parameters as displayed in table 4. The start and end of the observation period needs to be defined first. If the exposure duration is repeated this can entered with the number of repeated days.
The number of calculation steps per hour can be set as well as the number of reports per hour. It is recommended to use a small step size to increase accuracy of the model. Therefore at least 1000 steps per hour are recommended. Increasing the steps to 10,000 per hour will produce more precise results, but will slow down the numerical integration process. The number of report times per hour is also optional. The number of 1 means one pointvalue per hour, a number of 60 means a pointvalue for every minute. The higher the number the more details are presented. This might be important in the initial period of exposure and at the end of exposure, when levels in body fluids are rapidly changing.

Table 4. The Excel-file IndusChemFate for entry of simulation settings and the exposure period(s).



	A	B	C	D
1	Parameters Airborne Exposure			
20	Setting of simulation parameters			
21	Select human or experimental animal subject (1-14)	1		
22	Length of exposure period (in days)	7		
23	Number of shifts/periods with exposure in a week (1 to 7)	5		
24				
25	Observation settings			
26	Time of start of observation (time in hours)	0		
27	Time of end of observation (time in hours)	196		
28	Number of steps per hour	1000		
29	Report times per hour	1		
30				
31				

3.2.2 Exposure period(s)

The entry of periods with exposure is more flexible in version 2.0. Three items govern the exposure time and period:

- Duration per day in number of hours/day. Duration of inhalation exposure and and dermal exposure can be set independent. Oral intake is modeled as bolus-dose;
- Length of exposure period in number of days;

- Number of shifts/periods with exposure per week (from 1 to 7).

The entry cells are B4, B11, B22 and B23 (as displayed in table 4)

3.2.3 Selection of human or animal subject

The IndusChemFate model holds preselected subjects: 12 human options (male, female, child; normal or obese; in rest or light activity) and 2 experimental animal options (rat or mouse in rest) [1]. The predefined human subjects and experimental animals are shown in table 5. Human physiology parameters of male, female humans and children with normal weight and obese are used to dimension organ and tissue volumes and blood flows through these tissues were scaled relative to the total body weight. Standards for human physiological parameters were used from several reference documents [30, 32, 40-42]. The REACH guidance recommends values for organ volumes and blood flows through the organs for PBTK-modelling [31]. The organ volumes and organ blood flows, as recommended by Appendix IVC of the European Technical Guidance Documents 2nd edition 2003, are used in our model. The values for each of the 12 human subjects and 2 experimental animals are presented in appendix 1.

Table 5. Standardised subjects which can be selected in IndusChemFate.

No.	*Standardised subject*
1.	Normal man (in rest)
2.	Obese man (in rest)
3.	Normal man (light work)
4.	Obese man (light work)
5.	Normal woman (in rest)
6.	Obese woman (in rest)
7.	Normal woman (light work)
8.	Obese woman (light work)
9.	Normal child (in rest)
10.	Obese Child (in rest)
11.	Normal child (playing)
12.	Obese child (playing)
13.	Mouse (experimental animal)
14.	Rat (experimental animal)

Tissue volumes

All tissues except the bones are assumed to have a relative density similar to water (1 g/cm^3). For bones a relative density of 1,92 g/cm^3 is taken [30]. Tissue volumes are expressed in liters.

[1] Physiological parameters of rat and mouse were taken from the tabels of REACH Guidance 7c. The partitioning for man and experimental animals is different.

Blood flows
Blood flows through tissues are implemented as a fraction of the total cardiac output in correspondence with most other PBPK models. It is assumed that arterial and venous blood are distributed in a 30%:70% relation at all times [43-45].

Human types
Human subject are 70, 60 and 16 kg for male, female and child, respectively. The obese types with some overweight have an increased weight, e.g. 80, 70 and 20 kg, respectively.

Exercise levels
The two preset levels of exercise are rest and light work (~ 50W, up to a maximum heartbeat of 100 per minute). Table 6 shows the alveolar ventilation and cardiac output at the two exercise levels. At light work the relative blood flows to liver and kidney decrease whereas relative blood flows to muscles increase. Relative blood flows to other model compartments does not change.

Table 6. Cardiac output and alveolar ventilation per exercise level (liter/hour).

Parameter	*Value at rest*	*Value light work*
Cardiac output (L/h)	390	640
Alveolar ventilation (L/h)	530	1350

3.2.4 Entry of compound specific data

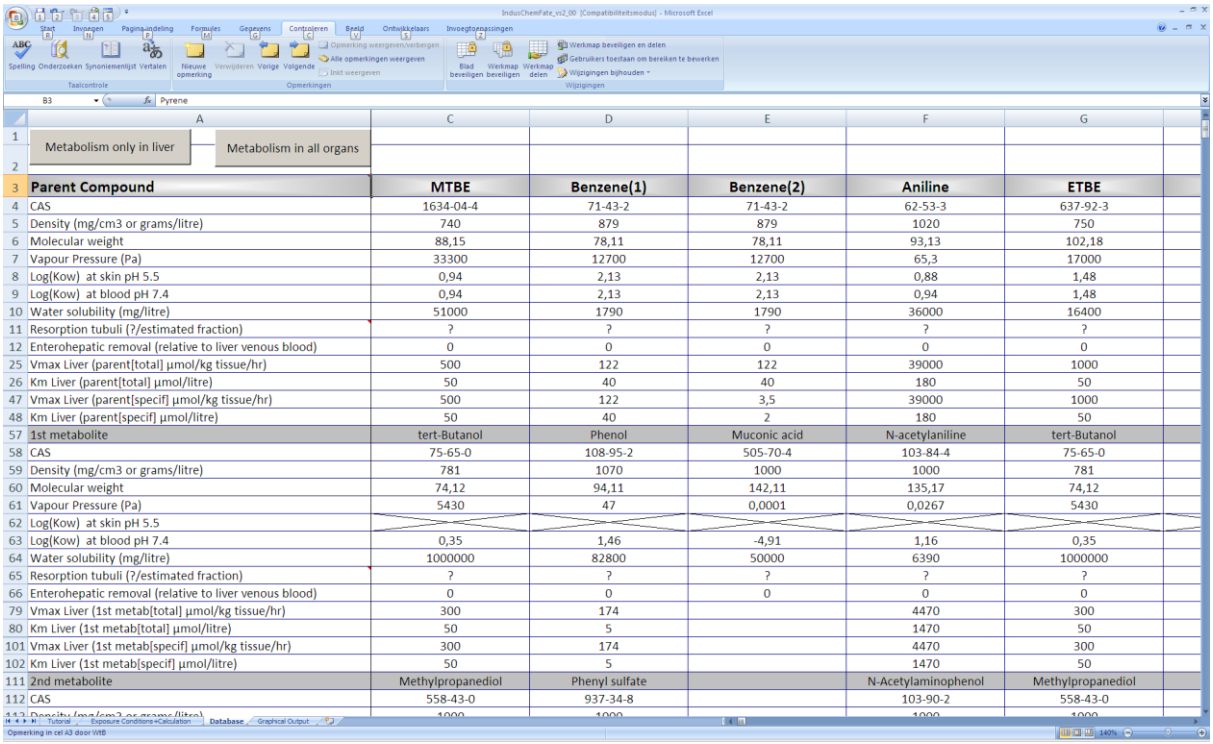
The chemical-specific properties of the parent compound and the metabolite(s) of interest for running the model IndusChemFate have to be entered in the sheet 'Database'. Table 7 lists the required compound-specific parameters for running the model.
When the data of the chemical and metabolites under study are already available in the sheet 'Database' of the Excel-file, these can be automatically entered in the model by pushing the cell with name of the chemical in the sheet '*Database*'. All data of the selected chemical (and corresponding metabolites) will be copied from the database to the wsheet '*Exposure Conditions+ Calculation*'.
In the case that the data of the chemical are not yet available in the sheet '*Database*', the required data should be entered in the next empty column of this sheet before transferring. The simulation will be restricted to the number of metabolites that have been entered.

3.2.4.1 Physico-chemical input data

Required physico-chemical properties are molecular weight, density, vapor pressure, water solubility and the log(octanol/water partition ratio) at pH 7.4 and at pH 5.5, respectively the pH of blood and skin. The log(octanol/water partition ratio) is controlled by the pH in case weak organic acids and organic bases. For selecting unknown physical-chemical parameters, the user is recommended to consult EPA's EpiSuite [33] or ChemSpider [34] in case of pH dependent log(octanol/water partition ratio). These databases contain experimental data on

physico-chemical properties of many substances, which are needed to derive the partition ratios of the parent and metabolites via QSPRs or QSPRs. Searching by CAS-number and/or by SMILES code is needed to get access to the substance properties. Other freely available internet resources of interest include PubChem [35], ChemSketch [46] and Wikipedia [47].

Table 7. Physical-chemicaql and biochemical properties of the parent substance and metabolites of chemicals to be entered into the Excel-file IndusChemFate.



Metabolism only in liver | Metabolism in all organs

Parent Compound	MTBE	Benzene(1)	Benzene(2)	Aniline	ETBE
CAS	1634-04-4	71-43-2	71-43-2	62-53-3	637-92-3
Density (mg/cm3 or grams/litre)	740	879	879	1020	750
Molecular weight	88,15	78,11	78,11	93,13	102,18
Vapour Pressure (Pa)	33300	12700	12700	65,3	17000
Log(Kow) at skin pH 5.5	0,94	2,13	2,13	0,88	1,48
Log(Kow) at blood pH 7.4	0,94	2,13	2,13	0,94	1,48
Water solubility (mg/litre)	51000	1790	1790	36000	16400
Resorption tubuli (?/estimated fraction)	?	?	?	?	?
Enterohepatic removal (relative to liver venous blood)	0	0	0	0	0
Vmax Liver (parent[total] µmol/kg tissue/hr)	500	122	122	39000	1000
Km Liver (parent[total] µmol/litre)	50	40	40	180	50
Vmax Liver (parent[specif] µmol/kg tissue/hr)	500	122	3,5	39000	1000
Km Liver (parent[specif] µmol/litre)	50	40	2	180	50
1st metabolite	tert-Butanol	Phenol	Muconic acid	N-acetylaniline	tert-Butanol
CAS	75-65-0	108-95-2	505-70-4	103-84-4	75-65-0
Density (mg/cm3 or grams/litre)	781	1070	1000	1000	781
Molecular weight	74,12	94,11	142,11	135,17	74,12
Vapour Pressure (Pa)	5430	47	0,0001	0,0267	5430
Log(Kow) at skin pH 5.5					
Log(Kow) at blood pH 7.4	0,35	1,46	-4,91	1,16	0,35
Water solubility (mg/litre)	1000000	82800	50000	6390	1000000
Resorption tubuli (?/estimated fraction)	?	?	?	?	?
Enterohepatic removal (relative to liver venous blood)	0	0	0	0	0
Vmax Liver (1st metab[total] µmol/kg tissue/hr)	300	174		4470	300
Km Liver (1st metab[total] µmol/litre)	50	5		1470	50
Vmax Liver (1st metab[specif] µmol/kg tissue/hr)	300	174		4470	300
Km Liver (1st metab[specif] µmol/litre)	50	5		1470	50
2nd metabolite	Methylpropanediol	Phenyl sulfate		N-Acetylaminophenol	Methylpropanediol
CAS	558-43-0	937-34-8		103-90-2	558-43-0

3.2.4.2 Biochemical input data

Metabolism

Metabolism can be simulated in all organs of the PBTK model. Serial metabolism can be entered (parent compound into 1st -metabolite, followed by biotransformation into the 2nd – metabolite, and so on to the 4th metabolite). The maximum velocity of metabolism (V_{max}) and the Michaelis-Menten constant (K_m) are the parameters, controlling the metabolic clearance. The required parameters for each step are listed in table 8. Conversion of experimental V_{max} units to the proper units is given in the addendum of table 8.

Table 8. Metabolic input parameters for the PBTK-model IndusChemFate.

Variable	***Unit***	***Description***
V_{max} liver (compound[total])	µMole/kg/hr*	Removal of parent compound
K_m liver (compound[total])	µMole/L	
V_{max} liver (compound[specif])	µMole/kg/hr*	For production of specific metabolite
K_m liver (compound[specif])	µMole/L	

*Most V_{max} values are reported in µMole/mg microsomal protein/min. To convert this value to µMole/kg liver/hour, it can be assumed that the microsomal fraction of liver tissue is about 4% [69] and that the average human liver weighs 2 kg. K_m values are mostly reported in µM.

The model considers both removal of the parent compound and production of metabolite as separate steps. That means the biotransformation of the parent compound occurs for only x% into the metabolite of interest and for (100-x)% into other (unknown) metabolites. That is how the stoichiometric yield of each step is set.
Metabolism can be modeled in all tissues or in liver only. Default is metabolism in liver only. It is recommended to start initially with only metabolism in liver by pushing the button:

Metabolism only in liver

When other tissue(s) are involved in the metabolism, V_{max} and K_m values of these tissue(s) should be entered in the proper input cells.
If an input cell remains empty, metabolism is not considered.

Tubular resorption
The input parameter 'resorption tubuli' refers to the tubular resorption fraction. It may be calculated by a internal QSPR-algorithm or it may be entered directly. If the user leaves the decision on tubular resorption to the internal QSPR-algorithm, a question mark should be entered. In case of measured tubular resorption the true value may be entered. In such a case the algorithms are overruled.

Enterohepatic recirculation
Fase II-metabolites may undergo enterohepatic recirculation. Enterohepatic recirculation is entered as 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. If the removal ratio from the liver by enterohepatic recirculation is set to 1, 50% of the total amount that leaves the liver per unit of time is excreted to blood and 50% to the intestinal lumen via bile (see table 9).

Table 9. Enterohepatic circulation ratio as adopted in the PBTK-model IndusChemFate.

