Consultancy & Services

IndusTox

IndusChemFate

A PBTK-model in MS-Excel applicable for worker and consumer exposure to multiple chemicals

User manual

version 1.5

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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 tool in MS- Excel.
Three uptake routes are considered (inhalation, dermal and/or oral) as well as two built-in exercise levels. The model assumes a reference human of 70 kilograms. 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). All human physiological parameters such as blood flows, tissue dimensions, cardiac output and alveolar ventilation are adopted from reference documents. 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 model contains published and in-house developed algoritmes (QSPRs = Quantitative Structure-Property Relationships) for blood:air and tissue:blood partitioning. That is why the model can be used even when experimental partition characteristics of a compound are lacking.
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 organs/tissues 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.
Model 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 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.
This document serves two goals: It is a manual of the software-tool and it is a background document of the PBTK-model.

Content

1 Introduction

Computational toxicology is an emerging field between toxicology, chemistry, biostatistics and computer sciences. It may enhance the capacity to estimate risks associated with exposure to contaminants. 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 some published generic PBPK models

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

A drawback of some generic models is that they are not built in easily accessible software although the initiatives of Cahill et al and Beliveau et al present alternatives in MS Excel. Also the use of QSPRs (Quantitative Structure-Property Relationships) is an advantage, especially for generic models.
Generic models allows to join with the so-called tiered approach principle for risk assessment that has been introduced in the past years. 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 moment.

Demands for the new generic model

The aim of this project was to develop a *(first tier)* screening tool for data-poor substances that supports the internal exposure assessment of chemicals as a result of external exposure. The generic tool should have a high level of transparency and should require a minimum of input data. This resulting PBTK-model should be able to estimate blood- and urine concentrations of various chemicals, given a certain exposure scenario. The model should be available as freeware with a open source code.

2 Program features and limitations

The PBTK-model IndusChemFate has been developed 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 assumes a reference human of 70 kilograms. The layout of the model (figure 1) is in line with most PBPK models.

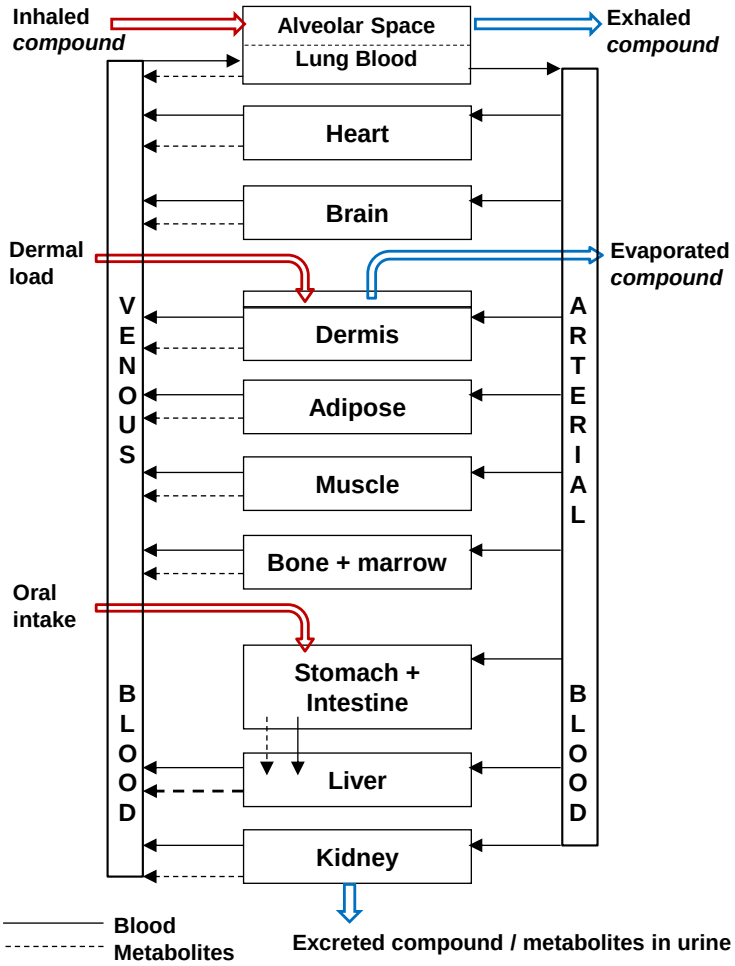


Figure 1. The outline of the PBPK-model as applied in the IndusChemFate tool

The model contains 11 body compartments (Lung, Heart, Brain, Skin, Adipose, Muscles, Bone, Bone marrow, Stomach & Intestines (lumped), Liver and Kidney). All human 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.

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 program is unprotected and can be consulted or easily adjusted and/or expanded by its users.

3 Running the model

3.1 Instruction in short

The PBTK-model IndusChemFate is programmed as a macro in Visual Basic and runs in MS Excel. The Excel-file has 4 sheets:

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

Start: After startup of the MS Excel file the macro needs to be enabled.

Entering data:
Initial settings of the modeling and the exposure conditions are entered in the sheet 'Exposure Conditions + Calculation' in column B. 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 worksheet '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. Metabolism can be modeled in all tissues or in liver only. It is recommended to start initially with only metabolism in liver by pushing the button: Metabolism only in liver.

Running the model: When all the required data of the substance and the exposure scenario are entered, press the button: Calculate. The predited 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.

3.2 Extended instructions

The initial settings of the model simulation can be determined by the parameters as displayed in table 2. 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 IndusChemFate model holds two preset levels of exercise; rest and light work (by entering 1, respectively 2). The user of the model decides what level is applicable [1]. 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 a little 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 2. Selection of simulation characteristics in the PBTK-model IndusChemFate.

Selection of model parameters	
Select model (1=hum. rest, 2=hum. light act. 3=mouse 4=rat)	**2**
Repeating exposure for how many days?	**5**
Observation settings	
Start of observation (time in hours)	**0**
End of observation (time in hours)	**168**
Number of steps per hour	**1000**
Report times per hour	**1**

3.2.1 Physiological basis of two built-in exercise levels

The PBTK-model IndusChemFate holds a large number of parameters. Most parameters have selected fixed values that have been incorporated in the model are embedded in the source code (visible via the VBA editor), others are displayed in the active worksheet.
Standardized human physiology parameters 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 default body weight is set to 70 kg. Standards for human physiological parameters were used from several reference documents [30, 32, 40-42]. The REACH

[1] The program can also be used for mice and rats. By selecting code 3 or 4, respectively. Phsiological parameters of rat and mouse were taken from the tabels of REACH Guidance 7c. The partitioning for animals is set as equal to man.
This option requires in most case a number of report times of 10.000. It should be stressed that this option with modeling of experimental animals is still in the stage of Beta-version; not sufficient tested.

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. Adopted tissue volumes and blood flows can be found in appendix 2.

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.

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].

Exercise levels

The two preset levels of exercise are rest and light work (~ 50W, up to a maximum heartbeat of 100 per minute). Table 3 shows that light exercise increases alveolar ventilation and cardiac output change significantly. Also the relative blood flows to liver and kidney decrease whereas relative blood flows to muscles increase. Relative blood flows to other model compartments change only to a very limited extent (see Appendix 2.3).

Table 3. 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

3.2.2 Entry of compound specific data

The chemical-specific input parameters for running the model IndusChemFate have to be entered in the sheet 'Database'. Data of the parent compound and the metabolite(s) of interest are needed. Table 4 lists the required compound-specific parameters for running the model.

3.2.2.1 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 pH5.5, 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 4. Field with the required input parameters of the parent substance and the primary metabolite(s) for the generic PBTK-model IndusChemFate.

	Column B
Parent Compound	
CAS	
Density (mg/cm3 or grams/litre)	
Molecular weight	
Vapour Pressure (Pa)	
Log(Kow) at skin pH 5.5	
Log(Kow) at blood pH 7.4	
Water solubility (mg/litre)	
Resorption tubuli (y/n/?)	
Enterohepatic removal (relative to liver venous blood)	
Vmax Liver (parent[total] µMol/kg/hr)	
Km Liver (parent[total] µMol/litre)	
Vmax Liver (parent[specif] µMol/kg/hr)	
Km Liver (parent[specif] µMol/litre)	
1st metabolite	
CAS	
Density (mg/cm3 or grams/litre)	
Molecular weight	
Vapour Pressure (Pa)	
Log(Kow) at skin pH 5.5	
Log(Kow) at skin pH 5.5	
Water solubility (mg/litre)	
Resorption tubuli (y/n/?)	
Enterohepatic removal (relative to liver venous blood)	
Vmax Liver (1st metab[total] µMol/kg/hr)	
Km Liver (1st metab[total] µMol/litre)	
Vmax Liver (1st metab[specif] µMol/kg/hr)	
Km Liver (1st metab[specif] µMol/litre)	

3.2.2.2 Metabolic enzymatic kinetics

The maximum velocity of metabolism (V_{max}) and the Michaelis-Menten constant (K_m) are the parameters, controlling the metabolic rate in nMols per minute per mg microsomal protein per gram organ tissue. These parameters are substance- and tissue- specific and are derived from experimental studies with animal and human tissue, mostly from the liver.
However, metabolism is not limited to the liver. Depending on the tissue(s) in which metabolism takes place, V_{max} and K_m values should be entered by the user in the proper input cells. If an input cell remains empty, metabolism is not considered. Metabolism can be

simulated in all organs of the PBTK model, but is restricted to serial reactions of the parent compound into 1st -metabolite, followed by biotransformation into the 2nd –metabolite, and so on. The metabolism pathway setting of [total] and [specific] in the model makes it possible that the formation [specific] can be set for a certain x% of [total] parent removal. To keep the mass in balance the rest fraction of (100-x)% is introduced as 'undefined metabolites'. This is needed to account the balance between metabolised, exhaled and excreted mass against absorbed mass of the substance.

As The PBTK model IndusChemFate is primarily developed for data-poor substances, the V_{max} and K_m may not be available from human data. In some cases animal data may be available although extrapolating these data to humans can be problematic. QSPRs for metabolism kinetics are available, although mostly limited to a specific group of substances [48-50]. Otherwise experimental human V_{max} and K_m values for known substances could be used in case of structure similarities. Also the availability of iso-enzymes (cytochrome P450) per tissue type [51, 52] could be a starting point when specific enzymatic biotransformation steps are known for the substance of interest or substances with a similar chemical structure.

3.2.2.3 Additional toxicokinetic input data

The additional input parameters concern the tubular resorption (or renal reabsorption) and/or enterohepatic circulation. These processes are described in detail in §4.3.2 and §4.2.2.

3.2.3 Entry of exposure conditions

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 5 shows the required exposure conditions 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 5. Exposure conditions for three routes in the generic PBTK-model IndusChemFate.

Parameters Airborne Exposure	column B
Concentration parent compound (mg/m3)	**80**
Start of airborne exposure (hours)	**0**
Duration of airborne exposure (hours)	**8**
Respiratory protection factor (=> 1)	**1**
Dermal protection factor (air tight clothing => 1)	**1**
Parameters Dermal exposure to parent compound	
Skin deposition pure substance mg/cm2/hour	**0,00E+00**
Start of skin exposure (hours)	**0**
Duration of skin exposure (hours)	**0**
Skin temperature (centigrade)	**25**
Affected skin area (cm2)	**2000**
Parameters of oral absorption	
Bolusdose to stomach of parent compound (mg/kg bwt)	**0,00E+00**
Time of application (time in hours)	**0**
Absorption rate into intestinal tissue (1/hour)	**0,4**
Selection of model parameters	
Select model (1=hum. rest, 2=hum. light act. 3=mouse 4=rat)	**1**
Repeating exposure for how many days?	**1**
Observation settings	
Start of observation (time in hours)	**0**
End of observation (time in hours)	**24**
Number of steps per hour	**1000**
Report times per hour	**1**

3.3 Model Output

3.3.1 Numerical output data

After running , a large number of time-series of output parameters is listed for both the parent compound and its metabolite(s) in the sheet 'Exposure conditions + Calculation' (table 6). This listing includes amounts, concentrations and rates in all tissues or fluids. After every simulation the output-parameters are listed in column F to CP.

Table 6 shows the listed parameters of output by default. 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.

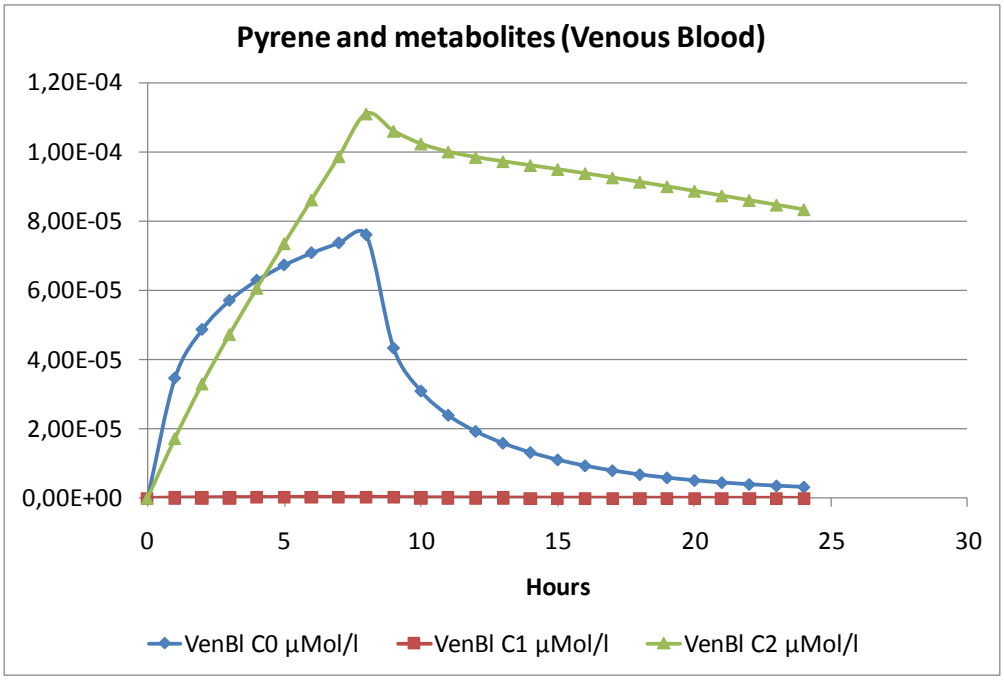
Table 6. Output parameters of The PBTK-model IndusChemFate

Model Parameter	**Unit**	**Column**
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*
Organ concentrations	(in µMole/L)	*AN to CP*

3.3.2 Graphical output of urine and blood concentration

After running the model, the predicted concentrations in blood, urine and exhaled air are presented as graphs in the sheet 'Graphical Output'. Figure 2 shows an example.

Figure 2. Example of graphical output of the PBTL model-IndusChemFate. Inhalation during 8 h , observation periode was 24 h. C0 is parent compound, C1 = first metabolite, C2 is second metabolite. VenBl = venous blood.



3.3.3 Saving simulation data

To save the input data and numerical output tables, press the button Save calculations 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 'Exp cond + Calc'). In the header time and date of saving are printed.
The results can directly be viewed by pressing the button View text file.

3.3.4 Mass balance

Additionally, the data of the mass balance of the model simulation is presented by default (as in table 7). 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 active sheet.

Table 7. Example of log table of data with estimated mass balance of a specific simulation.

Parent and	Metabolites		Parent	Absorbed	
Sum Tissues	3,16E+00	µMol/KgBwt	Sum Inhaled	1,37E+01	µMol/KgBwt
Sum Exhaled	9,14E+00	µMol/KgBwt	Sum Skin Air	6,46E-02	µMol/KgBwt
Sum Blood	4,09E-01	µMol/KgBwt	Sum Skin Liq	0,00E+00	µMol/KgBwt
Sum Urin	1,16E+00	µMol/KgBwt	Sum Oral Abs	0,00E+00	µMol/KgBwt
Sum Hep. Circ.	0,00E+00	µMol/KgBwt	Total Absorb	1,38E+01	µMol/KgBwt
Sum Metab Undef	0,00E+00	µMol/KgBwt			
Sum Total	1,39E+01	µMol/KgBwt			

3.3.5 Calculated partition coefficients

The log-file of 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 8). The log table of partition coefficients is presented under the data-listing, in the active sheet.

Table 8. Example of log table with estimated partition coefficients of a specific parent compound and two metabolites.

Part.Coeff	C0	C1	C2
Blood/Air	3.27E+06	4.15E+09	5.60E+18
Adipose tissue/Blood	1.42E+02	1.42E+02	1.00E-01
Bone/Blood	5.24E+00	5.15E+00	7.39E-01
Brain/Blood	1.44E+01	1.17E+01	7.75E-01
Heart/Blood	5.24E+00	5.15E+00	7.39E-01
Kidney/Blood	7.60E+00	6.71E+00	7.71E-01
Intestine/Blood	8.27E+00	8.12E+00	5.15E-01
Liver/Blood	8.27E+00	8.12E+00	5.15E-01
Lung/Blood	5.24E+00	5.15E+00	7.39E-01
Muscle/Blood	5.24E+00	5.15E+00	7.39E-01
Skin/Blood	5.24E+00	5.15E+00	7.39E-01
BoneMarrow/Blood	1.44E+01	1.17E+01	7.75E-01

4 Basics of the model

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.

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 has been derived in house from a comparison of experimental and estimated values of a large series number of chemicals (n=106). The resulting algoritm is using the dimensionless Henry coefficient and the octanol:air partition coefficient (Koa) as dependant variables. The total group of 106 substances was divided 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 Koa 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 9. These formulas are implemented as blood:air partitioning QSPR in the model IndusChemFate, dependant from log(Henry_DL).

Table 9. QSPR in the PBTK-model IndusChemFate to derive blood-air partition coefficients.

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

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 (Koa) is calculated from log Kow and Log(Henry_DL) using the formula: log(Koa) = log(Kow)- Log(Henry_DL).

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 3) 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.

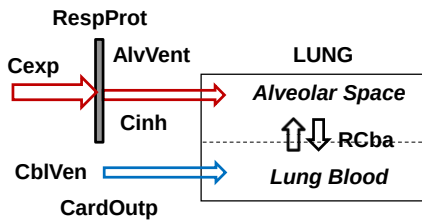


Figure 3. Use of respiratory protection

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.

Skinperm distinguishes 2 pathways of permeation through the skin; trans-cellular and inter-cellular. The physiological model of skinperm considers the following processes (figure 4):

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

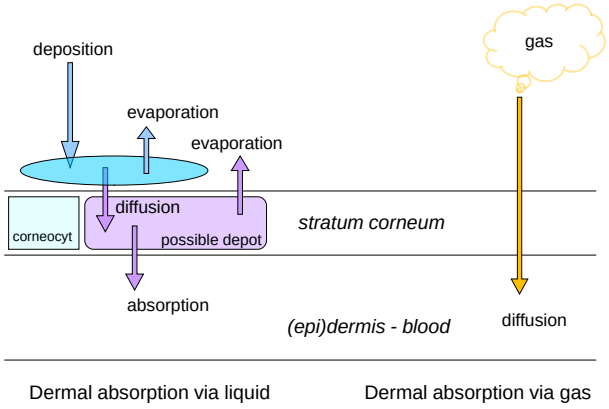


Figure 4. Schematic of dermal absorption processes in the model.

Both liquid absorption after dermal contact and absorption from the gas phase are considered.

Dermal absorption via liquids and soluble 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, as developed by ten Berge [38].

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.

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. Thus, the application of a protection factor of 2 halves the exposure concentration of the skin to air.

4.1.3 Oral & Intestinal 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 and the release rate are the required input parameters for oral uptake in the PBTK-model IndusChemFate (see table 10).

Table 10. Oral exposure parameters of the PBTK-model IndusChemFate

Parameters	***Value***
Bolusdose stomach (µMole)	
Release rate (1/hour)	

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

Once a substance is absorbed into the blood, it is distributed over the different tissues. The PBTK-model IndusChemFate holds 11 body compartments that all have their specific tissue:blood partitioning coefficient (p.c.). The different p.c. and the tissue specific (arterial) blood flows determine the supply and discharge of the substance over the body and therefore also the tissue concentrations in the various model compartments. As with blood:air partitioning, tissue:blood partitioning is substance specific. Furthermore p.c. vary from tissue to tissue. Tissue partitioning is simulated in the PBTK-model IndusChemFate by application of a QSPR as decribed by De Jongh et al [37]. This QSPR was derived from reported human and rat p.c. from organic compounds. The developed QSPR is based on the octanol:water partition coefficient (Kow) only. For every tissue a specific formula is adopted in the PBTK-model IndusChemFate based on the regression analysis as performed by De Jongh. As De Jongh presents algorithms for only 5 tissue types and the PBTK-model

IndusChemFate consists of 11 tissues, some tissues share the same partitioning algorithm (table 11). This aggregation of tissues is based on the lipid fraction of the tissue [63].

Table 11. Assumed tissue similarities for partitioning as adopted in the PBTK-model The PBTK-model IndusChemFate based on the QSPR as developed by De Jongh et al.

Tissue	***Assumed tissue similarity in the PBTK-model IndusChemFate***
Fat	Fat
Liver	Liver, Intestine
Muscle	Muscle, Bone, Heart, Lung, Skin
Kidney	Kidney
Brain	Brain, Bone marrow

When the Kow of a substance is outside the domain of the QSPR (when the equation turns into negative numbers) the Kow is set to equal the lowest measured tissue:blood value. The QSPR's algorithms are part of the model syntax. Example 1 shows the brain:blood partition coefficient algorithm.

Example 1. Algorithm of the brain:blood partition coefficient.

$$RCtisbl = \frac{0.133 * Kow^{0.48} + 0.775}{0.0056 * Kow^{0.48} + 0.83} - 0.21$$

RCtisbl = partition coefficient tissue:blood for brain tissue
Kow = octanol:water partition coefficient

No specific attention is paid to protein or plasma binding. It is expected that it is included in the used algorithms for blood/air and blood/tissue partitioning. For renal excretion the model takes into account only the fraction of the substance dissolved in water and ignores the fraction present in the blood lipids.