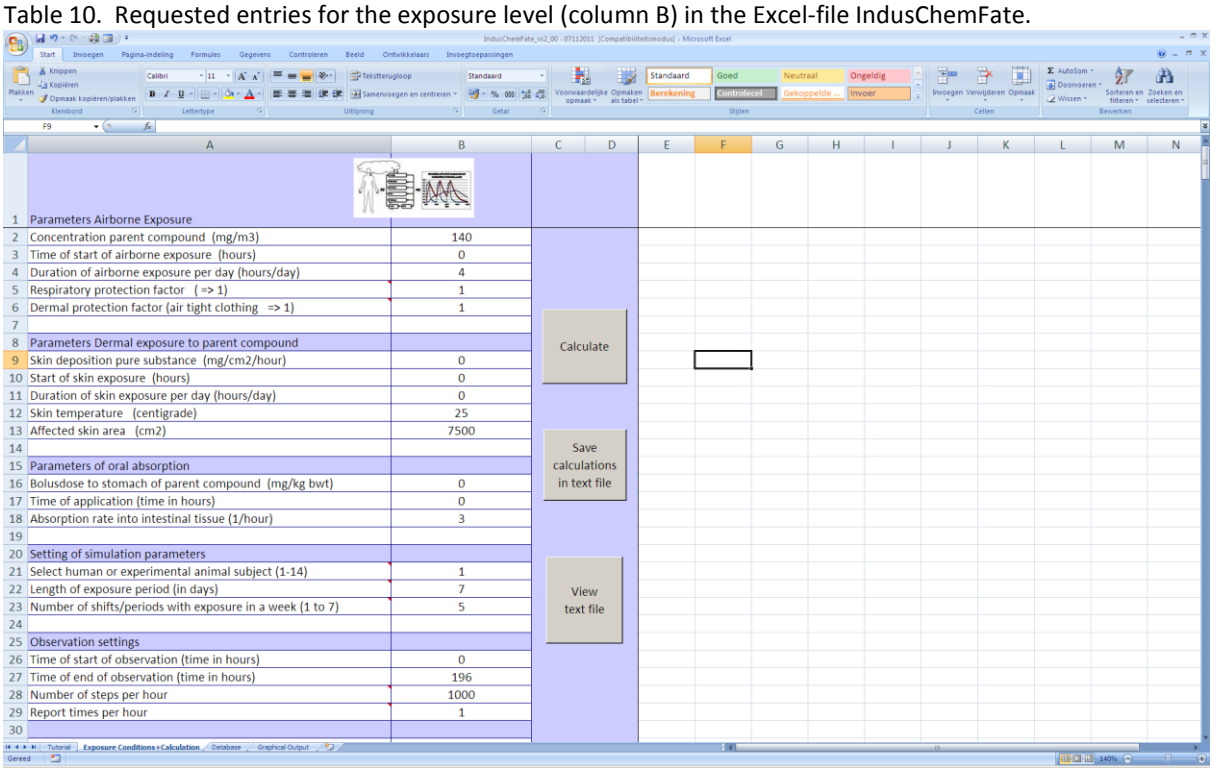
Ratio of excretion to bile versus liver venous blood	*Percentage excreted in bile*
0	0%
1	50%
2	66.7%
9	90%

3.2.5 Entry of exposure level and duration

Three routes of exposure can be defined: inhalation, dermal uptake, oral uptake. Also a combination is possible. If any of the input fields is not applicable it can be left empty. Table

10 shows the required exposure entries for simulation of inhalation, dermal uptake and oral uptake. When respiratory protective equipment is used, the protection factor can be entered. Default value of no protection = 1.
Skin exposure might occur via deposition of the substance on the skin. Skin uptake of the neat substance requires log (Kow) at pH of skin (= 5,5). If deposition or direct skin contact occurs skin uptake may still play a role via dermal uptake of vapor. This is also considered by the model and is linearly related to the air concentration. When gas tight protective clothing is worn, the skin uptake of vapour will be decreased. The protection factor of protective clothing can also be entered. Default value of no protection = 1.

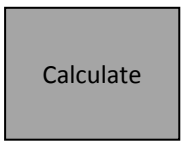
Table 10. Requested entries for the exposure level (column B) in the Excel-file IndusChemFate.



	A	B
1	Parameters Airborne Exposure	
2	Concentration parent compound (mg/m3)	140
3	Time of start of airborne exposure (hours)	0
4	Duration of airborne exposure per day (hours/day)	4
5	Respiratory protection factor (=> 1)	1
6	Dermal protection factor (air tight clothing => 1)	1
7		
8	Parameters Dermal exposure to parent compound	
9	Skin deposition pure substance (mg/cm2/hour)	0
10	Start of skin exposure (hours)	0
11	Duration of skin exposure per day (hours/day)	0
12	Skin temperature (centigrade)	25
13	Affected skin area (cm2)	7500
14		
15	Parameters of oral absorption	
16	Bolusdose to stomach of parent compound (mg/kg bwt)	0
17	Time of application (time in hours)	0
18	Absorption rate into intestinal tissue (1/hour)	3
19		
20	Setting of simulation parameters	
21	Select human or experimental animal subject (1-14)	1
22	Length of exposure period (in days)	7
23	Number of shifts/periods with exposure in a week (1 to 7)	5
24		
25	Observation settings	
26	Time of start of observation (time in hours)	0
27	Time of end of observation (time in hours)	196
28	Number of steps per hour	1000
29	Report times per hour	1
30		

3.3 Run the model

When all the required data of the substance and the exposure scenario are entered, press the button:



The predicted concentrations and amounts in body fluids and in body compartments over time will appear in identified columns of the active sheet. When the calculations are done, a part of the data will be presented as three graphs in the sheet 'Graphical Output'. The graphs will automatically appear and they present the concentrations of the substance and its metabolites in exhaled air, venous blood and urine.

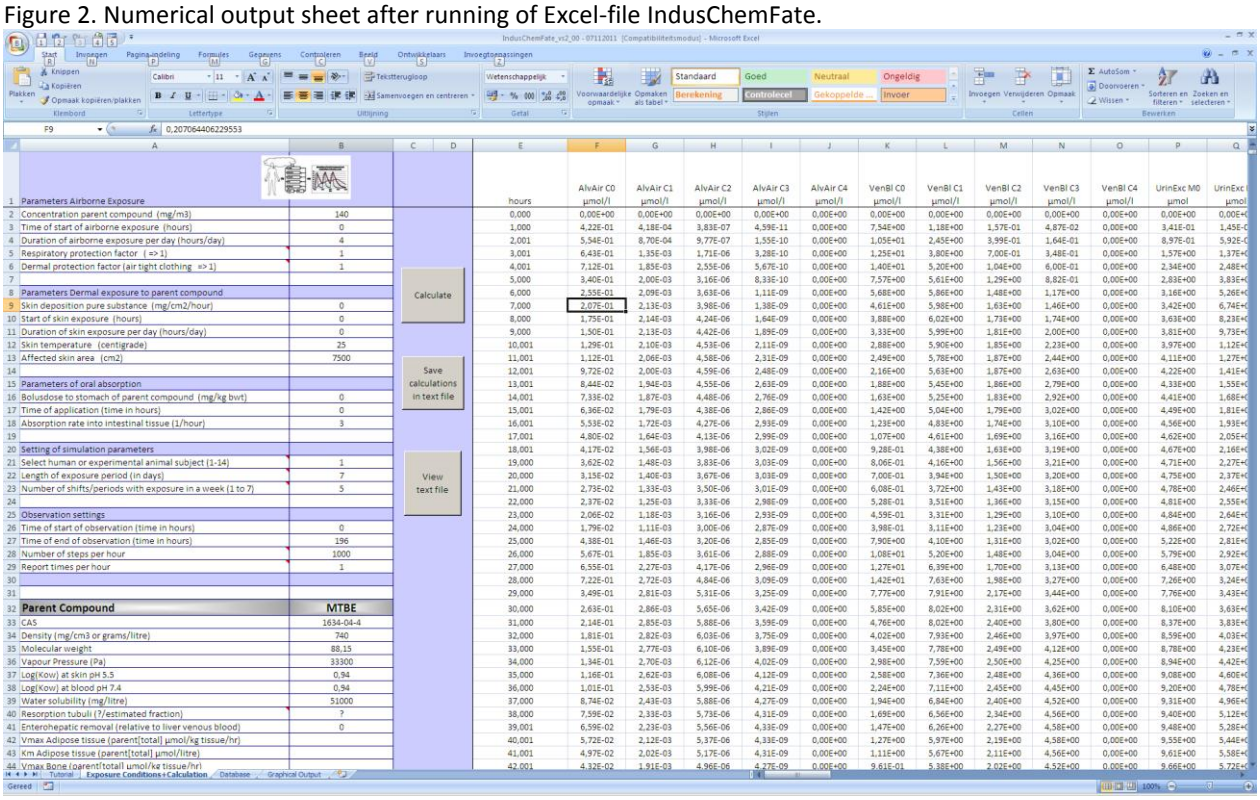
Depending on the defined scenario criteria (and the specification of your computer) the running will take a period of a few seconds to tens of seconds.

3.4 Model Output

3.4.1 Numerical output data

After running , a large number of time-series of output parameters is listed for both the parent compound (code 0) and its metabolite(s) (code 1 to 4) in the sheet '*Exposure conditions + Calculation*'. See figure 2.

Figure 2. Numerical output sheet after running of Excel-file IndusChemFate.



The listing includes mass (code M), concentrations (code C) and rates. After every simulation the output-parameters are listed in column E to CP (see overview of output columns in table 11). Column AG to AK give insight in the dermal absorption process.
Concentrations in all 11 organs , both for the parent compound and metabolite(s), are calculated (column AN to CP). This might be relevant if for instance the target tissue concentrations are of special interest. For example the bone marrow concentration is relevant when assessing benzene, as this substance is causing leukemia.
Columns presenting masses (in µMol) have 'M' included in the column title. Concentrations (in µMol/L) are indicated by 'C' in the title.

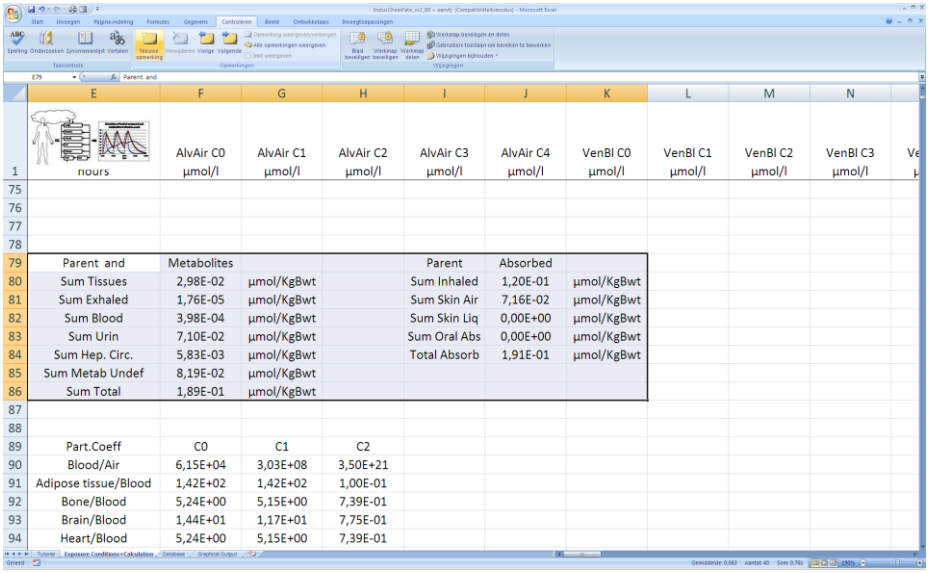
Table 11. Output parameters of The PBTK-model IndusChemFate.

Model Parameter	*Unit*	*Column*
Time	(in hours)	E
Alveolar air concentration	(in µMole/L)	F, G, H, I, J
Venous blood concentration	(in µMole/L)	K, L, M, N, O
Urinary excreted mass	(in µMole)	P, Q, R, S, T
Exhaled amount	(in µMole)	U, V, W, X, Y
Urinary concentration	(in µMole/L)	Z, AA, AB, AC, AD
Dermal deposition rate	(in mg/cm^2/hr)	AG
Mass on skin	(in mg/cm^2)	AH
Evaporated from skin	(in mg/cm^2)	AI
Mass in stratum corneum	(in mg/cm^2)	AJ
Dermally absorbed mass	(in mg/cm^2)	AK
Concentration in 11 different tissues	(in µMole/L)	From AN to CP

3.4.2 Mass balance data

A mass balance is an internal check for loss of mass. After every model simulation a mass balance is calculated. Absorbed amounts per route are summarized and compared with the total of excreted amounts, amounts in tissues and amounts to undefined metabolites, not assigned to the metabolic route considered. The total absorbed mass of the parent compound ("sum inhaled" + "sum skin air" + "sum skin liquid" + "sum oral absorption" = "total absorbed") must be equal to the sum of mass of parent + metabolites in all model compartments plus the mass excreted in urine and/or exhaled air ("sum tissues" + "sum air" + "sum blood" + "sum urine" + "sum hep circ" + "sum metabolites lost" = "sum total").
The mass balance of each simulation is presented in the active worksheet. All input and output of the model is on molar basis and taken up in the mass balance (as in table 12). The total absorbed mass of the parent compound is presented. This mass must be equal to the sum of mass (parent + metabolites) in all model compartments plus the mass excreted in urine and/or exhaled air. The log table of mass balance data is presented under the data-listing, in the sheet '*Exposure conditions + Calculation*'.

Table 12. Example of log table of data with estimated mass balance of a specific simulation of Excel-file IndusChemFate.

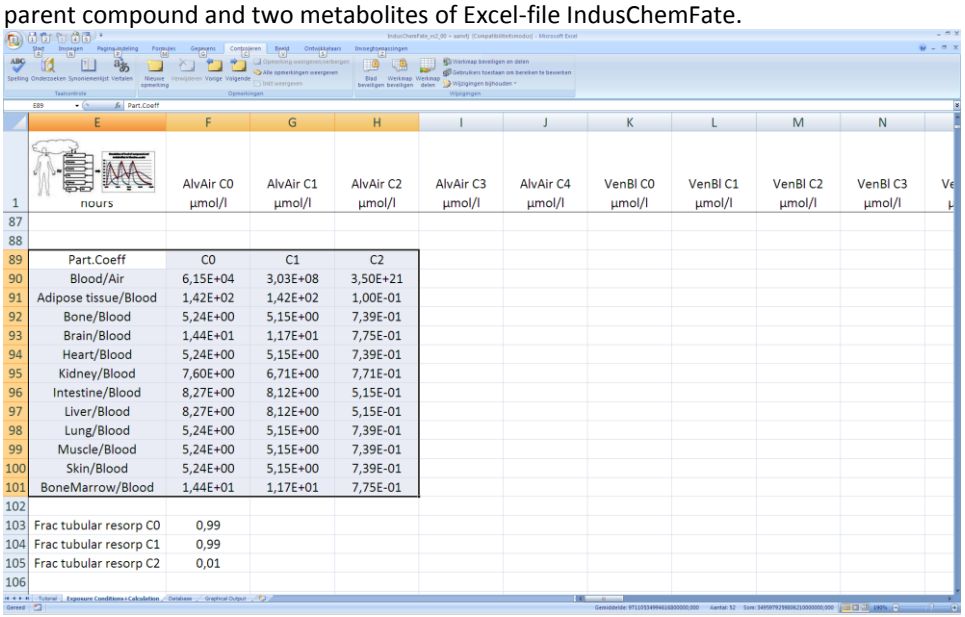


	E	F	G	H	I	J	K	L	M	N
1	nours	AlvAir C0 µmol/l	AlvAir C1 µmol/l	AlvAir C2 µmol/l	AlvAir C3 µmol/l	AlvAir C4 µmol/l	VenBl C0 µmol/l	VenBl C1 µmol/l	VenBl C2 µmol/l	VenBl C3 µmol/l
79	Parent and	Metabolites			Parent	Absorbed				
80	Sum Tissues	2,98E-02	µmol/KgBwt		Sum Inhaled	1,20E-01	µmol/KgBwt			
81	Sum Exhaled	1,76E-05	µmol/KgBwt		Sum Skin Air	7,16E-02	µmol/KgBwt			
82	Sum Blood	3,98E-04	µmol/KgBwt		Sum Skin Liq	0,00E+00	µmol/KgBwt			
83	Sum Urin	7,10E-02	µmol/KgBwt		Sum Oral Abs	0,00E+00	µmol/KgBwt			
84	Sum Hep. Circ.	5,83E-03	µmol/KgBwt		Total Absorb	1,91E-01	µmol/KgBwt			
85	Sum Metab Undef	8,19E-02	µmol/KgBwt							
86	Sum Total	1,89E-01	µmol/KgBwt							
89	Part.Coeff	C0	C1	C2						
90	Blood/Air	6,15E+04	3,03E+08	3,50E+21						
91	Adipose tissue/Blood	1,42E+02	1,42E+02	1,00E-01						
92	Bone/Blood	5,24E+00	5,15E+00	7,39E-01						
93	Brain/Blood	1,44E+01	1,17E+01	7,75E-01						
94	Heart/Blood	5,24E+00	5,15E+00	7,39E-01						

3.4.3 Predicted partition coefficients

The log-file of QSPR-predicted partition coefficients is listed, summarizing the blood:air and tissue:blood partition coefficients for all tissues that are calculated by the model and were used in the simulation. This includes both the partition coefficients for the parent compound and all metabolites of interest (see table 13). The log table of partition coefficients is presented under the data-listing, in the active sheet '*Exposure conditions + Calculation*'.

Table 13. Example of log table with estimated partition coefficients of a specific parent compound and two metabolites of Excel-file IndusChemFate.

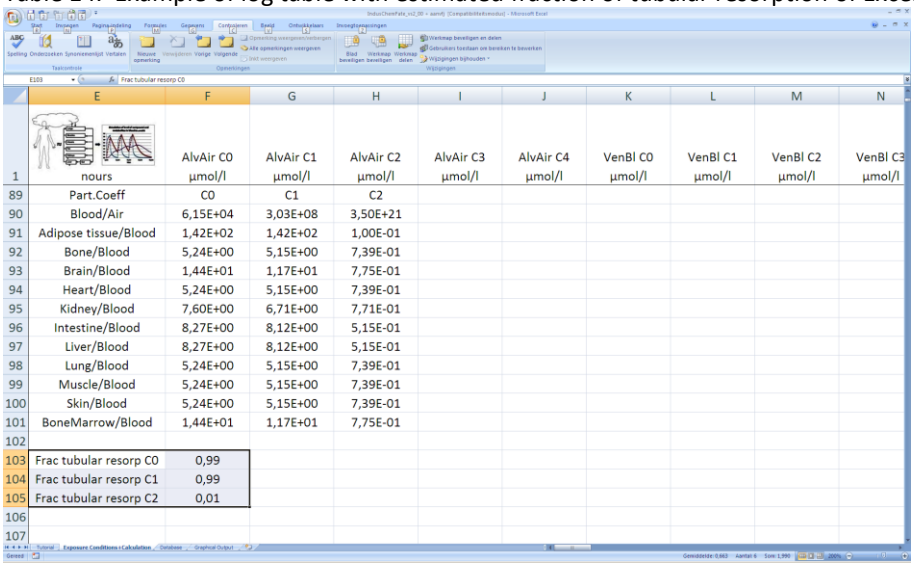


	E	F	G	H	I	J	K	L	M	N
1	nours	AlvAir C0 µmol/l	AlvAir C1 µmol/l	AlvAir C2 µmol/l	AlvAir C3 µmol/l	AlvAir C4 µmol/l	VenBl C0 µmol/l	VenBl C1 µmol/l	VenBl C2 µmol/l	VenBl C3 µmol/l
89	Part.Coeff	C0	C1	C2						
90	Blood/Air	6,15E+04	3,03E+08	3,50E+21						
91	Adipose tissue/Blood	1,42E+02	1,42E+02	1,00E-01						
92	Bone/Blood	5,24E+00	5,15E+00	7,39E-01						
93	Brain/Blood	1,44E+01	1,17E+01	7,75E-01						
94	Heart/Blood	5,24E+00	5,15E+00	7,39E-01						
95	Kidney/Blood	7,60E+00	6,71E+00	7,71E-01						
96	Intestine/Blood	8,27E+00	8,12E+00	5,15E-01						
97	Liver/Blood	8,27E+00	8,12E+00	5,15E-01						
98	Lung/Blood	5,24E+00	5,15E+00	7,39E-01						
99	Muscle/Blood	5,24E+00	5,15E+00	7,39E-01						
100	Skin/Blood	5,24E+00	5,15E+00	7,39E-01						
101	BoneMarrow/Blood	1,44E+01	1,17E+01	7,75E-01						
103	Frac tubular resorp C0	0,99								
104	Frac tubular resorp C1	0,99								
105	Frac tubular resorp C2	0,01								

3.4.4 Predicted fraction of tubular resorption

The log-file of the predicted fractional tubular resorption of the parent compound (C0) and all metabolites (C1, C2 and so on) of interest is listed in sheet '*Exposure conditions + Calculation*', as in table 14.

Table 14. Example of log table with estimated fraction of tubular resorption of Excel-file IndusChemFate.

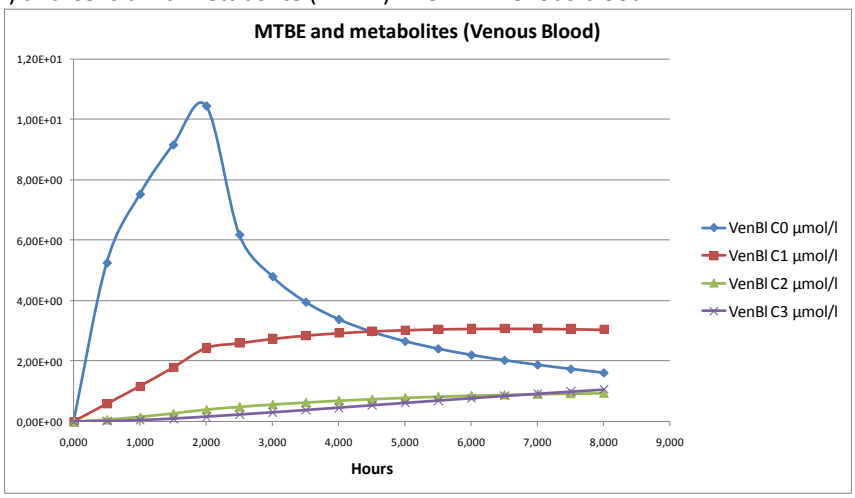


	E	F	G	H	I	J	K	L	M	N
1	nours	AlvAir C0 µmol/l	AlvAir C1 µmol/l	AlvAir C2 µmol/l	AlvAir C3 µmol/l	AlvAir C4 µmol/l	VenBl C0 µmol/l	VenBl C1 µmol/l	VenBl C2 µmol/l	VenBl C3 µmol/l
89	Part.Coeff	C0	C1	C2						
90	Blood/Air	6,15E+04	3,03E+08	3,50E+21						
91	Adipose tissue/Blood	1,42E+02	1,42E+02	1,00E-01						
92	Bone/Blood	5,24E+00	5,15E+00	7,39E-01						
93	Brain/Blood	1,44E+01	1,17E+01	7,75E-01						
94	Heart/Blood	5,24E+00	5,15E+00	7,39E-01						
95	Kidney/Blood	7,60E+00	6,71E+00	7,71E-01						
96	Intestine/Blood	8,27E+00	8,12E+00	5,15E-01						
97	Liver/Blood	8,27E+00	8,12E+00	5,15E-01						
98	Lung/Blood	5,24E+00	5,15E+00	7,39E-01						
99	Muscle/Blood	5,24E+00	5,15E+00	7,39E-01						
100	Skin/Blood	5,24E+00	5,15E+00	7,39E-01						
101	BoneMarrow/Blood	1,44E+01	1,17E+01	7,75E-01						
102										
103	Frac tubular resorp C0	0,99								
104	Frac tubular resorp C1	0,99								
105	Frac tubular resorp C2	0,01								
106										
107										

3.4.5 Graphical output of alveolair air, urine and blood concentrations

After running the model, the predicted concentrations in blood, urine and exhaled air are presented as graphs in the sheet 'Graphical Output'. Figure 3 shows an example of time course of venous blood concentrations.

Figure 3. Example of graphical output of the PBTK model-IndusChemFate. Inhalation of 140 mg/m3 MTBE during 2 h , observation periode was 8h. C0 is parent compound MTBE, C1 = first metabolite TBA, C2 is second metabolite (MPD) and C3 is third metabolite (=HiBA) . VenBl = venous blood.



3.4.6 Saving data

To save the input data and numerical output tables, press the button:

Save calulations in text file

in the sheet *'Exposure Conditions+ Calculation'*. The data are written to a simple notepad textfile that will appear in the active directory with the filename of the substance (filename taken from cell B31 of sheet *'Exposue conditions + Calculation'*). In the header time and date of saving are printed. After saving the results can directly be viewed by pressing the button:

View Text file

3.4.7 Example

Appendix 2 shows results of simulations of exposure of volunteers and rats to MTBE using IndusChemFate version 2.00. Simulation results are compared with experimental results of an exposure study that encompass both dosimetry and biomonitoring.

4 Basics of the model

The PBTK-model IndusChemFate is written in Visual Basic. The full source code with algorithms and equations is available and readable with the VBA editor of MS Excel.

4.1 Exposure routes

4.1.1 Inhalation

Inhalation absorption in the PBTK-model IndusChemFate is typically controlled by the concentration of the compound in the breathing zone, the alveolar ventilation and the blood:air partition coefficient. The result of inhalation absorption is a change in arterial blood concentration and followed by a change in the (target) tissues and the venous blood concentrations.

4.1.1.1 Human blood-air partitioning

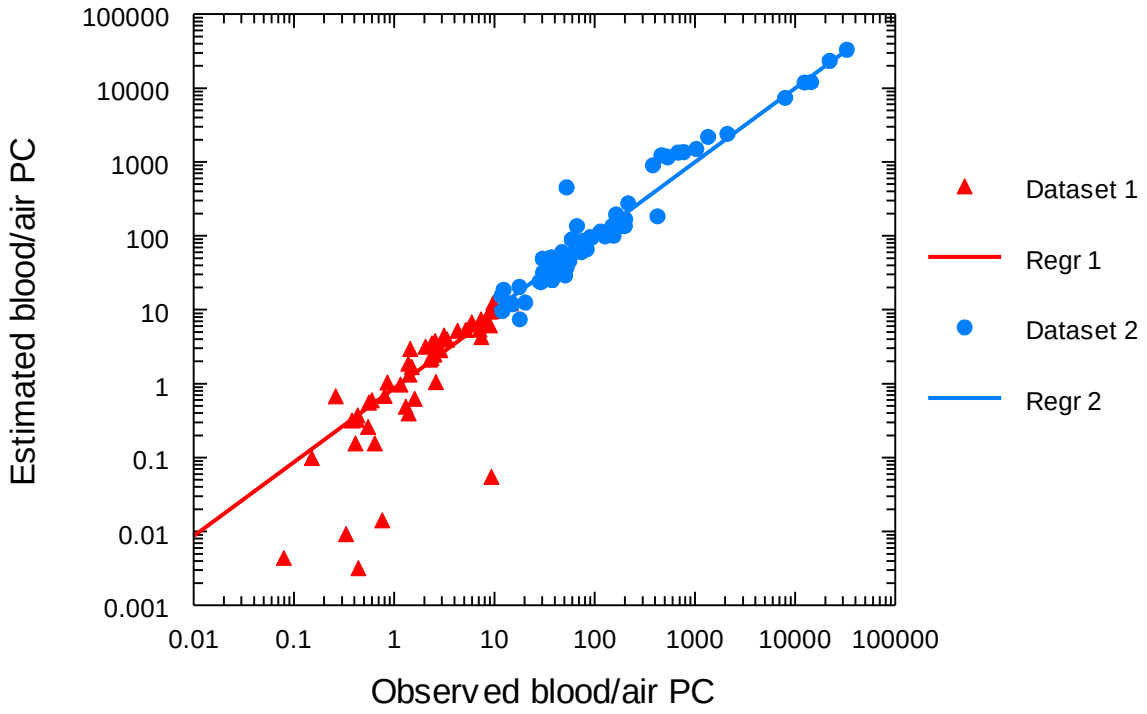
The blood:air partition coefficient (RCba) is substance specific and plays a crucial role in the uptake of a compound that has reached the alveoli where it forms a rapid equilibrium with the capillary blood. Blood:air partitioning also plays a role in the possible exhalation of the substance as molecules may also exchange from blood to air. The PBTK-model IndusChemFate holds a QSPR to estimate the blood:air partition coefficient. The QSPR for human subjects has been derived by regression analysis of experimental human values of a large series number of chemicals (n=106) as published by Meulenberg & Vijverberg (2000). The resulting algoritm is using the dimensionless Henry coefficient and the octanol:air partition coefficient (Koct:air) as independent variables. The total group of 106 substances was split in two separate groups based on the Log Henry coefficient (dimensionless = DL). The split was based on the value of -1 for the Log Henry coefficient (dimensionless). For both subsets a multiple regression analysis was conducted with both the Koct:air and the Henry coefficient as independent variables and the blood:air partition ratio as the dependent variable. This resulted in two different regression formulas as presented in table 15. The proper regression equation is selected in the model IndusChemFate, related to the log(Henry_DL).

Table 15. QSPR algoritm in the PBTK-model IndusChemFate to derive human blood-air partition coefficients.

Condition	*Formula*
Log(Henry_DL) < -1	P (blood : air) = 0.4445 x (1/Henry_DL) + 0.0052 x (K oct:air)
Log(Henry_DL) ≥ -1	P (blood : air) = + 0.8355 x (1/Henry_DL) + 0.0058 x (K oct:air)

A plot of experimental human partition coefficients blood:air and QSPR-estimated values is presented in figure 4. Ninety estimates of the 106 experimental partition coefficients were within a factor two and 99 were within a factor three of the experimental results.

Figure 4. Comparison of the human experimental blood:air partition coefficients with the QSPR-derived estimates. Regression of two subsets of volatile organic compounds are presented



The dimensionless Henry coefficient is calculated from other properties of the chemical (Henry coef dim less = Vapour Pressure * Mol Weight/ (Water solubility *gas constant * Temp K).
The octanol:air partition coefficient (Koct:air) is calculated from log Kow and Log(Henry_DL) using the formula: log(Koct:air) = log(Kow)- Log(Henry_DL).

4.1.1.2 Rat blood-air partitioning

The QSPR for the blood:air partition coefficient of experimental rats has equally been derived from regression analysis of 92 rat blood:air values as compiled by Meulenberg and Vijverberg (2000). The resulting algoritm is using the dimensionless Henry coefficient and the octanol:air partition coefficient (Koct:air) as independent variables. Two regression equations were derived: One for a log (Henry_DL) larger than -0.7 and one for smaller than -0.7. This resulted in two different regression formulas as presented in table 16. These formulas are implemented as blood:air partitioning QSPR in the model IndusChemFate, dependant from log(Henry_DL).

Table 16. QSPR algoritm in the PBTK-model IndusChemFate to derive rat blood-air partition coefficients.

Condition	Formula
Log(Henry_DL) < -0.7	P (blood : air) = 0.1933 x (1/Henry_DL) + 0.002592 x (K oct:air)
Log(Henry_DL) ≥ -0.7	P (blood : air) = 2.32 x (1/Henry_DL) + 0.00674 x (K oct:air)

4.1.1.3 Mouse blood-air partitioning

Experimental partitioning data in mice were not available. Therefore, blood:air partition coefficient in mice was regarded as equal to the value in rats. Scientific support for this choice was based on the similar reported lipid contents in organs of mice and rats (Cunnane et al 1986, Kalén et al 1989).