4.2.2 Enterohepatic circulation

Substances that undergo enterohepatic circulation are metabolized in the liver, excreted in the bile and passed to the intestinal lumen, from where they are reabsorbed across the intestinal mucosa and return to the liver via the portal circulation (figure 5) . The enterohepatic circulation generally involves compounds with a phase II metabolism step - that is conjugation in the liver to a polar group such as glucuronic acid, sulfate, taurine, glycine, or glutathione. Conjugation increases the solubility of the metabolite in bile, but the conjugated compounds are poorly absorbed by the intestinal mucosa. Enzymes produced by

intestinal bacteria—such as ß-glucuronidase, sulfatase, and various glycosidases—deconjugate these compounds, releasing the parent compounds after which these are readily reabsorbed across the intestinal wall.

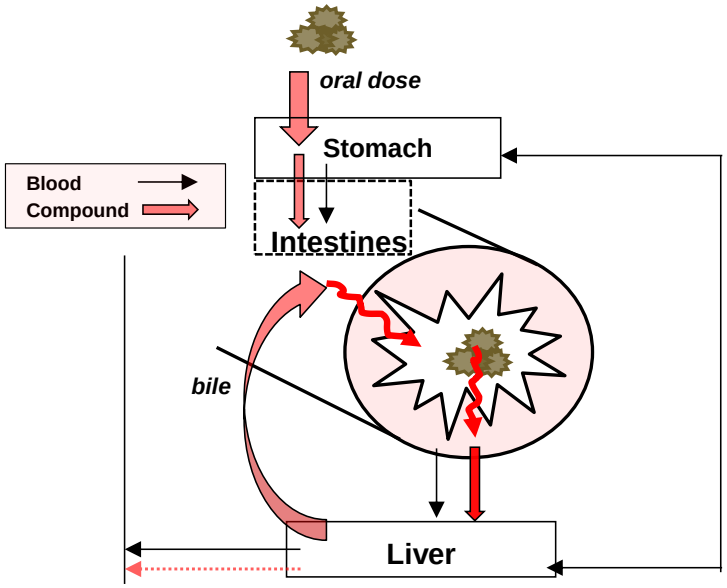


Figure 5. Schematic of the Enterohepatic circulation

Only few published PBPK model structures consider enterohepatic circulation [64, 65]. These models require experimental data for transfer rates or are based on an educated guess. The PBTK-model IndusChemFate has a different approach: it incorporates enterohepatic circulation by defining the excretion (or removal) to bile relative to excretion via the venous liver blood. 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 circulation 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 tabel 12).
Whether enterohepatic ciculation occurs in reality depends on physico-chemical properties of the substance. Fase II-metabolites of glucuronic acid start enterohepatic circulation [51, 66, 67] from a molecular weight of 325 and higher.

Table 12. 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%

4.3 Elimination

The xenobiotic to which the human body is exposed will in most cases be eliminated by metabolism (or biotransformation) and excretion. Excretion routes are exhalation and urinary excretion.

4.3.1 Metabolism

Biotransformation is described by Michaelis-Menten saturable metabolism following the mathematical algorithms as described by Ramsey and Andersen [68]. The (parent) compound is metabolized by (a set of) (iso)enzymes and usually one or more metabolite(s) are produced, possibly in different consecutive steps. Metabolites may either undergo further metabolism or will be excreted.
Contrary to many PBPK models the occurrence of metabolism is not limited to a specific model compartment, but can be considered in any (of the 11) model compartment(s). However, the default is metabolism only in liver.

Considering of metabolism in the PBTK-model IndusChemFate

The maximum velocity of metabolism (V_{max}) and the Michaelis-Menten constant (K_m) are the parameters, controlling the metabolic rate in nMols per minute per mg microsomal protein per gram organ tissue. Metabolism can be simulated in all organs of the PBTK model, but is restricted to serial reactions.
In order to gain flexibility metabolism is introduced as two different steps: (1) removal of the parent compound and (2) formation of metabolite.
The first step estimates the removal of a parent compound by metabolism based on the V_{max} and K_m of removal of parent compound (defined as “Quantity of Parent Compound Removed”). The second step estimates the appearance of a (consecutive) metabolite, depending on V_{max} and K_m values for production of the metabolite (defined as “Quantity of Compound Produced”). When parallel metabolic pathways are involved, the V_{max} and K_m values for metabolite production are different from those for removal. If not, the production and removal parameters are equal.
The model still considers that the biotransformation occurs for only x% into the metabolite of interest and for (100-x)% into other metabolites. This is needed to account the balance between metabolised, exhaled and excreted mass against absorbed mass of the substance.

Required input parameters for metabolism

The required parameters for a single metabolism step in liver are listed in table 13.

Table 13. Required metabolic parameters to run the PBTK-model IndusChemFate

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

* 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.

4.3.2 Urinary excretion

Substances can be excreted via urine, either unchanged (as parent compound), changed (as metabolite) or both. Metabolism usually makes the compound more hydrophilic and subsequently the renal clearance and thus urinary excretion are increased [66]. Not all PBPK models aim to quantify urinary excretion as the elimination of the parent compound by metabolism may be the main point of interest [65, 68, 70]. Models that do quantify renal excretion often use measured (renal clearance) data [9, 65, 71]. DeWoskin et al published a paper in which renal clearance is modeled in great detail [72]. The required input transcends application in a generic model.

The PBTK-model IndusChemFate takes into account the renal clearance of substances by means of ultrafiltration in the glomeruli and possible resorption to the blood in the tubuli (figure 6) . Tubular secretion, excretion by means of active transport, is not considered. The total renal clearance is depicted as the glomerular filtration minus the resorption in the tubuli.

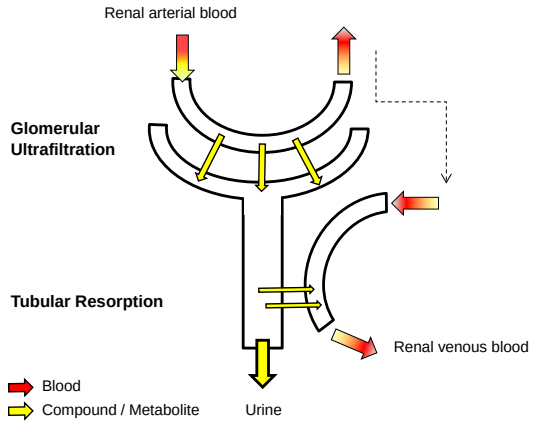


Figure 6. Schematic of renal clearance

Although scarcely considered in PBPK models for industrial chemicals, tubular resorption may require experimental data on (saturable) resorption [73]. The PBTK-model the PBTK-model IndusChemFate has adopted a rule of thumb for resorption: the user of the model can decide whether tubular resorption takes place by selecting 'yes' or 'no'. Resorption restricts the renal clearance to 1% of the glomerular filtrate. This factor (0.01) is arbitrary but in line with literature [51]. If it is unknown whether resorption occurs (user input '?'), the model selects either 'yes' or 'no' based on the log octanol-water partition coeffient (LogKow) at pH 7.4 of the substance or metabolite of interest. The cut-off is arbitrary set to a LogKow value of -1,5 at pH 7.4. This is close to the LogKow of water (-1.38). Water is always resorbed by the kidney. Very soluble substances with a LogKow < -1.5 are assumed not to be resorbed ('no' for resorption in the model, maximal renal clearance).

Example 3. pH dependence of LogKow for hippuric acid

LogKow	**Reference**
0.31	*Episuite [33]*
-1.61 (at pH 5.5)	*ACD Labs via Chemspider[34]*
-3.17 (at pH 7.4)	*ACD Labs via Chemspider[34]*

The calculation of urinary excretion also takes into account lipophilicity of substances, assuming that lipophilic substances are less water soluble and therefore excreted via urine to a lower extent. A QSPR (as developed by De Jongh et al [37]) that calculates the solubility in blood based on lipid and water fractions in blood, is therefore adopted in the syntax of the model (QSPR 2).

Example 4. QSPR 2 for the calculation of solubility in blood, based on De Jongh et al

$$FrWsol = \frac{0.993}{0.993 + 0.007 * 10^{LogKow}}$$

FrWsol = water (=blood) solubility of the compound
LogKow= octanol:water partition coefficient

De Jongh et al [37] assumes 0.7% lipids in human blood. SimCyp, an online (freely available) calculation tool also calculates the unbound fraction in plasma [74]. Table 12 shows a comparison of the calculated blood solubility for some industrial chemicals by means of the mentioned algorithms.

Table 12. Calculated solubility in blood for some compounds using different resources

Compound	***LogKow***	***Calculated solubility in blood***	
		De Jongh [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%

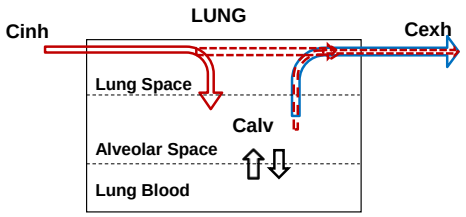
It should be noted that SimCyp intends to predict the unbound fraction of pharmaceuticals, whereas the QSPR by De Jongh mostly focus on industrial chemicals.

The glomulaire filtration rate in the the PBTK-model IndusChemFate model is set at 0.08 of the renal bloodflow [2].

The total urine production per day (24 hours) is set to 1.44 liters (based on an hourly urine production of 0.06 liter/h). The PBTK-model IndusChemFate does provide all urinary concentrations in micromole per liter urine which can be easily recalculated to any other unit, for example the often used microgram/gram creatinine of micromol /mol creatinine assuming a certain 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 7) is determined by the blood concentration in the (arterial) lung blood and the blood:air partition coefficient.



[2] Glomulaire filtration for mouse and rat is set at 0.16. Attention, the IndusChemFate PBPK-mdel for experimental animals is still at the stage of beta-version.

Figure 7. Schematic of exhalation elimination principle

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). Only the exhaled dose is calculated, not accounting for the inhaled air concentration.

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 environmental and occupational contaminants, for which inhalation and dermal exposure is the main exposure route .

4.4 Mass balance

A mass balance is an internal check for loss of mass. 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”). After every model simulation a mass balance is presented in the active worksheet. All input and output of the model is on molar basis and therefore verifiable in the mass balance.

5 Algorithms

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)

Intermittent Liquid Exposure is explained in detail in Appendix 1

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 an adult (18000 cm^2)

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)

$$\frac{dAm_{kidney}[j]}{dt} = \frac{dAm_{kidney}[j]}{dt} - 0.3 * Fr_{watersoluble}[j] * Remov_{Kidney}[j] * Q_{kidney} * C_{art}[j]$$

$Fr_{watersoluble}[j]$ = 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 (input parameter). 0.01 in case of tubular re-absorption into the blood (assumption log(Kow) at pH 7.4 > -1.5). 0.99 in the absence of tubular re-absorption into the blood (assumption log(Kow) at pH 7.4 ≤ -1.5). See also §4.2.3.

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)

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

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

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

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

$Am_{OrgVen}[j]$ = 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.

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, the PBTK-model IndusChemFate supports first tier or screening purposes, especially when little is known from the toxicokinetics of a (data-poor) compound. For the derivation of human biomonitoring equivalent guidance values (BEGV) one should realise that model outcomes are to be interpreted within an order of magnitude. Especially when the quality of input data is limited the model outcomes become more uncertain.