4.1.1.4 Respiratory protection

The PBTK-model IndusChemFate can take into account the protection of respiratory protective equipment (RPE). The reduction factor of the applied RPE is inserted as a respiratory protection factor (default value of no RPE = 1). The air concentration during exposure (Cexp in figure 5) is divided by the reduction factor to calculate the inhaled concentration (Cinh). Therefore, the application of a reduction factor of 2 halves the inhaled concentration. The air concentration is limited; the maximum air concentration (Cexp) is equal to the concentration at which the air is saturated.

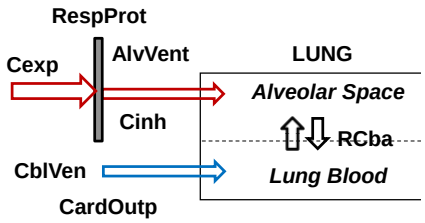


Figure 5. Respiratory protection in IndusChemFate.

4.1.2 Dermal absorption via liquid and gas

In the last two decades it has become clear that dermal absorption (both from environmental and occupational exposure) may be significant. This has lead to the development of PBPK models with an integrated dermal compartment [54, 55] as well as dermal only PBPK models [16, 55-61]. These models typically require many (experimentally determined) input parameters. In our model a modified version of the Skinperm algorithm as developed by Ten Berge [38, 39] has been incorporated. This is a diffusion based physiological model that predicts absorption based on physico-chemical properties of the substance. Two independent pathways of permeation through the skin are taken into account; trans-cellular and inter-cellular. The physiological model of skinperm considers the following three processes (figure 6):

(1) dermal deposition of a substance (liquid) on the skin;
(2) diffusion to the stratum corneum (SC);
(3) absorption to the dermis / blood flow.

Dermal absorption of chemicals in rat and mice is higher than in humans. The dermal permeation coefficient of rat and mouse was set on 3-fold the value of the human permeation coefficient, as a rough estimate of the lower boundary (Ravenzwaay and Leibold 2004). Both liquid absorption after dermal contact and absorption from the gas phase are considered (see next paragraphs).

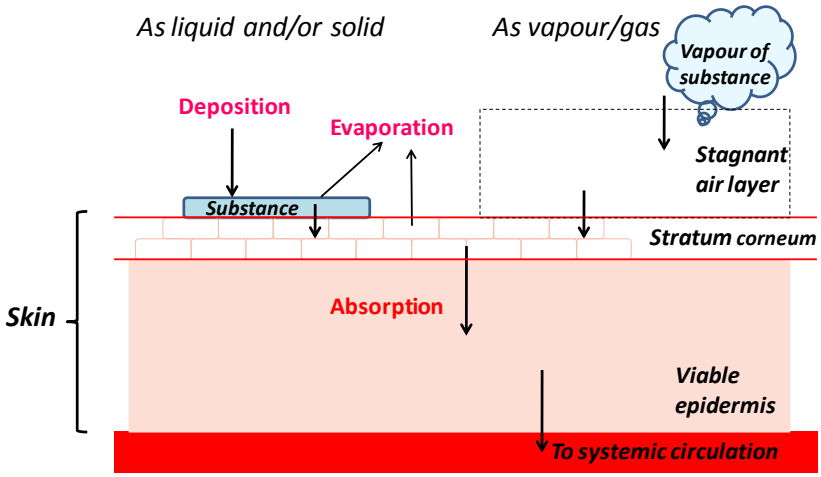


Figure 6. Schematic of dermal absorption processes in the model.

4.1.2.1 Dermal absorption via liquids and solids

After or during deposition of a liquid or solid substance on the skin, evaporation of the substance and dermal absorption occur simultaneously. Furthermore, a depot may be formed in the stratum corneum in case a substance diffuses easily into the stratum corneum but is slowly absorbed by the dermis. This depot can cause continuation of the absorption process although deposition on the skin has stopped. The above phenomena are simulated by IndusChemFate. Critical parameters are the aqueous dermal permeation coefficient and the stratum corneum/air partition coefficient. Both parameters are estimated by means of QSPRs derived from human experimental values, as developed by ten Berge [38].

4.1.2.2 Dermal absorption via gas

Also dermal uptake from air (vapour phase) may take place by diffusion. The aqueous permeation coefficient is transformed to a dermal air permeation coefficient as described by Wilschut et al [39], considering vapour pressure and resistance of a stagnant air layer. Depending on the physical activity of the subject this layer is estimated to be 3 cm (= at light work) or 10 cm (= at rest) thick. The total body surface of subjects is presented in table 17. For the estimation of the aqueous permeation coefficient the log octanol:water partition coefficient (at pH 5.5, similar to the pH of the stratum corneum) and , the molecular weight are required. The LogKow may vary at different pH, especially for organic acids and bases. This may effect the skin absorption dramatically (see example of nicotine [62]). The pH dependent LogKow can be obtained freely via Chemspider [34] or puchased via commercial databases.
The use of air tight clothing can be accounted for as a dermal protection factor against

dermal vapor uptake. The application of a protection factor of 2 will halve the exposure concentration of the skin to air.

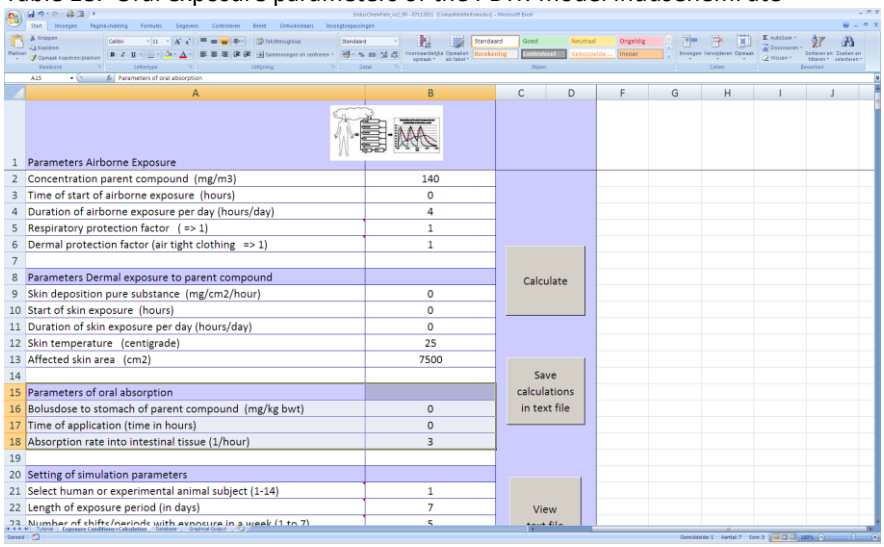
Table 17. Total body surface of subjects in IndusChemFate.

No. Standardised subject	*Total body surface (in cm2)*
1. Normal man (in rest)	18,000
2. Obese man (in rest)	18,000
3. Normal man (light work)	18,000
4. Obese man (light work)	18,000
5. Normal woman (in rest)	16,000
6. Obese woman (in rest)	16,000
7. Normal woman (light work)	16,000
8. Obese woman (light work)	16,000
9. Normal child (in rest)	6,000
10. Obese Child (in rest)	6,000
11. Normal child (playing)	6,000
12. Obese child (playing)	6,000
13. Mouse (experimental study)	450
14. Rat (experimental study)	100

4.1.3 Oral absorption

IndusChemFate simulates oral intake of compounds as a bolus dose that is applied to the intestinal lumen (via the stomach) and then released to the intestinal tissue at a first order rate. From the intestines the compound is released to the blood stream towards the liver (portal vein). The first order release rate is defined as the velocity at which the oral dose is absorbed by the intestinal tissue (as a fraction of the dose in the lumen per hour). Stomach and intestines are lumped in the model. The oral dose, time of dosing and the absorption rate are the required input parameters for oral uptake in the PBTK-model IndusChemFate (see table 18). Also repeated dosing (1-7 days) over week(s) can be entered in the simulation.

Table 18. Oral exposure parameters of the PBTK-model IndusChemFate



4.2 Distribution

Storage of a compound in the body is strongly influenced by the partitioning over blood and certain tissues. Enterohepatic circulation of a compound may increase the halflife in the body.

4.2.1 Tissue partitioning

4.2.1.1 Human tissue:blood

For the blood:tissue partitioning the QSPR algorithm as described by De Jongh et al. (1997) has been applied . They describe the distribution of compounds between blood and human body tissues as a function of water and lipid content of tissues and the n-octanol:water partition coefficient (K_{ow}). Experimental octanol:water partition coefficients of the 24 compounds were used for calibration of the model for human tissue-blood partition coefficients. As De Jongh et al. presented algorithms for only 5 tissue types and the IndusChemFate model consists of 11 tissue compartments, some compartments of the model share the same partitioning algorithm (see table 19). This selection is based on the lipid fraction of the tissue (Woodard and White, 1986).

Table 19. Aggregation of tissues for the prediction of tissue:blood partitioning.

Tissue	*Adopted tissue similarity in IndusChemFate model*
Fat	Fat
Liver	Liver, Intestine
Muscle	Muscle, Bone, Heart, Lung, Skin
Kidney	Kidney
Brain	Brain, Bone marrow

As an example, the algorithm for the brain:blood partition coefficient is shown as formula 5.

$$PCtb = \frac{0.133 * K_{OW}^{0.48} + 0.775}{0.0056 * K_{OW}^{0.48} + 0.830} - 0.21 \qquad (5)$$

With:
PCtb = brain:blood partition coefficient
K_{ow} = octanol:water partition coefficient

The octanol:water partition coefficients were taken at the physiological pH of 7.4. This considers the speciation of extent of ionization of the substance. These data were retrieved from databases of physical-chemical properties of chemicals {EPI suite database of US-EPA (2009), the Chemspider database (RCS, 2009)}.
Application of this equation to adipose tissue results into negative partition coefficients in case of Log(K_{ow}) < 0.4. This has no scientific meaning. So if the partition coefficient adipose

tissue:blood is estimated to be smaller than 0.1, the adipose tissue:blood partition coefficient is fixed to 0.1.

4.2.1.2 Rat tissue:blood

The rat tissue:blood partition coefficients were derived in a different way. Information on lipid and water content was only available for 3 organs. Therefore an alternative QSPR was developed. The logarithm of the ratio between rat organ tissue:air and rat blood:air partition coefficients (Meulenberg et al 2000) was plotted against the log(Kow). Above a log(Kow) of 5 and below a log(Kow) of -1 the organ tissue/blood partition coefficient did not change any more. At a log(Kow) larger than 5, the partition coefficient is controlled by the ratio of fat content between tissue and blood. Equally, below a log(Kow) of -1 the tissue/blood partition coefficient does not decrease and is probably controlled by the ratio between the water content of organ tissue and blood. So regression equations were derived for the organ tissue:blood partition coefficient, in which the independent variable log(Kow) predicts the log(partition organ tissue:blood). In case of adipose tissue a polynomial equation of log(Kow) of the second degree was used to predict the log(adipose tissue/blood) ratio. At a log(Kow)<-1, the tissue:blood ratio was used as predicted by log(Kow)=-1. At a log(Kow) > 5, the tissue:blood ratio was used as predicted by log(Kow)= 5

4.2.1.3 Mouse tissue:blood

Experimental partitioning data of tissue to blood in mice were not available. Therefore, tissue:blood partition coefficient in mice was regarded as equal to value in rats because of comparable lipid contents of organ tissue between rats and mice.

4.2.2 Enterohepatic recirculation

Phase II metabolism with conjugation of metabolites generally increases the solubility. Enzymes produced by intestinal bacteria - such as ß-glucuronidase, sulfatase, and various glycosidases - deconjugate these compounds in the intestines, releasing the parent compounds after which these are readily reabsorbed across the intestinal wall to the blood. This results in enterohepatic circulation of conjugated metabolites (see fig. 7).

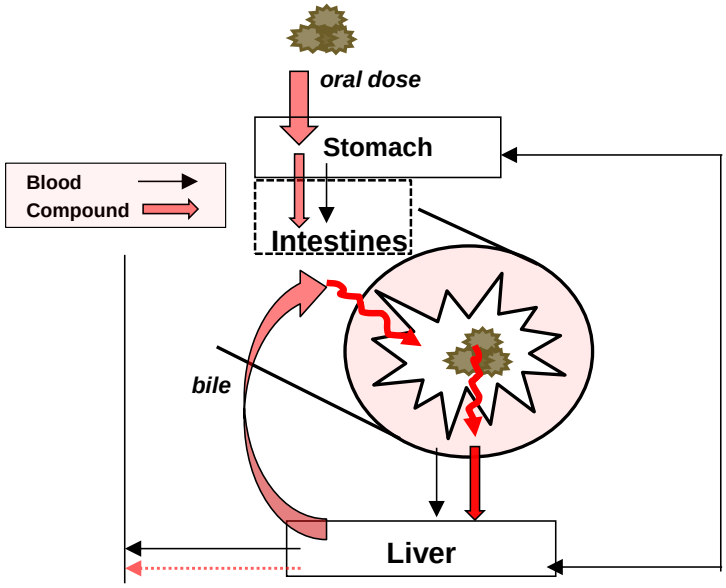


Figure 7. Schematic of the Enterohepatic circulation

Only few published PBPK models consider enterohepatic recirculation (Teeguarden et al., 2005; Collins et al., 1999) . These models require experimental data for transfer rates. In our model we applied a generic approach: it incorporates enterohepatic recirculation 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.
This ratio is defined as the fraction of the amount in liver tissue that is excreted to the intestinal lumen via bile, divided by the fraction that leaves the liver via venous blood at the same time. If the removal ratio from the liver by enterohepatic recirculation is set to 1, 50% of the total amount that leaves the liver per unit of time is excreted to blood and 50% to the intestinal lumen via bile.

4.2.3 Protein or plasmabinding

No specific attention is paid to protein or plasma binding. It is considered that it is included to some extent in the QSPR algorithms for blood:air and blood:tissue partitioning, since these are derived from experimental opservations.

4.3 Elimination

The chemical in the human body is eliminated in the model by two processes: metabolism (or biotransformation) and direct excretion in air or urine.

4.3.1 Metabolism

Biotransformation is described by Michaelis-Menten saturable metabolism following the mathematical algorithms as described by Ramsey and Andersen (1984). The (parent)

compound is metabolised by a set of (iso)enzymes. Usually one or more metabolite(s) are produced. Metabolites may either undergo further metabolism or may be excreted.
The occurrence of metabolism is not limited to the liver compartment, but can be considered in any of the 11 model compartments. However, the default setting is metabolism in the liver only. Metabolic kinetic parameters are the maximum velocity of metabolism (= V_{max} in µmol/(kg tissue*h)) and the Michaelis-Menten constant (=k_M in µM). Preferably these values are taken form experimental data with human tissue. When parallel metabolic pathways are involved, the V_{max} and k_M -values for a specific metabolite production can be set as different from those the parent compound. That is possible because the model considers both removal of the parent compound and production of metabolite as separate steps. That means the biotransformation of the parent compound occurs for only x% into the metabolite of interest and for (100-x)% into other (unknown) metabolites. That is how the stoichiometric yield of each step is set.
V_{max} and k_M metabolism constants of a series of VOC have recently been summarized (Aylward et al., 2010).

4.3.2 Urinary excretion

Substances can be excreted via urine, either unchanged as parent compound or as a metabolite. A scheme of renal clearance of chemicals is shown in figure 8. DeWoskin and Thompson (2008) published a paper in which renal clearance is modeled in great detail, however, the required input data transcends the application in a generic model. Manga et al (2003) showed that log Kow is a predictive determinant of urinary excretion of drugs in humans, among other determinants.

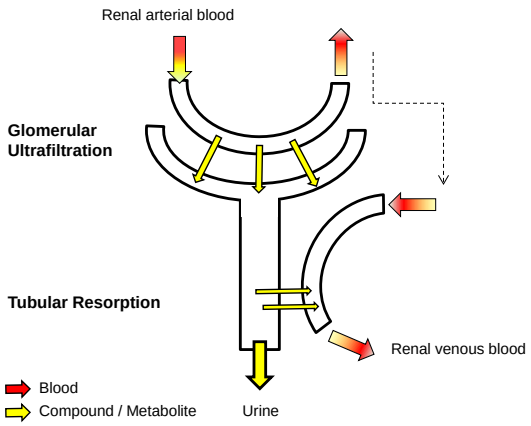


Figure 7. Scheme of renal clearance of chemicals.

In the PBTK-model IndusChemFate, the renal excretion is estimated based on the log(Kow) using 3 algoritms (with log(Kow) at pH 7.4):

1. It affects the soluble fraction in blood. We used the QSPR as developed by DeJongh et al. (1997) that calculates the solubility in blood based on lipid fractions in blood. This is adopted in the syntax of the model. It is assumed that human blood contains 0.7% lipids. See formula 6.

$$FrWsol = \frac{0.993}{0.993 + 0.007 * 10^{Log(Kow)}} \qquad (6)$$

With:
FrWsol = blood solubility of the compound
Log(K_{ow}) = Log(octanol:water partition coefficient)

2. It estimates the tubular resorption according to the following equation:

Fraction tubular resorption = Normal Distr(log(Kow), (-0.5, 0.5) (7)
With:
Log(K_{ow}) = Log(octanol:water partition coefficient)
Sigmoide resorption curve with Log(Kow) = -0.5 as midpoint and with SD = 0.5

Fig. 9 shows the graph of this sigmoide relation of tubular resorption as dependant from log (Kow).

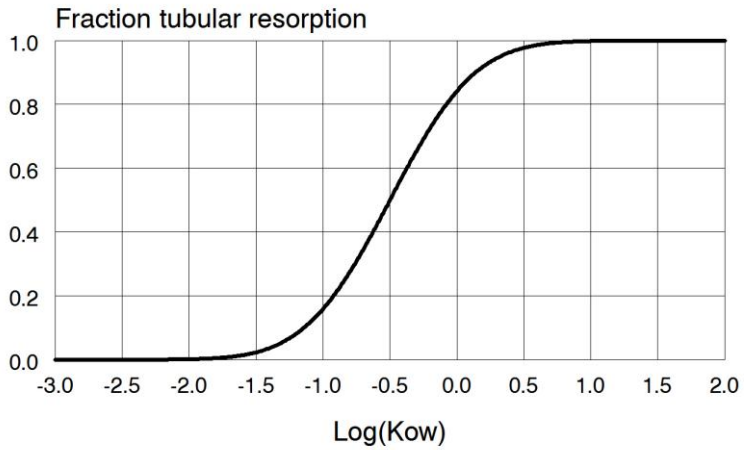


Figure 9. Fraction of the tubular resorption as dependant from Log (Kow).

3. If the estimated tubular resorption is smaller than 0.01, the value is fixed to 0.01. If the estimated tubular resorption is larger than 0.99, the value is fixed to 0.99.

SimCyp, an online calculation tool also calculates the unbound fraction in plasma [74]. Table 20 shows the values of the estimated blood solubility according to DeJong et al (1997) and SimCyp for some industrial chemicals. It should be noted that SimCyp intends to predict the

unbound fraction of pharmaceuticals, whereas the QSPR by De Jongh mostly focus on industrial chemicals.

Table 20. Calculated solubility in blood for some compounds using different resources.

Compound	*Log(Kow)*	*Calculated solubility in blood*	
		DeJongh [37]	*SimCyp [74]*
Acetaldehyde	-0.16	99.5%	78.0%
Benzene	2.22	46.1%	23.0%
Butadiene	1.86	66.2%	31.0%
Ethanol	-0.19	99.5%	79.0%
Methanol	-0.82	99.9%	88.0%
MTBE (Methyl-tert-butyl-ether)	1.15	90.9%	48.0%
NMP (N-methyl-pyrrolidone)	-0.31	99.7%	81.0%
Propene	1.84	67.2%	31.0%
Pyrene	5.17	0.1%	1.4%
Toluene	2.68	22.9%	16.0%

The glomulaire filtration rate in the the PBTK-model IndusChemFate model is set at 0.08 of the renal bloodflow. Glomulaire filtration for mouse and rat is set at 0.16.

The urine production of humans is set to 0.0214 L/kg BW/24 h (= 1.5 L/day). The urine production of rats is set to 0.2 L/kg BW/24 h. The value for mice is 0.05 ml/kg BW/24 h. The urinary concentrations are reported in micromole per liter urine. This unit can be recalculated to microgram/gram creatinine or micromol /mol creatinine using the amount of daily excretion of creatinine [75].

4.3.3 Exhalation

The parent compound (or any metabolite) can be exhaled and may then play a role in elimination. The exhaled concentration consists of a mixture of the inhaled air concentration (air that has not reached the alveoli) and alveolar air. The concentration of a compound in the alveolar space of the lungs (Calv in figure 10) is determined by the blood concentration in the (arterial) lung blood and the blood:air partition coefficient.

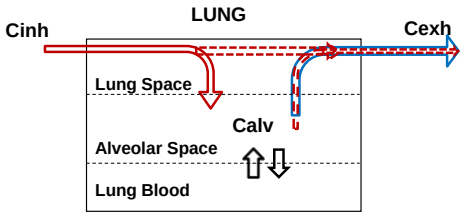


Figure 10. Scheme of exhalation of chemicals.

The amount of a compound (parent and/or metabolite) that is exhaled, is calculated by multiplying the alveolar concentration (Calv) by the alveolar ventilation (=inhalation rate in liters per hour).

4.3.4 Bile and Fecal excretion

Fecal excretion has not been modeled as a separate excretion pathway in the PBTK-model IndusChemFate. It is considered as a minor excretion pathway of volatile and semi-volatile environmental and occupational contaminants, for which inhalation and dermal exposure is the main exposure route .

5 Differential equations

Each tissue compartment in the IndusChemFate model is, similar to all PBPK models, described with a mass-balance ordinary differential equation. These equations describe the change of amount of chemical per tissue over time. The equations used in the PBTK-model IndusChemFate are in accordance with the generally accepted mathematical representation of PBPK modeling, although some abbreviations are different from those reported elsewhere [41, 68].

5.1 Absorption by inhalation

$$C_{lung_art}[j] = \frac{Q_{flow} * C_{LungVen}[j] + AlvVent * C_{inh}[j]}{Q_{flow} + AlvVent / R_{blood/air}[j]} \qquad eq.\ 1$$

$$C_{alv.air}[j] = \frac{C_{LungArt}[j]}{R_{blood/air}[j]} \qquad eq.\ 2$$

$C_{LungArt}[j]$ = Concentration substance[j] in arterial blood flowing from lung (µMol/litre)
$C_{LungVen}[j]$ = Concentration of substance[j] in venous blood entering lung (µMol/litre)
Q_{flow} = Cardiac output (litres/hour)
AlvVent = Alveolar ventilation (litres/hour)
$C_{inh}[j]$ = Concentration of substance [j] in inhaled air (µMol/litre)
$R_{blood/air}[j]$ = Blood/air partition ratio of substance [j], estimated by QSPR [36].
$C_{alv.air}[j]$ = Conc substance [j] in alveolar air

5.2 Mass flows

The equations below describe the mass change of the parent compound (j=0) and its succeeding metabolites (j=1 to 4) in time. These mass changes apply to all organs considered and relate to the fraction of the cardiac output to the specific organ Q_{org} and to the metabolic removal and formation of the parent and its metabolites.

5.2.1 Generic mass flow in organs

Parent substance (j=0) (eq.3)

$$\frac{dAm_{org}[0]}{dt} = Q_{org}\left(C_{art}[0] - \frac{C_{org}[0]}{R_{org/ven}[0]}\right) - \frac{Vmax_{rem}[0] * V_{org} * C_{org}[0]}{Km_{rem}[0] + C_{org}[0]}$$

Metabolites (j=1 to 4) (eq.4)

$$\frac{dAm_{org}[j]}{dt} = Q_{org}\left(C_{art}[i] - \frac{C_{org}[j]}{R_{org/ven}[j]}\right) - \frac{Vmax_{rem}[j]*V_{org}*C_{org}[j]}{Km_{rem}[j]+C_{org}[j]} + \frac{Vmax_{form}[j-1]*V_{org}*C_{org}[j-1]}{Km_{form}[j-1+C_{org}[j-1]}$$

$Am_{org}[j]$ = Mass of substance j in organ (µMol)
Q_{org} = Arterial blood flow to organ (fraction of cardiac output) in litres/hour
$C_{art}[j]$ = Concentration substance[j] µMol/litre in arterial blood.
$C_{org}[j]$ = Concentration substance [j] µMol/kg in organ tissue (density tissue is 1 kg/ litre)
$R_{org/ven}[j]$ = Organ tissue/blood partiton coefficient of substance [j], estimated by QSPR [37]
$Vmax_{rem}[j]$ = Maximum biotransformation in µMol/kg tissue/hour in subst[j+1] from substance [j]
V_{org} = Volume of organ (litre)
$Km_{rem}[j]$ = Concentration substance [j] µMol/kg tissue, at which the biotransformation rate into substance [j+1] from substance [j] is half maximum
$Vmax_{form}[j-1]$ = Maximum biotransformation in µMol/kg tissue/hour in substance [j] from substance [j-1]
$Km_{form}[j-1]$ = Concentration substance [j] µMol/kg tissue, at which the biotransformation rate into substance [j] from substance [j-1] is half of the maximum

However, there are some organs that deserve special attention and additional source and removal contributing to mass changes have to be formulated.

5.2.2 Mass change in liver tissue additional to generic mass change

The formula below *(eq.5)* should be added to the generic mass flow description *(eq.4)*.

Mass change in the liver tissue (eq.5)

$$\frac{dAm_{liver}[j]}{dt} = [eq4] + Q_{intestines} * \frac{C_{intestines}[j]}{R_{intestines/ven}[j]} - Q_{intestines} * \frac{C_{liver}[j]}{R_{liver/ven}[j]} - Removal_{Bile} * AM_{liver}[j]$$

$Am_{liver}[j]$ = Mass of substance j in the liver (µMol)
$Q_{intestines}$ = Arterial blood flow to the intestines in litres/hour
$C_{intestines}[j]$ = Concentration substance [j] (µMol/kg) in the intestines
$R_{intestines/ven}[j]$ = Intestine tissue/blood partiton coefficient of substance [j]
$C_{liver}[j]$ = Concentration substance [j] (µMol/kg) in the liver tissue
$R_{liver/ven}[j]$ = Liver tissue/blood partiton coefficient of substance [j]
$AM_{liver}[j]$ = Mass of substance [j] (µMol) metabolised by the liver tissue

The removal from the liver into the bile ($Removal_{Bile}$) is expressed as the fraction of the mass [j] in the liver per hour, discharged via the bile in the intestinal lumen. In the input information for running the program, this removal via the bile has to be indicated relative to

the removal of the mass in the liver via the venous blood flow out of the liver. The software assigns the proper value to the removal from the liver into bile as the fraction of the mass[j] in the liver per hour.

5.2.3 Mass change in the lumen of the intestines

Mass change in the intestinal lumen (eq.6)

$$\frac{dAm_{intest-lumen}[j]}{dt} = Removal_{Bile} * AM_{liver}[j] - 0.3 * Am_{intest-lumen}[j]$$

$Am_{intest-lumen}[j]$ = Mass of substance j in the intestinal lumen (µMol)

It is assumed that the mass [j] in the intestinal lumen, released via the bile, is re-absorbed in the intestinal tissue with a rate of 0.3 per hour.

5.2.4 Mass change in intestinal tissue additional to generic mass change

The formula below *(eq.7)* should be added to the generic mass flow description *(eq.4)*.

Mass change in the intestinal tissue (eq.7)

$$\frac{dAm_{intestines}[j]}{dt} = \frac{dAm_{intestines}[j]}{dt} + AbsRate * Bolus[j] + 0.3 * Am_{lumen}[j]$$

$Am_{intestines}[j]$ = Mass of substance j in the intestinal tissue (µMol)

It is assumed, that the mass in the intestinal tissue is increased by an oral dose (*Bolus[i] µMoles*), which is absorbed form the intestinal lumen in the intestinal tissue with the rate *AbsRate* (1/hour) and by the re-absorption of the mass[i] from the intestinal lumen, released via the bile, with a rate of 0.3 per hour.

5.2.5 Mass change in dermal tissue additional to generic mass change

Dermal absorption is generally assumed to occur for the parent compound (j=*0*) and is dependent on:

The dermal absorption flux consists of two parts:
Intermittent dermal exposure to liquid.
Dermal exposure from the ambient air.

The formula below *(eq.8)* should be added to the generic mass flow description *(eq.4).*

Mass change in the dermal tissue (eq.8)

$$\frac{dAm_{skin}[j]}{dt} = \frac{dAm_{skin}[j]}{dt} + [Intermittent\ Liquid\ Exposure] + [Rate_{Skin-Air}[j]$$

$Am_{skin}[j]$ = Mass of substance [j] in the skin (µMol)

Dermal exposure from ambient air (eq.9)

$$Rate_{Skin-Air}[j] = Kp_{air-x}[j] * Surface_{Body} * C_{air}[j]/1000$$

$$K_{air-rest}[j] = 36 * \sqrt{\frac{76}{Mw[j]}} \qquad K_{air-light-activity}[j] = 120 * \sqrt{\frac{76}{Mw[j]}} \qquad K_{wa}[j] = \frac{R*T*W_{solub}[j]}{Mw[j]*Vp[j]} \qquad (eq.\ 9)$$

$$Kp_{air-x}[j] = \frac{1}{\frac{1}{Kp_{aq}[j]*K_{wa}[j]} + \frac{1}{K_{air-x}[j]}} \qquad (eq.\ 10)$$

$Rate_{Skin-Air}[j]$ = Dermal absorption rate of substance [j] from air into the skin [dermis] (mg/cm^2/hour)

$Kp_{air-x}[j]$ = Dermal permeation coefficient of substance [j] through the skin from air as vehicle

$K_{air-x}[j]$ = Permeation coefficient of substance [j] through air layer around the skin, dependent on $K_{air-x}[j]$,related to worker activity (cm/hour)

$K_{wa}[j]$ = Water/air partition coefficient of substance [j] to adapt $Kp_{aq}[j]$ to absorption from air

$Kp_{aq}[j]$ = Aqueous permeation coefficient of substance [j] in cm/hour, estimated by QSPR (ten Berge 2009 [38])

$C_{air}[j]$ = Concentration of substance [j] in air (µMol/litre)

$Surface_{body}$ = Surface area of the body of the study subject

R = Gas constant (8.314 Joule/Mol/°K)

T = Skin surface temperature (°K)

$W_{solub}[j]$ = Water solubility of substance [j] (gram/litre)

Mw[j] = Molecular weight of substance [j].