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Appendix 1 –QSPR Dermal absorption of liquid exposure

The dermal liquid exposure algorithm (SkinPerm) as developed by Ten Berge can be consulted additionally in a recent paper [38] and online [76].

Description of absorption into the stratum corneum and evaporation from the skin surface of liquid by intermittent contact with the skin

The dermal exposure is assumed to occur by deposition of aerosol of the liquid up on the skin. The liquid will be removed from the dermal surface by evaporation and absorption. The program takes into account the following conditions:

1. skin absorption and evaporation are fast and the skin remains dry, that means there is no liquid left on the skin.
2. skin absorption and evaporation are slow and a thin liquid film is left on the skin surface.

$$\frac{dLiqFilm_{neat}}{dt} = Deposition\ rate - Absorption\ rate(into\ SC) - Evaporation\ rate(LF) \qquad eq.\ 1$$

$$Absorption\ rate\ (into\ SC) = \left[\frac{Dens * Diff_{sc}}{h_{sc}} * \frac{M_{max} - M_{sc}}{M_{max}} \right] \qquad eq.\ 2$$

$$Diff_{sc} = \frac{Kp_{aq} * h_{sc}}{P_{sc/w}} \qquad Kp_{aq} = \frac{P_{sc/w} * Diff_{sc}}{h_{sc}} \qquad eq.\ 3$$

$$^{10}\log(Kp_{lipids}) = -2.59 + 0.732 *^{10}\log(K_{ow}) - 0.00683 * Mw \qquad eq.\ 4$$

$$^{10}\log(Kp_{corneocytes}) = -1.37 - 1.36 *^{10}\log(Mw) \qquad eq.\ 5$$

$$Kp_{aq} = Kp_{lipids} + Kp_{corneocytes} \qquad eq.\ 6$$

$$P_{sc/w} = 0.72 * K_{ow}^{0.43} \qquad eq.\ 7$$

Deposition rate, absorption rate and evaporation rate are all expressed in mg/cm^2/hour

SC = stratum corneum

Dens = density of liquid (mg/cm$^{3)}$

$Diff_{sc}$ = diffusivity of the liquid in the stratum corneum (cm^2/hour)

Kp_{aq} = overall aqueous dermal permeation coefficient through lipid and corneocyte part of stratum corneum (cm/hour)

Kp_{lipids} = aqueous permeation coefficient of lipid phase of the stratum corneum (QSPR, ten Berge 2009 [38])

$Kp_{corneocytes}$ = aqueous permeation coefficient of corneocyte phase of the stratum corneum (QSPR, ten Berge 2009)

$P_{sc/w}$ = stratum corneum/water partition coefficient (QSPR, ten Berge 2009)

K_{ow} = octanol/water partiton coefficient at the dermal pH=5.5 (pH adjustment needed for amines and acids)

Mw = molecular weight
h_{sc} = thickness stratum corneum (fixed to 0.002 cm)
M_{max} = h_{sc}*Dens (mg/cm^2, maximum possible mass is 20% of volume of stratum corneum filled with liquid)
M_{sc} = mass actually present in stratum corneum (mg/cm^2)

$$Evaporation\ rate(LF) = \frac{\beta * Mw * Vp}{R * T * 10} \qquad eq.\ 8$$

$$\beta = \frac{0.0111 * V^{0.96} * D_g^{0.19}}{\nu^{0.15} * X^{0.04}} \qquad eq.\ 9$$

$$D_g = 0.06 * \sqrt{\frac{76}{Mw}} \qquad eq.\ 10$$

Evaporation rate(LF), the evaporation from the liquid film (LF) is expressed in mg/cm^2/hour.
Mw = Molecular weight
Vp = Vapour pressure of the liquid at skin temperature in Pascal
R = Gas constant in J/Mol/°K
T = Skin temperature in °K
β = Coefficient of mass transfer in the vapour phase in meter/hour
V = Velocity of air (1080 meter/hour)
D_g = Diffusivity of the liquid in the gas phase in m2/hour
ν = Kinematic viscosity of air (0.054 m^2/hour)
X = Length of the area of evaporation (0.1 meter)

The evaporation rate(LF) as described above is part of the REACH Guidance for chemical safety assessment (REACH Guidance 2008 [77]).

Description of absorption in blood and evaporation from stratum corneum without liquid film

The basic assumption is, that the dermal absorption rate will never exceed the absorption from a saturated aqueous solution of the liquid in case of an undamaged skin. Direct and prolonged contact with lipophilic liquids may harm the lipid phase of the stratum corneum and increase the permeability of the skin.

$$Evaporation(dry\ SC) = Kp_{evap} * FrAq * W_{solub} \qquad eq.\ 11$$

$$Absorption(blood) = Kp_{aq} * FrAq * W_{solub} \qquad eq.\ 12$$

$$\frac{dM_{sc}}{dt} = (Kp_{evap} + Kp_{aq}) * FrAq * W_{solub} \qquad eq.\ 13$$

$$Kp_{evap} = \frac{1}{\frac{1}{Kp_{aq}} + \frac{K_{wa}}{K_{air}}} \qquad eq.\ 14$$

$$K_{air} = 120 * \sqrt{\frac{76}{Mw}} \qquad K_{wa} = \frac{R * T * Wsolub}{Mw * Vp} \qquad eq.\ 15$$

$$FrAq = \frac{M_{sc}}{M_{max\_aq}} \quad (If\ \ FrAq > 1\ \ then\ \ FrAq = 1) \qquad eq.\ 16$$

$$M_{max\_aq} = P_{sc/w} * h_{sc} * W_{solub} \qquad eq.\ 17$$

$$P_{sc/w} = 0.72 * Kow^{0.43} \qquad eq.\ 18$$

Evaporation (dry SC) = mg/cm^2/hour
Absorption(dermis) = mg/cm^2/hour

M_{sc} = mass actually present in stratum corneum (mg/cm^2)

Kp_{aq} = overall aqueous dermal permeation coefficient through lipid and corneocyte part of stratum corneum (cm/hour) estimated from QSPR (ten Berge 2009)

Kp_{evap} = evaporation coefficient from the stratum corneum without a film of deposited liquid on the stratum corneum (cm/hour)

K_{air} = permeation coefficient through air layer around the skin of uncovered skin (cm/hour)

K_{wa} = partition coefficient water/air needed to adapt K_{air} to permeation from stratum corneum matrix.

M_{max_aq} = maximum mass (mg/cm^2), that can be absorbed into the stratum corneum from a saturated aqueous solution. This is the mass in the stratum corneum, at which the absorption rate in the blood is maximum.

$P_{sc/w}$ = stratum corneum/water partition coefficient (QSPR, ten Berge 2009)

W_{solub} = water solubility (mg/cm^3)

In the modelling it is needed to take into account, that deposition of aerosol will not hit the same tiny surface of the skin all the time. The deposition is so small that a liquid film on the skin surface is not formed. This means that the sum of the rate of evaporation and the rate of absorption is much larger than the deposition rate. In this case the rates of evaporation and absorption become:

$$Absorption\ rate(into\ SC) = \frac{Absorption\ rate(into\ SC)}{Absorption\ rate(into\ SC) + Evaporation\ rate(LF)} * Deposition\ rate \quad eq.19$$

$$Evaporation\ rate\ (1) = \frac{Evaporation\ rate(LF)}{Absorption\ rate(into\ SC) + Evaporation\ rate(LF)} * Deposition\ rate \quad eq.20$$

$$Evaporation\ rate(2) = K_{evap} * FrAq * W_{solub} \quad eq.21$$

$$If\ \ Evaporation\ rate(1) > Evaporation\ rate(2) \quad then\ \ Evaporation\ rate = Evaporation\ rate(1) \quad eq.22$$

$$If\ \ Evaporation\ rate(2) > Evaporation\ rate(1) \quad then\ \ Evaporation\ rate = Evaporation\ rate(2) \quad eq.23$$

Appendix 2 – Model syntax in Visual Basic

The PBTK-model IndusChemFatel has been witten in Visual Basic. The syntax is not protected and can be viewed in the IndusChemFate Excel program file.
This appendix presents explanations of the main basics of the model.

A2.1 PBTK model in VB Syntax

All parameters needed for the calculations are either predefined in MS Visual Basics (VBA) or collected from the designated cells in the worksheet. The calculations are executed in 'for-next loops'. Each of these loops contain a number of calculations, for instance the QSPR that is applied for partitioning or the dermal uptake module (Skinperm). The big advantage is the speed at which the calculations (simultaneously) can be made.

The applied PBTK model itself is captured in 1 for-next loop. As the model makes calculations over a predefined time period every next (time) step results in a change (delta or d) compared to the previous step. The differential equations as summarized in chapter 5 are transcribed to Visual Basic syntax as follows:

Mathematical representation generic mass flow (eq.1 in §5.1.1):

$$\frac{dAm_{org}[j]}{dt} = Q_{org}\left(C_{art}[j] - \frac{C_{org}[j]}{R_{org/ven}[j]}\right) \quad \text{.....}$$

Visual Basic representation generic mass flow:
dAmnt(i, j) = FlowOrg (i) x (Cblart(j) – CblOrg(i,j))

A2- Table1. Explanation used abbreviation different representations model syntax

Description	***Abbreviation used***	
	Mathematical	***Visual Basic syntax***
change in mass of substance j in tissue i	$\frac{dAm_{org}[j]}{dt}$	dAmnt(i,j)
blood flow through tissue i	Q_{org}	FlowOrg (i)
arterial blood concentration of substance j	$C_{art}[j]$	Cblart(j)
tissue blood concentration of substance j in tissue i	$\frac{C_{org}[j]}{R_{org/ven}[j]}$	CblOrg(i,j)

Note: $CblOrg(I,J) = C_{Bl.Org}[j] = \frac{C_{Org}[j]}{R_{blood/air}[j]}$

Depending on the type of tissue for which the calculations are made the amount of the parent compound or metabolite can be increased (lungs – inhalation, skin – dermal absorption, stomach&intestines – oral intake) or decreased (liver – metabolic clearance, kidney – renal clearance).
The amount of a substance in a tissue therefore depends on the concentration in the tissue, the supply by arterial blood and the possible increase or decrease by absorption or excretion.
Furthermore multiple 'If – then statements' may alter calculation values for certain parameters, for instance for level of exercise (change of physiological parameters) or glomerular filtration (change of efficiency of renal clearance).

A2.2 Mass Flows

All separate calculations as described in the next paragraphs are finally linked to the PBTK model as described below.
The PBTK model calculates concentrations in blood and tissues over time, accounting for factors affecting these concentrations (increase or decrease of amount) by processes such as absorption, metabolism and excretion via (user defined) various pathways (lungs, skin, stomach&intestine, liver, kidney).

A2.2.1 Generic mass flow in organs (tissues)

Note: Applies to all tissues except kidney, stomach&intestine, liver, skin (Case I%= Else)

dAmnt#(I%, J%) = FlowOrg#(I%) * (Cblart#(J%) - CblOrg#(I%, J%))
+ dAmntMet#(I%, J%)

Where:

J%	= Substance of interest (parent or metabolite)
I%	= Tissue (organ) of interest
Amnt (I%, J%)	= Mass of substance J in tissue I - µMole
FlowOrg	= Blood flow to organ (fraction of cardiac output) - L/hour
Cblart	= Concentration of substance in arterial blood– µMole/L
CblOrg	= Concentration of substance in organ venous blood– µMole/L
AmntMet (I%, J%)	= Amount of metabolite J in tissue I - µMole

Metabolism equations are explained in greater detail in A2.6.

A2.2.2 Mass change in organ venous blood

All tissues except liver

AmntOrgVen#(J%) = AmntOrgVen#(J%) + FlowOrg#(I%) * CblOrg#(I%, J%)

Liver

AmntOrgVen#(J%) = AmntOrgVen#(J%) + (FlowOrg#(I% - 1) + FlowOrg#(I%)) * CblOrg#(I%, J%)

Where:

AmntOrgVen = amount of chemical in venous blood from organ – µMole
FlowOrg = blood flow from organ – L/hr

A2.2.3 Mass change in liver tissue

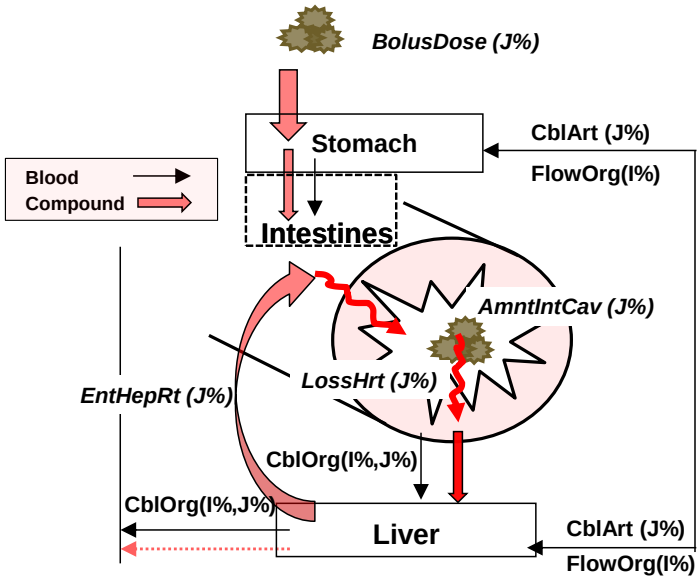
Liver (*Case I%= 7)*

dAmnt#(I%, J%) = FlowOrg#(I%) * Cblart#(J%) + FlowOrg#(I% - 1) * CblOrg#(I% - 1, J%) - (FlowOrg#(I%) + FlowOrg#(I% - 1)) * CblOrg#(I%, J%) + dAmntMet#(I%, J%) - EntHepRt#(J%) * Amnt#(I%, J%)

where:

Amnt (I%, J%) = Amount of substance J in tissue I - µMole
EntHepRt(J%) = Removal rate of mass [j] from the liver via bile (enterohepatic circulation, see next paragraph (§ A2.2.3) - 1/hour

A2.2.4 Mass change in stomach and intestines (+ hepatic circulation)



A2 –Figure 1. Schematic of Oral uptake and Enterohepatic circulation including syntax terminology

(**Case 6** : *l% = 6 = stomach & intestines)*

Oral dosing

dBolusdose#(J%) = LossHrt#(J%) * BolusDose#(J%)

BolusDose#(J%) = BolusDose#(J%) - dBolusdose#(J%) / StepNum#

Where:

BolusDose = Oral intake applied directly to the stomach - µMoles

LossHrt = Absorption rate into intestinal tissues

Enterohepatic circulation

EntHepRt#(J%) = (FlowOrg#(6) + FlowOrg#(7)) * FracHpRt#(J%) / (VolLiver# * RCtisbl#(7, J%))

Where:

EntHepRt#(J%) = Enterohepatic circulation rate – 1/hr

FracHpRt = Fraction of the mass of substance j in liver tissue that is excreted to the intestinal lumen via bile, relative to the mass of the substance in liver tissue that is excreted to blood

Mass change in intestinal lumen *(compare to eq.4 §5.1.3)*

dAmntIntCav#(J%) = EntHepRt#(J%) * Amnt#(7, J%) - 0.3 * AmntIntCav#(J%)

AmntIntCav#(J%) = AmntIntCav#(J%) + dAmntIntCav#(J%) / StepNum#

If AmntIntCav#(J%) < 0 Then AmntIntCav#(J%) = 0

Where:

AmntIntCav = Amount in intestinal lumen (internal cavity) - µMole

Mass change in intestinal tissue *(compare to eq.5 §5.1.4)*

dAmnt#(I%, J%) = FlowOrg#(I%) * (Cblart#(J%) - CblOrg#(I%, J%)) + dBolusdose#(J%)
+ dAmntMet#(I%, J%) + 0.3 * AmntIntCav#(J%)

Where:

Amnt (I%, J%) = Mass of substance J in intestinal tissue - µMole

It is assumed, that the mass in the intestinal tissue is increased by an oral dose (*BolusDose*), which is absorbed form the intestinal lumen in the intestinal tissue with the rate (*LossHrt*) and by the re-absorption of the mass from the intestinal lumen, released via the bile (*AmntIntCav*) , with a rate of 0.3 per hour.

A2.2.5 Mass change in dermal tissue

Skin (*Case I%= 10)* *(compare to eq.6 §5.1.5)*

dAmnt#(I%, J%) = FlowOrg#(I%) * (Cblart#(J%) - CblOrg#(I%, J%)) + dAmntMet#(I%, J%) + SkinFlux#(J%) + DermFact# * AmntDermAir#(J%)

where:

Skinflux = flux of dermally absorbed substance (to blood) from intermittent liquid exposure – µMole/h

AmntDermAir = dermal absorption from ambient air concentration– µMole/h

Dermal absorption is calculated by means of the SkinPerm QSPR [38] which is adopted to the VB syntax. Its algorithm is explained in greater detail in §A2.3.2 and Appendix 1.

A2.2.6 Mass change in kidney tissue

Kidney (*Case I%= 5)* *(compare to eq.10 §5.1.6)*

dUrinExcr#(J%) = 0.3 * RemovKdn#(J%) * FlowOrg#(I%) * Cblart#(J%) * FrWsol#(J%)

dAmnt#(I%, J%) = FlowOrg#(I%) * (Cblart#(J%) - CblOrg#(I%, J%)) - dUrinExcr#(J%) + dAmntMet#(I%, J%)

where:

UrineExcr	= Amount of excreted compound in urine – µMole
RemovKdn	= removal ratio of glomerular filtration = 0,01 or 1
FrWsol	= water solubility of compound

Urinary excretion equations are explained in greater detail in §A2.6.2.

A2.2.7 Mass balance

Check of mass transfers

For J% = 1 To NumSubs%

SumExh# = SumExh# + AmntExh#(J%)

SumUrin# = SumUrin# + UrinExcr#(J%)

SumCav# = SumCav# + AmntIntCav#(J%) + BolusDose#(J%)

SumBlood# = SumBlood# + AmtlungArt#(J%) + AmtlungVen#(J%)

For I% = 1 To 11

SumInt# = SumInt# + Amnt#(I%, J%) – sum in tissues

Where

SumExh = sum in exhaled air (µMole)

SumUrin = sum in excreted urine (µMole)

SumCav= sum in intestinal lumen (µMole)

SumBlood = sum in blood (µMole)

A2.3 Routes of exposure

A2.3.1 Absorption by inhalation

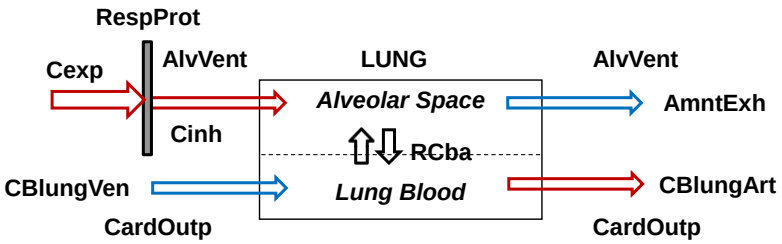
Inhalation exposure followed by uptake in the lungs increases the blood concentration in the lung tissue and consecutive distribution over the body. The arterial concentration of a substance determines the burden on the target tissues after distribution and also the venous concentration. This is calculated using the following equations (see also figure 9):

CBlungArt#(J%) = (CardOutp# * CBlungVen#(J%) + AlvVent# * Cinh#(J%)) / (CardOutp# + AlvVent# / RCba(J%))

(compare to eq.11 §5.2)

Where:

CBlungArt = Concentration of chemical (J%) in arterial blood from lung – µMole/L
CardOutp = Cardiac output – L/hr
CBlungVen = Concentration of chemical in venous blood to lungs– µMole/L
AlvVent = Alveolar ventilation – L/hr
Cinh = Inhaled concentration – µMole/L
Rcba = Blood : Air partition coefficient



A2- Figure 2. Scheme of the lungs

Calv#(J%) = CBlungArt#(J%) / RCba#(J%)

(compare to eq.12 §5.2)

Where:

Calv = Concentration of chemical (J%) in alveolar blood – µMole/L

Cinh#(J%) = Cexp#(J%) / MW#(J%) / RespProt#

Where:

Cinh = Inhaled concentration – µMole/L

Cexp = Concentration of chemical (J%) in ambient air – mg/m3
MW = Molecular Weight (J%)
RespProt = Respiratory protection factor against ambient air *(from worksheet)*

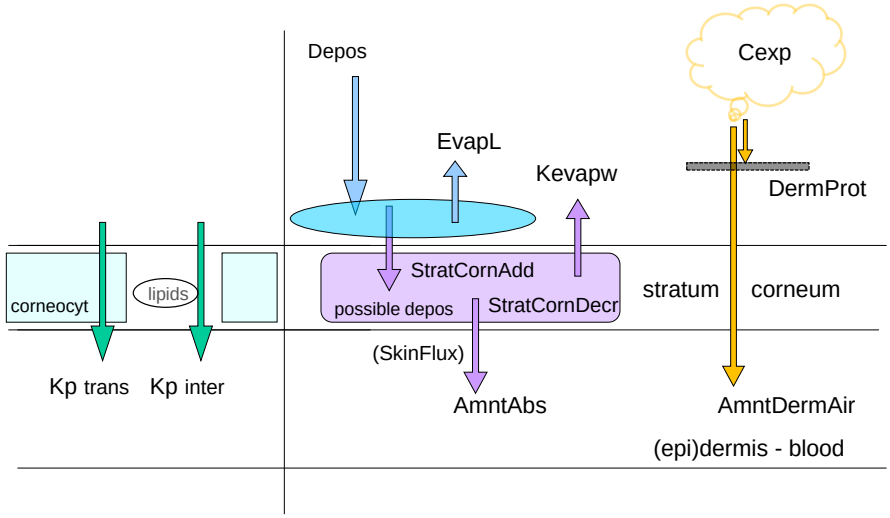
```
RespProt#      = Worksheets(Fina$).Cells(5, 2)
If RespProt# < 1# Then
RespProt# = 1#
End If

dAmntExh#(J%) = AlvVent# * Calv#(J%)
AmntExh#(J%) = AmntExh#(J%) + dAmntExh#(J%) / StepNum#
```

Where:

dAmntExh = Change in amount of exhaled chemical – µMole/hr
AmntExh = Amount of exhaled chemical – µMole
StepNum = Number of time steps per hour (every step equals 1 calculation)