Vp[j] = Vapour pressure of substance [j] at skin surface temperature (Pascal)

5.2.6 Mass change in kidney tissue additional to generic mass change

The formula below *(eq.11)* should be added to the generic mass flow description *(eq.4).*

Mass change in the kidney tissue (eq.11)

$$\frac{dAm_{kidney}[j]}{dt} = \frac{dAm_{kidney}[j]}{dt} - GlomFiltr * Fr_{watersoluble}[j] * Remov_{Kidney}[j] * Q_{kidney} * C_{art}[j]$$

GlomFiltr = Glomerulus filtrate as fraction of renal arterial folow
$Fr_{watersoluble}[j]$ = Fraction of substance [j] in arterial blood, that is dissolved in water
$Remov_{kidney}[j]$ = Fraction removed from the glomerulus filtrate and excreted with urine
$Remov_{kidney}[j]$ = 1 – Fraction tubular resorption

$$Fraction\ tubular\ resorption = \int_{-\infty}^{x} \frac{exp(-t^2/2)}{\sqrt{2\pi}}\ dt$$

$$x = 2*(\log(Kow) + 0.5)$$

If the estimated fraction of tubular resorption is smaller than 0.01, the value is fixed to 0.01.
If the estimated tubular resorption is larger than 0.99, the value is fixed to 0.99.
(See also §4.3.2.)
Q_{kidney} = Fraction of cardiac ouput to the kidney (litres/hour)
$C_{art}[j]$ = Concentration substance [j] µMol/litre in arterial blood

5.3 Concentration in arterial blood (entering the organs)

$$\frac{dAm_{art.vol}[j]}{dt} = Q_{flow} * (C_{lung_art}[j] - C_{art}[j]) \qquad eq.\ 12$$

$$C_{art}[j] = \frac{Am_{art.vol}[j]}{ArtVol} \qquad eq.\ 13$$

$Am_{art.vol}[j]$ = Mass of substance [j] in arterial blood volume
$C_{lung_art}[j]$ = Conc substance[j] in arterial blood flowing from lung (µMol/litre)
$C_{art}[j]$ = Conc subst[j] in arterial blood flowing to organs (µMol/litre)
Q_{flow} = Cardiac output (litres/hour)
ArtVol = Volume of arterial blood.

5.4 Concentration in venous blood (entering the lung)

$$Am_{OrgVen}[j] = \sum_{Sum\ all\ organs}^{Not\ liver,\ not\ intestines} Q_{org} * C_{org}[j] / R_{org/ven}[j] + (Q_{intestines} + Q_{liver}) * C_{liver}[j] / R_{liver/ven}[j] \quad eq.\ 14$$

$$C_{ven}[j] = Am_{OrgVen}[j] / Q_{flow} \quad eq.\ 15$$

$$\frac{dAm_{LungVen}[j]}{dt} = Q_{flow} * (C_{ven}[j] - C_{LungVen}[j]) \quad eq.\ 16$$

$$C_{LungVen}[j] = Am_{LungVen}[j] / VenVol \quad eq.\ 17$$

$Am_{OrgVen}[j]$ = Mass of substance [j] in blood flowing out of all organs
$C_{Ven}[j]$ = Concentration of substance [j] in mixed venous blood from all organs
$Am_{LungVen}[j]$ = Mass of substance [j] in venous blood volume entering the lung
$C_{LungVen}[j]$ = Concentration of substance [j] in venous blood entering the lung
VenVol = Volume of venous blood in the body

6 Interpretation of model outcomes

The PBTK-model IndusChemFate offers a free modeling platform based on well-known software that only requires easy available input parameters. Additional modeling or programming skills is not required, but basic knowledge of toxicokinetics is necessary to understand.

The accuracy of the model prediction is illustrated by simulating experimental observations. Published experimental inhalation and dermal exposure studies on a series of different chemicals (pyrene, N-methyl-pyrrolidone, methyl-tert-butylether, heptane, 2-butoxyethanol and ethanol) were selected to compare the observed data with the model-simulated data. The examples show that the model-predicted concentrations in blood and/or urine after inhalation and/or transdermal uptake have an accuracy of within an order of magnitude (Jongeneelen & ten Berge 2011a, 2011b).

Model simplifications as introduced for physiological processes (simple tubular resorption, gastrointestinal absorption, enterohepatic circulation, absence of fecal excretion, etc.) may decrease the precision of the model outcomes, but will make the use of the model easier due to the limited input data.

Simulations with the model result in single point estimates per unit of time. No bandwidth nor distribution with upper and lower confidence intervals are presented. The model is aimed to predict within one order of magnitude, corresponding to interindividual differences. As many parameter values are involved, the reliability of the model outcomes strongly depends on the quality (or reliability) of the input data but also the variability. Variability of input data is not automatically assessed as no distributions are used. Nevertheless the model calculates quick enough to use a range of relevant parameters to get some feeling of the impact of parameter variability.

The objectives of the assessment that is performed with the model IndusChemFate can be different. In terms of risk assessment a worst case estimate can be of interest as well as the assessment of the most probable exposure scenario. Also route to route extrapolation is possible with the model. The model can also support first interpretation of, for instance, blood or urine samples.

A simple PBTK-model that requires minimal input information will help the general understanding of toxicokinetics and can also be useful to educate students and scientists in the biomedical sciences on PBPK/PBTK-modelling.

In summary, we have demonstrated that the developed PBPK-model can be applied to multiple compounds with a degree of accuracy that can be regarded as a screening tool or as a first tier assessment. Such a first level model can exploit the results of environmental and

occupational monitoring and modelling programs to deduce the routes of uptake, tissue concentrations, and body burdens. This PBPK model can contribute to an improved assessment of uptake of environmental and occupational contaminants.

In conclusion, the PBTK-model IndusChemFate is a model for first tier or screening purposes, suitable in cases when only little is known of the toxicokinetics of a compound. One should realise that outcomes of the model should be interpreted within an accuracy of an order of magnitude. Especially when the quality of input data is limited the output of the model outcomes become more uncertain.

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Appendix 1: Physiology parameters of 12 human subjects and two experimental animals

Physiology of Subjects in InduschemFate 2.00

Case 1

BodyWt# = 70 'kg normal man
FrArt# = 0.3 'fraction arterial blood
FrVen# = 0.7 'fraction venous blood
VolBlood# = 0.0832 * BodyWt# 'litre
VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood
VolBlungVen# = FrVen# * VolBlood# 'volume venous blood
VolAdip# = 0.184 * BodyWt# 'litre
VolBone# = 0.0589 * BodyWt# 'litre
VolBrain# = 0.0192 * BodyWt# 'litre
VolHeart# = 0.0051 * BodyWt# 'litre
VolKidney# = 0.0046 * BodyWt# 'litre
VolIntest# = 0.0222 * BodyWt# 'litre
VolLiver# = 0.0225 * BodyWt# 'litre
VolLungs# = 0.0144 * BodyWt# 'litre
VolMuscle# = 0.4576 * BodyWt# 'litre
VolSkin# = 0.0553 * BodyWt# 'litre
VolMarrow# = 0.0729 * BodyWt# 'litre
TotSkin# = 18000 'body surface cm2
SkinFac# = 1 'ratio skin permeability
UrinFlow# = 0.0214 'litre/kgbw/day
GlomFiltr# = 0.08 'fraction of renal arterial flow

'Normal man in rest
CardOutp# = 6 * BodyWt# 'litres per hour
AlvVent# = 7 * BodyWt# 'litres per hour
FrAdip# = 0.08226 'fraction cardiac output rest
FrBone# = 0.04139 'fraction cardiac output rest
FrBrain# = 0.09814 'fraction cardiac output rest
FrHeart# = 0.10536 'fraction cardiac output rest
FrKidney# = 0.16886 'fraction cardiac output rest
FrLivVen# = 0.152 'fraction cardiac output rest
FrLivArt# = 0.0381 'fraction cardiac output rest
FrLung# = 0.0234 'fraction cardiac output rest
FrMuscle# = 0.13711 'fraction cardiac output rest
FrSkin# = 0.06783 'fraction cardiac output rest
FrMarrow# = 0.08515 'fraction cardiac output rest

Case 2

BodyWt# = 80 'kg obese man
FrArt# = 0.3 'fraction arterial blood
FrVen# = 0.7 'fraction venous blood
VolBlood# = 0.06787 * BodyWt# 'litre
VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood
VolBlungVen# = FrVen# * VolBlood# 'volume venous blood
VolAdip# = 0.33458 * BodyWt# 'litre
VolBone# = 0.04805 * BodyWt# 'litre
VolBrain# = 0.01563 * BodyWt# 'litre
VolHeart# = 0.0042 * BodyWt# 'litre
VolKidney# = 0.00373 * BodyWt# 'litre
VolIntest# = 0.01808 * BodyWt# 'litre
VolLiver# = 0.01831 * BodyWt# 'litre
VolLungs# = 0.01178 * BodyWt# 'litre
VolMuscle# = 0.37318 * BodyWt# 'litre
VolSkin# = 0.04513 * BodyWt# 'litre
VolMarrow# = 0.05948 * BodyWt# 'litre
TotSkin# = 18000 'body surface cm2
SkinFac# = 1 'ratio skin permeability
UrinFlow# = 0.0214 'litre/kgbw/day
GlomFiltr# = 0.08 'fraction of renal arterial flow

'Obese man in rest
CardOutp# = 6 * BodyWt# 'litres per hour
AlvVent# = 7 * BodyWt# 'litres per hour
FrAdip# = 0.08226 'fraction cardiac output rest
FrBone# = 0.04139 'fraction cardiac output rest
FrBrain# = 0.09814 'fraction cardiac output rest
FrHeart# = 0.10536 'fraction cardiac output rest
FrKidney# = 0.16886 'fraction cardiac output rest
FrLivVen# = 0.152 'fraction cardiac output rest
FrLivArt# = 0.0381 'fraction cardiac output rest
FrLung# = 0.0234 'fraction cardiac output rest
FrMuscle# = 0.13711 'fraction cardiac output rest
FrSkin# = 0.06783 'fraction cardiac output rest
FrMarrow# = 0.08515 'fraction cardiac output rest

Case 3

BodyWt# = 70 'kg normal man
FrArt# = 0.3 'fraction arterial blood
FrVen# = 0.7 'fraction venous blood
VolBlood# = 0.0832 * BodyWt# 'litre
VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood
VolBlungVen# = FrVen# * VolBlood# 'volume venous blood
VolAdip# = 0.184 * BodyWt# 'litre
VolBone# = 0.0589 * BodyWt# 'litre
VolBrain# = 0.0192 * BodyWt# 'litre
VolHeart# = 0.0051 * BodyWt# 'litre
VolKidney# = 0.0046 * BodyWt# 'litre
VolIntest# = 0.0222 * BodyWt# 'litre
VolLiver# = 0.0225 * BodyWt# 'litre
VolLungs# = 0.0144 * BodyWt# 'litre
VolMuscle# = 0.4576 * BodyWt# 'litre
VolSkin# = 0.0553 * BodyWt# 'litre
VolMarrow# = 0.0729 * BodyWt# 'litre

Case 4

BodyWt# = 80 'kg obese man
FrArt# = 0.3 'fraction arterial blood
FrVen# = 0.7 'fraction venous blood
VolBlood# = 0.06787 * BodyWt# 'litre
VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood
VolBlungVen# = FrVen# * VolBlood# 'volume venous blood
VolAdip# = 0.33458 * BodyWt# 'litre
VolBone# = 0.04805 * BodyWt# 'litre
VolBrain# = 0.01563 * BodyWt# 'litre
VolHeart# = 0.0042 * BodyWt# 'litre
VolKidney# = 0.00373 * BodyWt# 'litre
VolIntest# = 0.01808 * BodyWt# 'litre
VolLiver# = 0.01831 * BodyWt# 'litre
VolLungs# = 0.01178 * BodyWt# 'litre
VolMuscle# = 0.37318 * BodyWt# 'litre
VolSkin# = 0.04513 * BodyWt# 'litre
VolMarrow# = 0.05948 * BodyWt# 'litre

Physiology of Subjects in InduschemFate 2.00	
TotSkin# = 18000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Normal man light work CardOutp# = 9 * BodyWt# 'litres per hour AlvVent# = 18 * BodyWt# 'litres per hour FrAdip# = 0.06957 'fraction cardiac output rest FrBone# = 0.035 'fraction cardiac output rest FrBrain# = 0.08299 'fraction cardiac output rest FrHeart# = 0.1057 'fraction cardiac output rest FrKidney# = 0.14279 'fraction cardiac output rest FrLivVen# = 0.12888 'fraction cardiac output rest FrLivArt# = 0.03222 'fraction cardiac output rest FrLung# = 0.01978 'fraction cardiac output rest FrMuscle# = 0.25369 'fraction cardiac output rest FrSkin# = 0.05736 'fraction cardiac output rest FrMarrow# = 0.07201 'fraction cardiac output rest	TotSkin# = 18000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Obese man light work CardOutp# = 9 * BodyWt# 'litres per hour AlvVent# = 18 * BodyWt# 'litres per hour FrAdip# = 0.06957 'fraction cardiac output rest FrBone# = 0.035 'fraction cardiac output rest FrBrain# = 0.08299 'fraction cardiac output rest FrHeart# = 0.1057 'fraction cardiac output rest FrKidney# = 0.14279 'fraction cardiac output rest FrLivVen# = 0.12888 'fraction cardiac output rest FrLivArt# = 0.03222 'fraction cardiac output rest FrLung# = 0.01978 'fraction cardiac output rest FrMuscle# = 0.25369 'fraction cardiac output rest FrSkin# = 0.05736 'fraction cardiac output rest FrMarrow# = 0.07201 'fraction cardiac output rest
Case 5 BodyWt# = 60 'kg normal woman FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood VolBlood# = 0.0744 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.281 * BodyWt# 'litre VolBone# = 0.0491 * BodyWt# 'litre VolBrain# = 0.0216 * BodyWt# 'litre VolHeart# = 0.0054 * BodyWt# 'litre VolKidney# = 0.0047 * BodyWt# 'litre VolIntest# = 0.026 * BodyWt# 'litre VolLiver# = 0.0243 * BodyWt# 'litre VolLungs# = 0.0156 * BodyWt# 'litre VolMuscle# = 0.3774 * BodyWt# 'litre VolSkin# = 0.06 * BodyWt# 'litre VolMarrow# = 0.0606 * BodyWt# 'litre TotSkin# = 16000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Normal woman in rest CardOutp# = 5 * BodyWt# 'litres per hour AlvVent# = 5 * BodyWt# 'litres per hour FrAdip# = 0.1421 'fraction cardiac output rest FrBone# = 0.02963 'fraction cardiac output rest FrBrain# = 0.09523 'fraction cardiac output rest FrHeart# = 0.14512 'fraction cardiac output rest FrKidney# = 0.12849 'fraction cardiac output rest FrLivVen# = 0.16326 'fraction cardiac output rest FrLivArt# = 0.04081 'fraction cardiac output rest FrLung# = 0.02407 'fraction cardiac output rest FrMuscle# = 0.09523 'fraction cardiac output rest FrSkin# = 0.07709 'fraction cardiac output rest FrMarrow# = 0.05895 'fraction cardiac output rest	Case 6 BodyWt# = 70 'kg obese woman FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood VolBlood# = 0.05808 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.43872 * BodyWt# 'litre VolBone# = 0.0383 * BodyWt# 'litre VolBrain# = 0.01684 * BodyWt# 'litre VolHeart# = 0.00421 * BodyWt# 'litre VolKidney# = 0.00365 * BodyWt# 'litre VolIntest# = 0.02029 * BodyWt# 'litre VolLiver# = 0.01894 * BodyWt# 'litre VolLungs# = 0.01221 * BodyWt# 'litre VolMuscle# = 0.29463 * BodyWt# 'litre VolSkin# = 0.04686 * BodyWt# 'litre VolMarrow# = 0.04728 * BodyWt# 'litre TotSkin# = 16000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Obese woman in rest CardOutp# = 5 * BodyWt# 'litres per hour AlvVent# = 5 * BodyWt# 'litres per hour FrAdip# = 0.1421 'fraction cardiac output rest FrBone# = 0.02963 'fraction cardiac output rest FrBrain# = 0.09523 'fraction cardiac output rest FrHeart# = 0.14512 'fraction cardiac output rest FrKidney# = 0.12849 'fraction cardiac output rest FrLivVen# = 0.16326 'fraction cardiac output rest FrLivArt# = 0.04081 'fraction cardiac output rest FrLung# = 0.02407 'fraction cardiac output rest FrMuscle# = 0.09523 'fraction cardiac output rest FrSkin# = 0.07709 'fraction cardiac output rest FrMarrow# = 0.05895 'fraction cardiac output rest
Case 7 BodyWt# = 60 'kg normal woman FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood	Case 8 BodyWt# = 70 'kg Obese woman FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood

Physiology of Subjects in InduschemFate 2.00	
VolBlood# = 0.0744 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.281 * BodyWt# 'litre VolBone# = 0.0491 * BodyWt# 'litre VolBrain# = 0.0216 * BodyWt# 'litre VolHeart# = 0.0054 * BodyWt# 'litre VolKidney# = 0.0047 * BodyWt# 'litre VolIntest# = 0.026 * BodyWt# 'litre VolLiver# = 0.0243 * BodyWt# 'litre VolLungs# = 0.0156 * BodyWt# 'litre VolMuscle# = 0.3774 * BodyWt# 'litre VolSkin# = 0.06 * BodyWt# 'litre VolMarrow# = 0.0606 * BodyWt# 'litre TotSkin# = 16000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Normal woman light work CardOutp# = 8 * BodyWt# 'litres per hour AlvVent# = 13 * BodyWt# 'litres per hour FrAdip# = 0.12046 'fraction cardiac output rest FrBone# = 0.02512 'fraction cardiac output rest FrBrain# = 0.08073 'fraction cardiac output rest FrHeart# = 0.1441 'fraction cardiac output rest FrKidney# = 0.10892 'fraction cardiac output rest FrLivVen# = 0.1384 'fraction cardiac output rest FrLivArt# = 0.0346 'fraction cardiac output rest FrLung# = 0.02041 'fraction cardiac output rest FrMuscle# = 0.21193 'fraction cardiac output rest FrSkin# = 0.06535 'fraction cardiac output rest FrMarrow# = 0.04998 'fraction cardiac output rest	VolBlood# = 0.0832 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.184 * BodyWt# 'litre VolBone# = 0.0589 * BodyWt# 'litre VolBrain# = 0.0192 * BodyWt# 'litre VolHeart# = 0.0051 * BodyWt# 'litre VolKidney# = 0.0046 * BodyWt# 'litre VolIntest# = 0.0222 * BodyWt# 'litre VolLiver# = 0.0225 * BodyWt# 'litre VolLungs# = 0.0144 * BodyWt# 'litre VolMuscle# = 0.4576 * BodyWt# 'litre VolSkin# = 0.0553 * BodyWt# 'litre VolMarrow# = 0.0729 * BodyWt# 'litre TotSkin# = 16000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Obese woman light work CardOutp# = 8 * BodyWt# 'litres per hour AlvVent# = 13 * BodyWt# 'litres per hour FrAdip# = 0.12046 'fraction cardiac output rest FrBone# = 0.02512 'fraction cardiac output rest FrBrain# = 0.08073 'fraction cardiac output rest FrHeart# = 0.1441 'fraction cardiac output rest FrKidney# = 0.10892 'fraction cardiac output rest FrLivVen# = 0.1384 'fraction cardiac output rest FrLivArt# = 0.0346 'fraction cardiac output rest FrLung# = 0.02041 'fraction cardiac output rest FrMuscle# = 0.21193 'fraction cardiac output rest FrSkin# = 0.06535 'fraction cardiac output rest FrMarrow# = 0.04998 'fraction cardiac output rest
Case 9 BodyWt# = 16 'kg normal child FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood VolBlood# = 0.1014 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.2148 * BodyWt# 'litre VolBone# = 0.0694 * BodyWt# 'litre VolBrain# = 0.0965 * BodyWt# 'litre VolHeart# = 0.0076 * BodyWt# 'litre VolKidney# = 0.0062 * BodyWt# 'litre VolIntest# = 0.0264 * BodyWt# 'litre VolLiver# = 0.0347 * BodyWt# 'litre VolLungs# = 0.0167 * BodyWt# 'litre VolMuscle# = 0.3402 * BodyWt# 'litre VolSkin# = 0.0403 * BodyWt# 'litre VolMarrow# = 0.0458 * BodyWt# 'litre TotSkin# = 6000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Normal child in rest CardOutp# = 12 * BodyWt# 'litres per hour AlvVent# = 10 * BodyWt# 'litres per hour FrAdip# = 0.05611 'fraction cardiac output rest FrBone# = 0.0239 'fraction cardiac output rest	Case 10 BodyWt# = 20 'kg obese child FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood VolBlood# = 0.08022 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.37857 * BodyWt# 'litre VolBone# = 0.05495 * BodyWt# 'litre VolBrain# = 0.07637 * BodyWt# 'litre VolHeart# = 0.00604 * BodyWt# 'litre VolKidney# = 0.00495 * BodyWt# 'litre VolIntest# = 0.02088 * BodyWt# 'litre VolLiver# = 0.02747 * BodyWt# 'litre VolLungs# = 0.01319 * BodyWt# 'litre VolMuscle# = 0.26923 * BodyWt# 'litre VolSkin# = 0.03187 * BodyWt# 'litre VolMarrow# = 0.03626 * BodyWt# 'litre TotSkin# = 6000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Obese child in rest CardOutp# = 12 * BodyWt# 'litres per hour AlvVent# = 10 * BodyWt# 'litres per hour FrAdip# = 0.05611 'fraction cardiac output rest FrBone# = 0.0239 'fraction cardiac output rest

Physiology of Subjects in InduschemFate 2.00	
FrBrain# = 0.23763 'fraction cardiac output rest FrHeart# = 0.27724 'fraction cardiac output rest FrKidney# = 0.10891 'fraction cardiac output rest FrLivVen# = 0.12146 'fraction cardiac output rest FrLivArt# = 0.03036 'fraction cardiac output rest FrLung# = 0.0239 'fraction cardiac output rest FrMuscle# = 0.04951 'fraction cardiac output rest FrSkin# = 0.02578 'fraction cardiac output rest FrMarrow# = 0.04522 'fraction cardiac output rest	FrBrain# = 0.23763 'fraction cardiac output rest FrHeart# = 0.27724 'fraction cardiac output rest FrKidney# = 0.10891 'fraction cardiac output rest FrLivVen# = 0.12146 'fraction cardiac output rest FrLivArt# = 0.03036 'fraction cardiac output rest FrLung# = 0.0239 'fraction cardiac output rest FrMuscle# = 0.04951 'fraction cardiac output rest FrSkin# = 0.02578 'fraction cardiac output rest FrMarrow# = 0.04522 'fraction cardiac output rest
Case 11 BodyWt# = 16 'kg normal child FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood VolBlood# = 0.1014 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.2148 * BodyWt# 'litre VolBone# = 0.0694 * BodyWt# 'litre VolBrain# = 0.0965 * BodyWt# 'litre VolHeart# = 0.0076 * BodyWt# 'litre VolKidney# = 0.0062 * BodyWt# 'litre VolIntest# = 0.0264 * BodyWt# 'litre VolLiver# = 0.0347 * BodyWt# 'litre VolLungs# = 0.0167 * BodyWt# 'litre VolMuscle# = 0.3402 * BodyWt# 'litre VolSkin# = 0.0403 * BodyWt# 'litre VolMarrow# = 0.0458 * BodyWt# 'litre TotSkin# = 6000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Normal child playing CardOutp# = 15 * BodyWt# 'litres per hour AlvVent# = 30 * BodyWt# 'litres per hour FrAdip# = 0.04995 'fraction cardiac output rest FrBone# = 0.02128 'fraction cardiac output rest FrBrain# = 0.21155 'fraction cardiac output rest FrHeart# = 0.26708 'fraction cardiac output rest FrKidney# = 0.09696 'fraction cardiac output rest FrLivVen# = 0.10813 'fraction cardiac output rest FrLivArt# = 0.02703 'fraction cardiac output rest FrLung# = 0.02128 'fraction cardiac output rest FrMuscle# = 0.13354 'fraction cardiac output rest FrSkin# = 0.02295 'fraction cardiac output rest FrMarrow# = 0.04025 'fraction cardiac output rest	Case 12 BodyWt# = 20 'kg obese child FrArt# = 0.3 'fraction arterial blood FrVen# = 0.7 'fraction venous blood VolBlood# = 0.08022 * BodyWt# 'litre VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood VolBlungVen# = FrVen# * VolBlood# 'volume venous blood VolAdip# = 0.37857 * BodyWt# 'litre VolBone# = 0.05495 * BodyWt# 'litre VolBrain# = 0.07637 * BodyWt# 'litre VolHeart# = 0.00604 * BodyWt# 'litre VolKidney# = 0.00495 * BodyWt# 'litre VolIntest# = 0.02088 * BodyWt# 'litre VolLiver# = 0.02747 * BodyWt# 'litre VolLungs# = 0.01319 * BodyWt# 'litre VolMuscle# = 0.26923 * BodyWt# 'litre VolSkin# = 0.03187 * BodyWt# 'litre VolMarrow# = 0.03626 * BodyWt# 'litre TotSkin# = 6000 'body surface cm2 SkinFac# = 1 'ratio skin permeability UrinFlow# = 0.0214 'litre/kgbw/day GlomFiltr# = 0.08 'fraction of renal arterial flow 'Obese child playing CardOutp# = 15 * BodyWt# 'litres per hour AlvVent# = 30 * BodyWt# 'litres per hour FrAdip# = 0.04995 'fraction cardiac output rest FrBone# = 0.02128 'fraction cardiac output rest FrBrain# = 0.21155 'fraction cardiac output rest FrHeart# = 0.26708 'fraction cardiac output rest FrKidney# = 0.09696 'fraction cardiac output rest FrLivVen# = 0.10813 'fraction cardiac output rest FrLivArt# = 0.02703 'fraction cardiac output rest FrLung# = 0.02128 'fraction cardiac output rest FrMuscle# = 0.13354 'fraction cardiac output rest FrSkin# = 0.02295 'fraction cardiac output rest FrMarrow# = 0.04025 'fraction cardiac output rest

Physiology of Subjects in InduschemFate 2.00	
Experimental animals	
Case 13 'mouse experimental study	**Case 14 'rat experimental study**
BodyWt# = 0.03 'kg average mouse	BodyWt# = 0.3 'kg average rat
FrArt# = 0.3 'fraction arterial blood	FrArt# = 0.3 'fraction arterial blood
FrVen# = 0.7 'fraction venous blood	FrVen# = 0.7 'fraction venous blood
VolBlood# = 0.09 * BodyWt# 'litre	VolBlood# = 0.0963 * BodyWt# 'litre
VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood	VolBlungArt# = FrArt# * VolBlood# 'volume arterial blood
VolBlungVen# = FrVen# * VolBlood# 'volume venous blood	VolBlungVen# = FrVen# * VolBlood# 'volume venous blood
VolAdip# = 0.09 * BodyWt# 'litre	VolAdip# = 0.07 * BodyWt# 'litre
VolBone# = 0.07 * BodyWt# 'litre	VolBone# = 0.06 * BodyWt# 'litre
VolBrain# = 0.016 * BodyWt# 'litre	VolBrain# = 0.006 * BodyWt# 'litre
VolHeart# = 0.005 * BodyWt# 'litre	VolHeart# = 0.0035 * BodyWt# 'litre
VolKidney# = 0.017 * BodyWt# 'litre	VolKidney# = 0.007 * BodyWt# 'litre
VolIntest# = 0.041 * BodyWt# 'litre	VolIntest# = 0.027 * BodyWt# 'litre
VolLiver# = 0.05 * BodyWt# 'litre	VolLiver# = 0.04 * BodyWt# 'litre
VolLungs# = 0.007 * BodyWt# 'litre	VolLungs# = 0.005 * BodyWt# 'litre
VolMuscle# = 0.36 * BodyWt# 'litre	VolMuscle# = 0.4 * BodyWt# 'litre
VolSkin# = 0.16 * BodyWt# 'litre	VolSkin# = 0.2 * BodyWt# 'litre
VolMarrow# = 0.03 * BodyWt# 'litre	VolMarrow# = 0.03 * BodyWt# 'litre
TotSkin# = 100 'body surface cm2	TotSkin# = 450 'body surface cm2
SkinFac# = 3 'ratio skin permeability	SkinFac# = 3 'ratio skin permeability
UrinFlow# = 0.05 'litre/kgbw/day	UrinFlow# = 0.2 'litre/kgbw/day
GlomFiltr# = 0.16 'fraction of renal arterial flow	GlomFiltr# = 0.16 'fraction of renal arterial flow
CardOutp# = 60 * BodyWt# 'litres per hour mouse	CardOutp# = 22 * BodyWt# 'litres per hour rat
AlvVent# = 73 * BodyWt# 'litres per hour mouse	AlvVent# = 35 * BodyWt# 'litres per hour rat
FrAdip# = 0.045 'fraction cardiac output rest	FrAdip# = 0.0454 'fraction cardiac output rest
FrBone# = 0.056 'fraction cardiac output rest	FrBone# = 0.0454 'fraction cardiac output rest
FrBrain# = 0.039 'fraction cardiac output rest	FrBrain# = 0.0227 'fraction cardiac output rest
FrHeart# = 0.079 'fraction cardiac output rest	FrHeart# = 0.0567 'fraction cardiac output rest
FrKidney# = 0.202 'fraction cardiac output rest	FrKidney# = 0.1701 'fraction cardiac output rest
FrLivVen# = 0.225 'fraction cardiac output rest	FrLivVen# = 0.1927 'fraction cardiac output rest
FrLivArt# = 0.056 'fraction cardiac output rest	FrLivArt# = 0.0272 'fraction cardiac output rest
FrLung# = 0.006 'fraction cardiac output rest	FrLung# = 0.0227 'fraction cardiac output rest
FrMuscle# = 0.18 'fraction cardiac output rest	FrMuscle# = 0.3152 'fraction cardiac output rest
FrSkin# = 0.067 'fraction cardiac output rest	FrSkin# = 0.068 'fraction cardiac output rest
FrMarrow# = 0.045 'fraction cardiac output rest	FrMarrow# = 0.034 'fraction cardiac output rest

Appendix 2: Example of simulation with PBTK-model IndusChemFate 2.00

Simulation of MTBE and MTBE-metabolites in blood and urine of males, females and rat with the IndusChemFate PBTK-model – version 2.00. Comparison with experimental measured concentrations and amounts.