A2.3.2 Absorption by the skin

Concept of the Skinperm algorithm by Ten Berge:



A2 – Figure 3. Scheme of Skinperm including syntax terminology

The following equations are used to calculate the dermal absorbed dose:

Simple initial calculations

LKPSC# = -2.59 + 0.7318 * DermLKOW#(1) - 0.006832 * MW#(1)
= Log Permeation Coefficient Stratum Corneum Intercellular [38] – cm/hour (compare to eq.4 Appendix 1)

KPSC# = 10 ^ LKPSC#
= Permeation Coefficient Stratum Corneum– cm/hour

KPOL# = 0.043 / MW#(1) ^ 1.361
= Permeation Coefficient Transcellular [38] – cm/hour

Kpw0# = KPSC# + KPOL#
= skin permeation coefficient – cm/hr

Dsco# = 0.002
= thickness stratum corneum – cm

Depi# = 0.008
= thickness epidermis – cm

Kow# = 10 ^ LKOW#(1)
= Octanol:water partition coefficient

Fpart# = 0.72 * Kow ^ 0.43
= Bunge SC partition data wet skin with corrected LKow (compare to eq.7 & 18 Appendix 1)

If Fpart# < 0.2 Then Fpart = 0.2
Assumption Fpart =0.2 for all compounds completely miscible with water

MassLoad# = 0.4
= Maximum load of SC independent of water solubility – mg/cm2

MaxCap# = Fpart# * Dsco# * Wsol#(1) / 1000

= Maximum capacity of SC independent of water solubility – mg/cm²
(compare to eq.17 Appendix 1)

If MaxCap# > MassLoad# Then MaxCap# = MassLoad#

Tlag# = Dsco# * Fpart# / Kpw0# / 6
= Lag time in hours

DermCm2# = Kpw0# * Wsol#(1) / 1000
= Maximum flux – mg/cm2/hour

Kwa# = 8.314 * (Temp# + 273) * Wsol#(1) / (MW#(1) * VpPa#(1))
= ratio concentration water solubility/concentration in vapour
(compare to eq.15 Appendix 1)

Evaporation from liquid applied to the skin (EvapL) according to TGD 2nd Ed [30]

Vw# = 1080
= velocity of air – m/h

Kva# = 0.054396
= kinematic viscosity of air - m2/h

Le# = 0.1
= length of the area of evaporation in the direction of the air stream

Dair# = 0.06 * Sqr(76 / MW#(1))
=coefficient of diffusion, gas phase – m2/h
(compare to eq.10 Appendix 1)

Beta# = 0.0111 * (Vw# ^ 0.96) * (Dair# ^ 0.19) / (Kva# ^ 0.15) / (Le# ^ 0.04)
= coefficient of mass transfer – m/hr
(compare to eq.9 Appendix 1)

EvapL# = Beta# * MW#(1) * VpPa#(1) / (8.314 * (Temp# + 273) * 10)
= evaporation substance (liquid) administered to skin – mg/cm2/hr
(compare to eq.8 Appendix 1)

Evaporation substance from dry stratum corneum (Kevapw)

Kevapw# = 1 / (1 / Kpw0# + Kwa# / Kair#)
(compare to eq.14 Appendix 1)

Where:

Kevapw = evaporation substance from dry stratum corneum – cm/hr
Kpw0 = skin permeation coefficient – cm/hr
Kwa = ratio concentration water solubility/concentration in vapour

Kair# = 120 * Sqr(76 / MW#(1))
= transfer coefficient in the gas phase – cm/hr
(compare to eq.15 Appendix 1)

Dermal absorption from ambient air exposure

Kpa0# = 1 / (1 / (Kpw0# * Kwa#) + 1 / Kair#)
= permeation coefficient from ambient air vehicle – cm/hr
(compare to eq.9 §5.1.5)

- *wearing light clothes (total skin surface ~ 18.000* cm2*) (compare to eq.8 §5.1.5)*

Select Case ExerLev%
Case 1 *(1 = at rest)*
Kair# = 36 * Sqr(76 / MW#(1))
= stagnant air layer thickness 10 cm close to skin at rest – cm/hr
Case 2 *(2 = light exercise)*
Kair# = 120 * Sqr(76 / MW#(1))
= stagnant air layer thickness 3 cm close to skin at light work– cm/hr
End Select

- *Using dermal protection equipment (air tight clothing)*

AmntDermAir#(1) = Cexp#(1) / MW#(1) * Kpa0# * 18 / DermProt#

Where:

AmntDermAir = absorbed from air through about 18000 cm2 of skin – µMoles/h
Cexp = ambient air concentration workplace – mg/m3
DermProt = Dermal protection factor by air tight clothing

```
DermProt# = Worksheets(Fina$).Cells(6, 2)
If DermProt# < 1# Then
   DermProt# = 1#
   Worksheets(Fina$).Cells(6, 2) = DermProt#
   End If
```

Dermal absorption from intermittent liquid to skin exposure

```
Select Case Tim#
Case TimSkExp# + NH% To TimSkExp# + ExpSkDur# + NH%
DeposRate# = Depos# / StepNum#
Case Else
DeposRate# = 0
End Select
```

Where:

TimSkExp = Period of skin exposure – hour
ExpSkDur = duration of dermal exposure – hour
Depos = dermal deposition rate – mg/cm2/hour

Initially absorbed substance into SC (MassInitAbs)

```
MassInitAbs# = Dens#(J%) * Kpw0# * (MassLoad# - StratCorn#) /
               MassLoad# / (Fpart#)
```

Where:

MassInitAbs = Mass of substance initially absorbed by SC by diffusion – mg/cm2/hr
Dens = density of the administered substance – mg/cm3
Kpw0 = skin permeation coefficient – cm/hr
MassLoad = maximum capacity of SC independent of water solubility – mg/cm2
StratCorn = change of mass of substance in SC – mg/cm2/hr
Fpart = ratio concentration substance in SC / water

If MassInitAbs# + EvapL# > Depos# Then MassSurf# = 0
Select Case MassSurf#

Where:

EvapL = evaporation from substance (liquid) administered to skin – mg/cm2/hr (*see initial calculations*)
MassSurf = mass present at skin surface – mg/cm2

Change of mass in Stratum Corneum

Principle: StratCorn = StratCorn + dStratCornAdd – dStratCornDecr

Where:

StratCorn = change of mass of substance in SC – mg/cm2/hr
dStratCornAdd = increase of mass in SC (by deposition) – mg/cm2/hr
dStratCornDecr = decrease of mass in SC (by absorption) – mg/cm2/hr

Case Is > 0
dStratCornAdd# = MassInitAbs# / StepNum#
dEvapReal# = EvapL# / StepNum#
dStratCornDecr# = RtScMc# * Kpw0# * Wsol#(1) / 1000 / StepNum#
Case Else
Select Case DeposRate#

Case Is > 0
dStratCornAdd# = DeposRate# * MassInitAbs# / (EvapL# + MassInitAbs#)
dStratCornDecr# = RtScMc# * Kpw0# * Wsol#(1) / 1000 / StepNum#

dEvapReal# = DeposRate# * EvapL# / (EvapL# + MassInitAbs#)
dEvapSC# = Kevapw# * RtScMc# * Wsol#(1) / 1000 / StepNum#
If dEvapReal# < dEvapSC# Then
  dEvapReal# = dEvapSC#
  dStratCornAdd# = DeposRate# - dEvapReal#
End If
Case Else
dEvapSC# = Kevapw# * RtScMc# * Wsol#(1) / 1000 / StepNum#
dEvapReal = dEvapSC#
dStratCornDecr# = RtScMc# * (Kevapw# + Kpw0#) * Wsol#(1) / 1000 / StepNum#

End Select

Where:

MassInitAbs = mass of substance initially absorbed by SC (diffusion) – mg/cm2/hr
EvapL = evaporation from substance administered to skin – mg/cm2/hr
Kevapw = evaporated substance from dry stratum corneum – cm/hr
EvapSC = evaporated substance from stratum corneum – cm/hr
EvapReal = total evaporated (cumulative) – mg/cm2
RtScMc = ratio maximum water soluble capacity of SC to retain the substance
Deposrate = deposition of substance on the skin – mg/cm2/hr

End Select
EvapReal# = EvapReal# + dEvapReal#
dMassSurf# = DeposRate# - dEvapReal# - dStratCornAdd#
MassSurf# = MassSurf# + dMassSurf#
If MassSurf# < 0 Then MassSurf# = 0
StratCorn# = StratCorn# + dStratCornAdd# - dStratCornDecr#
If StratCorn# < 0 Then StratCorn# = 0
RtScMc# = StratCorn# / MaxCap#
If RtScMc# > 1 Then RtScMc# = 1

Dermally absorbed from Stratum Corneum to bloodstream (from liquid)

dAmtAbs# = RtScMc# * Wsol#(1) * Kpw0# / 1000
AmtAbs# = AmtAbs# + dAmtAbs# / StepNum#

Where:

AmntAbs = amount of absorbed substance (to blood)– mg/cm2/hr
Kpw0 = skin permeation coefficient – cm/hr
Wsol = water solubility – mg/L

SkinFlux(1) = 1000 * dAmtAbs# * SkinArea# / MW#(1)
End Select

Where:

Skinflux = flux of absorbed substance (to blood) – µMole/h
SkinArea = exposed skin area – cm2

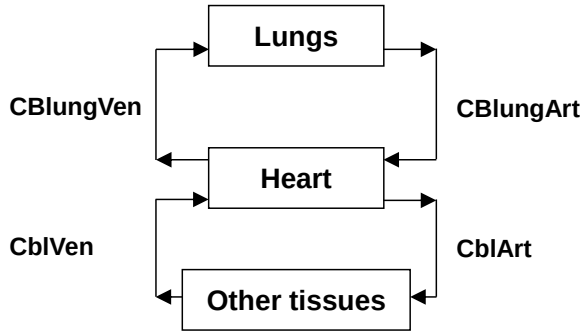
A2.3.3 Absorption after oral exposure

See §A2.2.3

A2.4 Concentrations

A2.4.1 Concentration in blood

App2 - Figure 4. Blood circulation in IndusChemFate



Arterial blood concentration

CBlungArt#(J%) = (CardOutp# * CBlungVen#(J%) + AlvVent# * Cinh#(J%)) / (CardOutp# + AlvVent# / RCba(J%)) *(compare to eq.11 §5.2)*

Calv#(J%) = CBlungArt#(J%) / RCba#(J%) *(compare to eq.12 §5.2)*

AmntlungArt#(J%) = AmntlungArt#(J%) + CardOutp# * (CBlungArt#(J%) – Cblart#(J%)) / StepNum#

Cblart#(J%) = AmntlungArt#(J%) / VolBlungArt *(compare to eq.14 §5.3)*

Venous blood concentration

Cblven#(J%) = AmtOrgVen#(J%) / CardOutp# *(compare to eq.16 §5.3)*

AmntlungVen#(J%) = AmntlungVen#(J%) + CardOutp# * (Cblven#(J%) – CBlungVen#(J%)) / stepNum# *(compare to eq.17 §5.3)*

CBlungVen#(J%) = AmntlungVen#(J%) / VolBlungVen# *(compare to eq.18 §5.3)*

Where:

J% = Chemical of interest (parent of metabolite)