Introduction

Six volunteers (3 male and 3 female) and 10 rats (5 male and 5 female) were exposed to a concentration of 40 ppm MTBE (methyl-tertiary-butylether) (= 144 mg/m^3) for 4 h in a dynamic exposure chamber (Amberg et al., 1999). The airborne MTBE concentration was determined at 15 min intervals. The measured level was 38.6 +/- 3.2 ppm = 140 mg/m^3.

In the body MTBE is metabolised to tert-butanol (Metabolite 1) . This metabolite is further metabolised, mainly to MPD (Metabolite 2), which is further oxidised to HiBA (Metabolite 3). Urine of the volunteers was collected at given intervals over 72h. Blood was sampled at the end of the 4h exposure period.

Methods

The experimental measured amounts and concentrations of MTBE and three metabolites in blood and urine was simulated with PBPK-model IndusChemFate (*version 2.00*) with the given scenario of exposure: 4 h exposure to a concentration of 140 mg/m^3. Different types of human subjects were entered.

Figure 1 shows the measured the time-course of urinary excretion of MTBE and the three main metabolites in human and rat urine.

Results

Figure 2 shows the simulation of the the time-course of urinary excretion of MTBE and the three main metabolites in a normal man in rest. HiBA (= metabolite 3) was the predominant metabolite. Fig 3 shows the variation of urinary excretion of the metabolite HiBA (= metabolite 3).

Figure 4 shows the simulation of the the time-course of urinary excretion of MTBE and the three main metabolites in rats, respectively, *assuming similar metabolism kinetics in man and rat*. Simulation in rats showed two predominant metabolites: MPD (Metabolite 2) and HiBA (Metabolite 3).

Partition coefficients of the QSPR-estimate of the simulation are presented in table 1 and 2, respectively.

Amberg et al. (1999) reported also the total amount of MTBE and metabolites excreted in urine over 72 h for man and rat.

In table 3 the experimental amounts of metabolites in urine are presented together with the model-predicted amounts for a male man in rest, an obese male in rest, a female in rest and for rats. Furthermore, blood levels were reported. The concentration of MTBE and t-butanol in blood were determined at the end of the 4-h exposure period. Table 4 shows the measured and predicted concentrations of MTBE and t-butanol in (veneous) blood of the three types of humans and of rats.

The differences within the human types are a little smaller (max 2 fold) than the difference of experimental values versus model-predicted concentration of MTBE and the metabolite t-butanol (max 4-fold).

All-over the model-predicted values are in the order of magnitude of the experimental values.

Conclusion

Although the species specific metabolism kinetics were not available, the tests of comparison of experimentally measured levels and amounts of MTBE and metabolites revealed encouraging and satisfying results.

Reference

Amberg A, Rosner E, Dekant W (1999). Biotransformation and kinetics of excretion of methyl-tert-butyl ether in rats and humans. Toxicol Sci; 51:1-8.

Figure 1. Experimentally measured data of Amberg et al 2001. MTBE and 3 metabolites in urine of humans and rats.

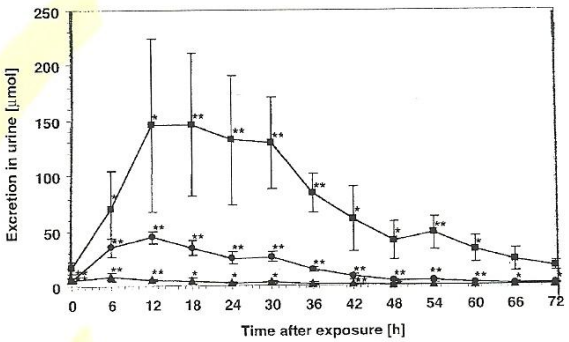

FIG. 2. Excretion in urine of 2-hydroxyisobutyrate (■), 2-methyl-1,2-propane diol (●), and t-butanol (▲) in 6 human volunteers exposed to 38.7 ± 3.2 ppm MTBE for 4 h in a dynamic exposure chamber. Numbers (mean ± SD) given represent total amounts of metabolite excreted in the urine samples collected at 6-h intervals. Each sample was analyzed in duplicate. Statistically significant differences as compared to background in controls: **, $p < 0.01$; *, $p < 0.05$.

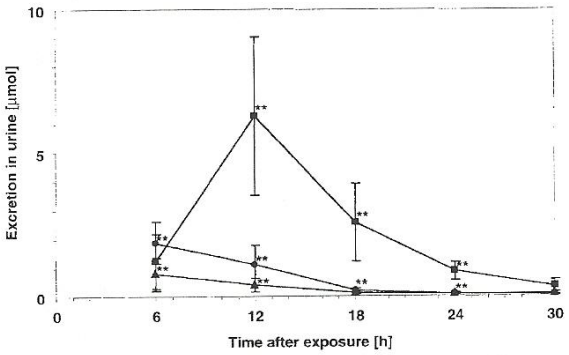

FIG. 4. Excretion in urine of 2-hydroxyisobutyrate (■), 2-methyl-1,2-propane diol (●), and t-butanol (▲) in 10 rats exposed to 38.7 ± 3.2 ppm MTBE for 4 h in a dynamic exposure chamber. Numbers (mean ± SD) given represent total amounts of metabolite excreted in the urine samples collected at 6-h intervals. Each sample was analyzed in duplicate. Statistically significant differences as compared to background in controls: **, $p < 0.01$; *, $p < 0.05$.

Figure 2. Prediction of MTBE and 3 metabolites in urine of a man in rest.

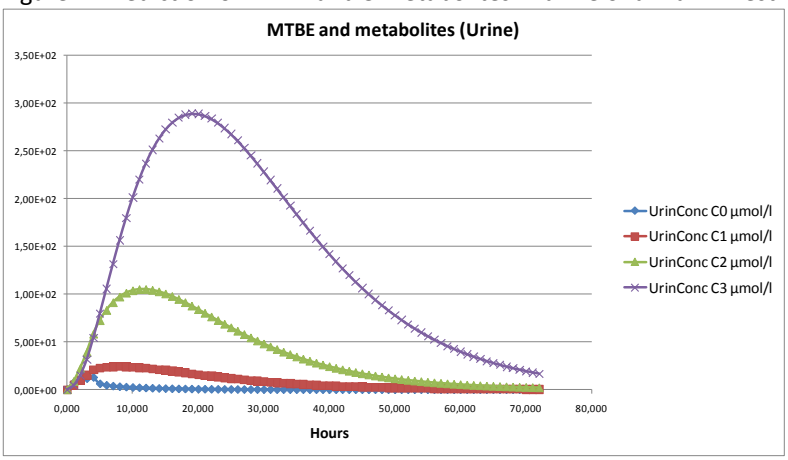

Table 1: Estimate of partition coefficients of MTBE and three metabolites in man.

Part.Coeff	MTBE (C0)	C1 (TBA)	C2 (MPD)	C3 (HiBA)
Blood/Air	2,11E+01	2,81E+03	4,09E+05	1,06E+09
Adipose tissue/Blood	8,28E+00	2,03E+00	1,00E-01	1,00E-01
Bone/Blood	9,06E-01	7,93E-01	7,43E-01	7,39E-01
Brain/Blood	1,15E+00	9,48E-01	7,99E-01	7,75E-01
Heart/Blood	9,06E-01	7,93E-01	7,43E-01	7,39E-01
Kidney/Blood	9,49E-01	8,46E-01	7,80E-01	7,71E-01
Intestine/Blood	8,03E-01	6,08E-01	5,22E-01	5,15E-01
Liver/Blood	8,03E-01	6,08E-01	5,22E-01	5,15E-01
Lung/Blood	9,06E-01	7,93E-01	7,43E-01	7,39E-01
Muscle/Blood	9,06E-01	7,93E-01	7,43E-01	7,39E-01
Skin/Blood	9,06E-01	7,93E-01	7,43E-01	7,39E-01
BoneMarrow/Blood	1,15E+00	9,48E-01	7,99E-01	7,75E-01

Figure 3. Prediction of variation of MTBE-metabolite HiBA (C3) in urine of 8 different male and female adults, normal weight and obese, in rest and with light activity.

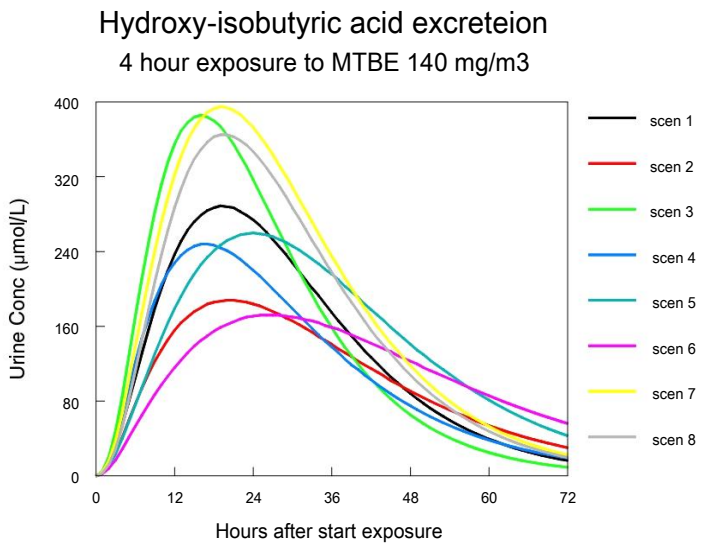

Figure 4. Prediction of MTBE and metabolites in urine of an experimental rat

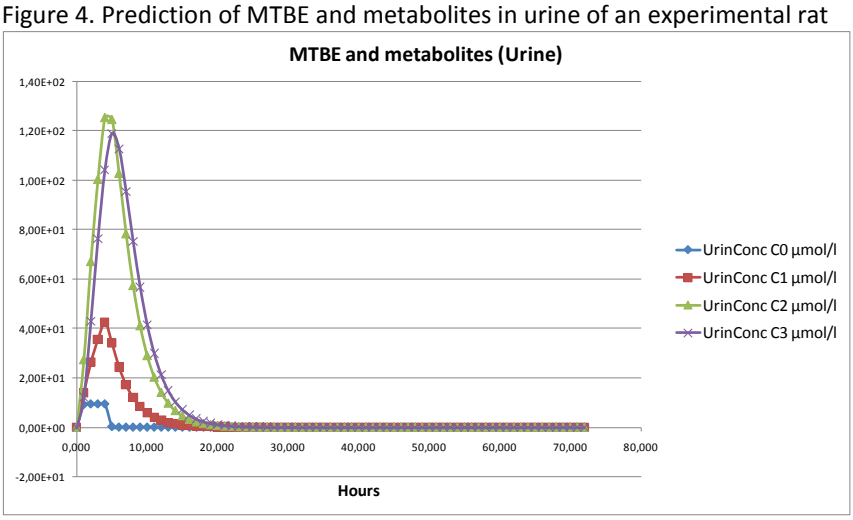

Table 2: Estimate of partition coefficients of MTBE and three metabolites in the rat.

Part.Coeff	MTBE (C0)	C1 (TBA)	C2 (MPD)	C3 (HiBA)
Blood/Air	9,30E+00	1,23E+03	1,78E+05	4,60E+08
Adipose tissue/Blood	3,01E+00	8,33E-01	5,99E-02	5,00E-02
Bone/Blood	6,68E-01	6,23E-01	5,52E-01	5,30E-01
Brain/Blood	1,17E+00	9,93E-01	7,52E-01	6,82E-01
Heart/Blood	6,68E-01	6,23E-01	5,52E-01	5,30E-01
Kidney/Blood	1,30E+00	8,53E-01	5,00E-01	5,00E-01
Intestine/Blood	1,07E+00	8,74E-01	6,18E-01	5,47E-01
Liver/Blood	1,07E+00	8,74E-01	6,18E-01	5,47E-01
Lung/Blood	6,68E-01	6,23E-01	5,52E-01	5,30E-01

Part.Coeff	MTBE (C0)	C1 (TBA)	C2 (MPD)	C3 (HiBA)
Muscle/Blood	6,68E-01	6,23E-01	5,52E-01	5,30E-01
Skin/Blood	6,68E-01	6,23E-01	5,52E-01	5,30E-01
BoneMarrow/Blood	1,17E+00	9,93E-01	7,52E-01	6,82E-01

Table 3. The excreted amount of MTBE and three MTBE-metabolites in urine after inhalation of 140 mg/m^3 over 72h. Measured and simulated values of human volunteers and experimental rats are presented.

Compound	***Human volunteers***				***Rats***	
	Measured	***PBTK Model-predicted***			***Measured***	***PBTK Model-predicted***
	Excreted amount in 72 h by a group with male & female - corrected for background) (in µmol)	***Excreted amount in 72 by normal male in rest (in µmol)***	***Excreted amount in 72 by obese male in rest (in µmol)***	***Excreted amount in 72 by normal female in rest (in µmol)***	***Excreted amount in in 72 h - corrected for background) (in µmol)***	***Excreted amount in in 72 h (in µmol***
MTBE (=C0)	2.0 +/- 1.3	5.0	6.0	2.4	n.d.	0.09
t-Butanol (TBA =C1))	29.3 +/- 9.4	37.9	43.8	18.7	0.9 +/- 0.7	0.59
2-methyl-1,2 propane-diol (MPD =C2)	205 +/- 15.6	181	200	101	2.7 +/- 1.0	2.05
2-hydroxy-isobutyrate (HiBA=C3)	722 +/- 332.6	620	555	581	9.0 +/- 2.8	2.09

Table 4. MTBE and t-butanol in blood after 4h of inhalation of 140 mg/m^3. Measured and simulated values of human volunteers and experimental rats are presented.

Substance	***Human volunteers***				***Rat***	
	Measured in blood of a group with male & female - after 4 h inhalation of 140 mg/m^3 MTBE - corrected for background) (in µmol/L)	***PBTK-model predicted in blood of normal male in rest after 4 h inhalation of 140 mg/m^3 MTBE (in µmol/L)***	***PBTK-model predicted in blood of obese male in rest after 4 h inhalation of 140 mg/m^3 MTBE (in µmol/L)***	***PBTK-model predicted in blood of normal female in rest after 4 h inhalation of 140 mg/m^3 MTBE (in µmol/L)***	***Measured in blood after 4 h inhalation of 140 mg/m^3 MTBE - corrected for background) (in µmol/L)***	***PBTK-model predicted in blood after 4 h inhalation of 140 mg/m^3 MTBE (in µmol/L)***
MTBE (=C0)	6.7 +/- 1.6	14.0	13.3	9.4	5.9 +/- 1.8	13.7
t-Butanol (TBA = C1)	20.9 +/- 3.7	5.2	4.2	3.7	35.0 +/- 10.8	13.6