CBlungArt = Concentration of chemical in arterial blood from lungs – µMole/L

CardOutp = cardiac output – L/hr

CBlungVen = Concentration of chemical in venous blood to lungs – µMole/L

AlvVen = alveolar ventilation – L/hr

Cinh = inhalation concentration – µMole/L

RCba = ratio concentration blood/air
Calv = alveolar concentration – µMole/L
Cblven = Concentration of chemical in venous blood from all organs – µMole/L
Cblart = Concentration of chemical in arterial blood from heart – µMole/L
AmntlungArt = Amount of chemical in arterial blood from heart – µMole
AmntlungVen = amount of chemical in venous blood to lungs – µMole
VolBlungVen = volume of venous blood – L

A2.4.2 Concentration in tissues

ConcOrg#(I%, J%) = Amnt#(I%, J%) / VolCmp#(I%)
CblOrg#(I%, J%) = ConcOrg#(I%, J%) / RCtisbl#(I%, J%)

Where:

ConcOrg = Concentration of chemical J in tissue I – µMole/L
CblOrg = Concentration of chemical J in tissue blood – µMole/L
RCtisbl = tissue:blood partition coefficient

Note: I% = 1,2,…11:
Organ$(1) = "Adipose tissue", Organ$(2) = "Bone", Organ$(3) = "Brain", Organ$(4) = "Heart", Organ$(5) = "Kidney", Organ$(6) = "Intestine", Organ$(7) = "Liver", Organ$(8) = "Lung", Organ$(9) = "Muscle", Organ$(10) = "Skin", Organ$(11) = "BoneMarrow"

A2.3 Human physiological parameters

The values of the physiological parameters are described in §3.1 and tabulated in table 14 and 15. The calculations below are not complete, but show the principle.

Tissue volumes

VolAdip# = 0.15 * BodyWt#

VolBone# = 0.07 * BodyWt#

Where:

VolAdip = volume of adipose tissue in liters

BodyWt = body weight = 70 kg

A2 – Table 2. Applied human physiological parameters – tissue volumes

Parameter	Abbreviation	Value
Adipose tissue volume	VolAdip	0,15
Bone tissue volume	VolBone	0,07
Brain tissue volume	VolBrain	0,021
Heart tissue volume	VolHeart	0,005
Kidney tissue volume	VolKidney	0,005
Intestines tissue volume	VolIntest	0,03
Liver tissue volume	VolLiver	0,025
Lung tissue volume	VolLungs	0,01
Muscle tissue volume	VolMuscle	0,47
Skin tissue volume	VolSkin	0,04
Bone marrow tissue volume	VolMarrow	0,02
Blood volume (arterial + venous)	VolBlood	0.09
		0,846

Blood volume

VolBlungArt# = FrArt# * VolBlood#

VolBlungVen# = FrVen# * VolBlood#

Where:

VolBlungArt = volume of arterial blood – L

VolBlungVen = volume of venous blood – L

FrArt = fraction arterial blood (=0.30)
FrVen = fraction venous blood (=0.70)

Blood flows to tissues

FlowOrg#(1) = FrAdip# * CardOutp#
FlowOrg#(2) = FrBone# * CardOutp#

Where:

FlowOrg = blood flow to tissue *X* – L/hr
FrAdip = fraction of adipose tissue
CardOutp = cardiac output – L/hr

Organ$(1) = "Adipose tissue", Organ$(2) = "Bone", Organ$(3) = "Brain", Organ$(4) = "Heart", Organ$(5) = "Kidney", Organ$(6) = "Intestine", Organ$(7) = "Liver", Organ$(8) = "Lung", Organ$(9) = "Muscle", Organ$(10) = "Skin", Organ$(11) = "BoneMarrow"

A2 – Table 3. Applied human physiological parameters – (tissue) blood flows

Parameter	**Abbreviation**	**Value at rest**	**Value light work**
Cardiac output	CardOutp	390 (absolute)	640 (absolute)
Alveolar ventilation	AlvVent	530 (absolute)	1350 (absolute)
Adipose tissue blood flow	FrAdip	0,053	0,0417
Bone tissue blood flow	FrBone	0,021	0,0128
Brain tissue blood flow	FrBrain	0,12	0,0731
Heart tissue blood flow	FrHeart	0,053	0,053
Kidney tissue blood flow	FrKidney	0,215	0,131
Liver venous blood flow	FrLivVen	0,215	0,131
Liver arterial blood flow	FrLivArt	0,053	0,0566
Lung tissue blood flow	FrLung	0,03	0,03
Muscle tissue blood flow	FrMuscle	0,15	0,3826
Skin tissue blood flow	FrSkin	0,05	0,05
Bone marrow tissue blood flow	FrMarrow	0,04	0,0381
sum		1,00	0,9999

All values are fractions of the cardiac output except the absolute values of the cardiac output and the alveolar ventilation

A2.5 Partitioning

Blood:Air partitioning

The QSPR that was developed for blood-air partitioning is described in §4.1.1. The QSPR has been derived from a comparison of experimental and estimated values of a large series of chemicals (n=106). The algoritm for P blood:air is based on the dimensionless Henry coefficient and the octanol:air partition coefficient (Koa).

A2 - Table 4. Results of the regression analysis that was used to derive blood-air partition coefficients

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

Henry coefficient - dimensionless was calculated as presented here:

Henry_DL= Vapour Pressure * Mol Weight/ (Water solubility *gas constant * Temp K)
With:
Vapour Pressure in Pa
Mol Weight in g* mol^{-1}
Water solubility in mg*L^{-1}
gas constant = 8.314472(15) in m^3 *Pa *K^{-1} *mol^{-1}
Temp K = Temp Kelvin = Temp (Celsius +273,15)

The VBA model syntax is displayed below:

```
For I% = 1 To NumSubs%
Select Case LHen(I%)
Case Is < -1
RCba#(I%) = 0.4445 * (1 / 10 ^ LHen#(I%)) + 0.005189 * 10 ^ LKoair(I%)
Case Is >= -1
RCba#(I%) = 0.8355 * (1 / 10 ^ LHen#(I%)) + 0.005804 * 10 ^ LKoair(I%)
End Select
```

Where:

LHen = Log Henry coefficient (dimensionless)
RCba = ratio concentration blood/air via QSPR
LKoair = Log octanol:air partition coefficient

Blood:Tissue partitioning (De Jongh et al [37]

```
Kow# = 10 ^ LKOW#(I%)
If Kow# < 0.1 Then Kow# = 0.1
RCtisbl#(1, I%) = (0.8 * Kow# ^ 1.03 + 0.2) / (0.0056 * Kow# ^ 1.03 + 0.83) - 0.38
```

If RCtisbl#(1, I%) < 0.1 Then RCtisbl#(1, I%) = 0.1

RCtisbl#(2, I%) = (0.031 * Kow# ^ 0.81 + 0.792) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.22
RCtisbl#(3, I%) = (0.133 * Kow# ^ 0.48 + 0.775) / (0.0056 * Kow# ^ 0.48 + 0.83) - 0.21
RCtisbl#(4, I%) = (0.031 * Kow# ^ 0.81 + 0.792) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.22
RCtisbl#(5, I%) = (0.053 * Kow# ^ 0.57 + 0.785) / (0.0056 * Kow# ^ 0.57 + 0.83) - 0.19
RCtisbl#(6, I%) = (0.049 * Kow# ^ 0.81 + 0.711) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.35
RCtisbl#(7, I%) = (0.049 * Kow# ^ 0.81 + 0.711) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.35
RCtisbl#(8, I%) = (0.031 * Kow# ^ 0.81 + 0.792) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.22
RCtisbl#(9, I%) = (0.031 * Kow# ^ 0.81 + 0.792) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.22
RCtisbl#(10, I%) = (0.031 * Kow# ^ 0.81 + 0.792) / (0.0056 * Kow# ^ 0.81 + 0.83) - 0.22
RCtisbl#(11, I%) = (0.133 * Kow# ^ 0.48 + 0.775) / (0.0056 * Kow# ^ 0.48 + 0.83) - 0.21

Where:

Kow = octanol:water partition coefficient
RCtisbl = ratio tissue:blood via QSPR

A2.6 Elimination

A2.6.1 Metabolism

The principle of metabolism in IndusChemFate is described in §4.3.1
The model first assumes the removal of the parent compound by tissue metabolism
(= Quantity Parent compound Decreased):

dAmnt#(I%, J%) = dAmnt#(I%, J%) - QntDecr# 'micromole/hour

Secondly it assumes the production of the metabolite
(= Quantity Metabolite Increased):

dAmntMet#(I%, J% + 1) = QntIncr# 'micromole/hour

where:

Amnt (I%, J%) = Amount of substance J in tissue I - µMole
AmntMet (I%, J%) = Amount of metabolite J in tissue I - µMole

Removal of the parent compound J% by tissue I%:

Retrieve Vmax and Km values from active worksheet:

```
Vmax#(I%, J%) = Worksheets(Fina$).Cells(I% + 15, 2)
Kmime#(I%, J%) = Worksheets(Fina$).Cells(I% + 16, 2)

If Kmime#(I%, J%) > 0 Then
   QntDecr# = Vmax#(I%, J%) * VolCmp#(I%) * ConcOrg#(I%, J%) / (Kmime#(I%, J%) +
              ConcOrg#(I%, J%))
   Else
   QntDecr# = 0
   End If
```

A2-Table 4. Explanation used abbreviation in different representations of model syntax for removal of parent substance [j] / [0] by biotransformation

Description	***Abbreviation used***	
	Mathematical *(eq. 1 §5.1, Parent subst (j=0))*	***Visual Basic syntax***
Maximum biotransformation in µMol/kg tissue/hour from substance [j] into subst[j+1]	$Vmax_{rem}[0]$	Vmax
Concentration substance [j] in tissue (µMol/kg), at which the biotransformation rate into substance [j+1] from substance [j] is half maximum	$Km_{rem}[0]$	Kmime
Quantity decreased (parent) compound (µMole/hr)	$\frac{Vmax_{rem}[0]*V_{org}*C_{org}[0]}{Km_{rem}[0]+C_{org}[0]}$	QntDecr
Volume of tissue I (liter)	V_{org}	VolCmp (I)
Concentration of chemical J in tissue I (µMol/kg)	C_{org}	ConcOrg (I,J)

Production of metabolite(s):

Retrieve Vmax and Km values from active worksheet:

```
 VmaxSp#(I%, J%) = Worksheets(Fina$).Cells(I% + 37, 2
KmimeSp#(I%, J%) = Worksheets(Fina$).Cells(I% + 38, 2

If KmimeSp#(I%, J%) > 0 Then
   QntIncr# = VmaxSp#(I%, J%) * VolCmp#(I%) * ConcOrg#(I%, J%) / (KmimeSp#(I%,
              J%) + ConcOrg#(I%, J%))
   Else
```

QntIncr# = 0
End If

A2- Table5. Explanation used abbreviation in different representations of model syntax for production of metabolite(s) [j=1 to 4] / [1,2,3] by biotransformation

Description	***Abbreviation used***	
	Mathematical *(eq. 2 §5.1, Metabolites (j=1 to 4))*	***Visual Basic syntax***
Maximum biotransformation in µMol/kg tissue/hour in substance [j] from substance [j-1]	$Vmax_{form}[j-1]$	VmaxSp
Concentration substance [j] µMol/kg tissue, at which the biotransformation rate into substance [j] from substance [j-1] is half of the maximum	$Km_{form}[j-1]$	KmimeSp
Quantity increased (parent) compound (µMole/hr)	$\frac{Vmax_{form}[j-1]*V_{org}*C_{org}[j-1]}{Km_{form}[j-1+C_{org}[j-1]}$	QntIncr

Calculation of amounts of (remaining) parent compound and metabolite (µMole/liter)

dAmnt#(I%, J%) = dAmnt#(I%, J%) - QntDecr#
dAmntMet#(I%, J% + 1) = QntIncr#
Amnt#(I%, J%) = Amnt#(I%, J%) + dAmnt#(I%, J%) / StepNum#
If Amnt#(I%, J%) < 0 Then Amnt#(I%, J%) = 0

A2.6.2 Urinary excretion

Renal clearance (glomerular filtration – resorption)

Select Case Resorbed$(I%)
Case "y"
RemovKdn#(I%) = 0.01 = removal fraction from glomerulus filtrate
Case "n"
RemovKdn#(I%) = 1 = removal fraction from glomerulus filtrate
Case "?"
Select Case LKOW#(I%)
Case Is < -1.5
RemovKdn#(I%) = 1 = removal ratio if LKOW#(I%)< 1.5
Case Is >= -1.5
RemovKdn#(I%) = 0.01 = removal ratio if LKOW#(I%)=> 1.5
End Select

Urinary excretion

Case 5 (= kidney) *(compare to eq.10 §5.1.6)*
dAmnt#(I%, J%) = FlowOrg#(I%) * (Cblart#(J%) - CblOrg#(I%, J%)) - dUrinExcr#(J%) + dAmntMet#(I%, J%)

dUrinExcr#(J%) = 0.08 * RemovKdn#(J%) * FlowOrg#(I%) * Cblart#(J%) * FrWsol#(J%)
UrinExcr#(J%) = UrinExcr#(J%) + dUrinExcr#(J%) / StepNum#

UrinVol# = UrinVol# + dUrinVol# / StepNum#
UrinConc#(J%) = dUrinExcr#(J%) / dUrinVol#

QSPR for calculation of water solubility (De Jongh et al [37])
FrWsol#(I%) = 0.993 / (0.993 + 0.007 * 10 ^ LKOW#(I%))

Where:

UrineExcr (J%) = urinary excretion of compound J – µMole
RemovKdn = Fraction removed from the glomerulus filtrate and excreted with urine (input parameter). 0.01 in case of tubular re-absorption into the blood (assumption log(Kow) at pH 7.4 > -1.5). 0.99 in the absence of tubular reabsorption into the blood (assumption log(Kow) at pH 7.4 ≤ -1.5).
FrWsol = water solubility of compound in arterial blood (De Jongh et al [37])
UrinVol = volume of produced urine (at 0,06 L/hr) – Liter
UrinConc = urinary concentration of compound - µMole/L

A2.6.3 Exhalation

dAmntExh#(J%) = AlvVent# * Calv#(J%)
AmntExh#(J%) = AmntExh#(J%) + dAmntExh#(J%) / StepNum#
= exhaled amount of compound J% - micromoles

A2.7 Legenda

A2 –Table 6. Legenda of variables of the VBA IndusChemFate model.

Variable in VBA syntax	Description
AlvVent	Pulmonary / Alveolar ventilation
Amnt(i,j)	Amount of substance J in tissue I
AmntDermAir(j)	Amount of substance J absorbed from air concentration by skin tissue (not from direct skin contact) – µMole
AmntIntCav(j)	Amount of substance J in intestinal lumen – µMole
AmntMet(i,j)	Amount of metabolite of substance J in tissue I – µMole
AmntOrgVen	Amount of substance in venous blood from organ – µMole
AmtAbs	Amount of dermal absorbed substance (to blood)– mg/cm2
AmtExh	Amount of substance in exhaled air – µMole
AmtlungArt	Amount of substance in arterial blood from heart – µMole
AmtlungVen	Amount of substance in venous blood to lungs – µMole
Beta	Coefficient of mass transfer (in evaporation calculation) – m/hr
BodyWt	Body Weight - kg
BolusDose	Amount of substance J that is applied directly to the stomach– µMole
Calv(j)	Concentration of substance J in alveolar air - µMol/m^3
CardOutp	Cardiac blood flow - L/h
CAS$	CAS-number
Cblart(j)	Concentration J in arterial blood– µMole/L
CblOrg(i,j)	Concentration of substance J in venous blood leaving tissue I– µMole/L
CBlungArt	Concentration of substance J in arterial blood from lung – µMole/L
CBlungVen	Concentration of substance in venous blood to lungs– µMole/L
Cblven(j)	Concentration of substance J in venous blood – µMole/L
Cexp	Concentration in ambient air – mg/m3
Chem$	Name of substance or metabolite
Cinh(j)	Concentration of substance J in inhaled air – mg/m3
ConcOrg(i,j)	Concentration of substance J in tissue I – µMole/L
Csat	Concentration in air when maximal suturated – mg/m3
Dair	coefficient of diffusion, gas phase – m2/h
dAmtAbs	Rate of dermal absorption in mg/cm2/hour

dBolusdose	Decrease of Bolusdose in intestines µMol/hour
DecrBolusRt	Absorption rate of BolusDose into intestinal tissue 1/hour
Dens	density of the administered substance – mg/cm3
Depos	deposition of substance on the skin – mg/cm2/hr
DermFact	This factor is 1 in case of airborne exposure to vapour, this factor is zero if airbornexposure is zero
DermLKow	Log(Kow) at skin pH 5.5
DermProt	Dermal protection factor by air tight clothing
Dsco	thickness stratum corneum – cm
EndTim	endtime of model simulation - hr
EntHepRt(j)	Excretion rate of substance J into bile 1/hr
EvapL	evaporation substance (liquid) deposited to skin – mg/cm2/hr
EvapReal	total evaporated (cumulative) – mg/cm2
dEvapReal	evaporated substance substance from skin mg/cm2/hr
dEvapSC	evaporated substance from stratum corneum – mg/cm2/hr
ExerLev	Exercise level (1 = rest, 2 = light work)
ExpSkDur	duration of dermal exposure to solid or liquid – hour
ExpWrDur	exposure duration ambient air in hours
FlowOrg#(1)	Blood flow to Adipose tissue – L/hr
FlowOrg#(2)	Blood flow to Bone tissue – L/hr
FlowOrg#(3)	Blood flow to Brain tissue – L/hr
FlowOrg#(4)	Blood flow to Heart tissue – L/hr
FlowOrg#(5)	Blood flow to Kidney tissue – L/hr
FlowOrg#(6)	Blood flow to Liver tissue (from intestines) – L/hr
FlowOrg#(7)	Blood flow to Liver tissue (arterial) – L/hr
FlowOrg#(8)	Blood flow to Lung tissue – L/hr
FlowOrg#(9)	Blood flow to Muscle tissue – L/hr
FlowOrg#(10)	Blood flow to Skin tissue – L/hr
FlowOrg#(11)	Blood flow to Bonemarrow tissue – L/hr
FlowOrg(i)	Blood flow to tissue I
Fpart	ratio concentration substance in SC / water (Bunge)
FrAdip	Fraction of cardiac output flowing to adipose tissue
FrArt	Fraction arterial blood relative to total blood volume
FrBone	Fraction of cardiac output flowing to bone tissue
FrBrain	Fraction of cardiac output flowing to brain tissue
FrHeart	Fraction of cardiac output flowing to heart tissue
FracHpRt(j)	Ratio between removal via bile and removal via venous blood from liver (parameter to be set) for substance (j)
FrKidney	Fraction of cardiac output flowing to kidney tissue
FrLivArt	Liver arterial blood flow as fraction of cardiac output

FrLivVen	Liver venous blood flow as fraction of cardiac output
FrLung	Fraction of cardiac output flowing to lung tissue
FrMarrow	Fraction of cardiac output flowing to bone marrow tissue
FrMuscle	Fraction of cardiac output flowing to muscle tissue
FrSkin	Fraction of cardiac output flowing to skin tissue
FrVen	Fraction venous blood relative to total blood volume
FrWsol(j)	Fraction of substance J that is dissolved in water
GlomFiltr#	Glomerular filtration as fraction of renal arterial flow (species specific)
I	Tissue I
J	substance J (parent: j=0, metabolites j=1 to 4)
Kair	permeation coefficient through air layer around the skin of uncovered skin (cm/hour)
Kevapw	evaporation coefficient from dry stratum corneum – cm/hr
Kmime(i,j)	Concentration in organ i, at which the removal rate of of substance J is half of Vmax µMoles/litre
KmimeSp(i,j)	Concentration in organ i, at which the formation rate of substance j+1 is half of Vmax µMoles/litre
Kow	Octanol :water partitioning coefficient
Kpa0	permeation coefficient of stratum corneum from substance vapour in ambient air – cm/hr
KPOL	Permeation Coefficient via the transcellular route of Stratum corneum – cm/hour
KPSC	Permeation Coefficientvia the intercellular route of Stratum corneum cm/hour
Kpw0	Total permeation coefficient of Stratum corneum – cm/hr
Kva	kinematic viscosity of air - m2/h
Kwa	ratio between the concentration in water and the concentration in air at specific temperature
Le	length of the area of evaporation in the direction of the air stream - m
Lhen	Log Henry coefficient
Lkoair	Log octanol:air partition coefficient
LKOW	Log Octanol :water partition coefficient
LKPSC	Log Permeation Coefficient via the intercellular route of Stratum Corneum
MassInitAbs	Initial absorption rate of neat substance into SC by diffusion – mg/cm2/hr
MassLoad	Maximum amount to be absorbed into SC as neat substance mg/cm2
MassSurf	mass present at skin surface – mg/cm2
MaxCap	Maximum amount in stratum corneum in equilibrium with a saturated aqueous solution of the substance – mg/cm^2
MgBolus	Instantaneous bolus dose in milligrams per kg bodyweight
MW	Molecular Weight

NumSubs	Number of substances (including metabolites)
Organ$(1)	Adipose tissue
Organ$(2)	Bone tissue
Organ$(3)	Brain tissue
Organ$(4)	Heart tissue
Organ$(5)	Kidney tissue
Organ$(6)	Intestine tissue
Organ$(7)	Liver tissue
Organ$(8)	Lung tissue
Organ$(9)	Muscle tissue
Organ$(10)	Skin tissue
Organ$(11)	Bonemarrow tissue
QntDecr	Quantity of substance removed by metabolism (intermediate parameter)
QntIncr	Quantity of produced metabolite of substance (intermediate parameter)
RCba(j)	Blood:Air partition coefficient for substance J
Rctisbl(i,j)	tissue:blood partition coefficient of substance j for tissue i
RemovKdn(j)	Removal ratio of substance J from the glomerulus filtrate by urinary excretion
Repeat$	Repeat of worker exposure for 5 consectutive days (yes/no)
Resorbed$	Resorption in tubuli kidney (yes/no)
RespProt	Respiratory protection factor against ambient air
RtScMc	ratio maximum water soluble capacity of SC to retain the substance
SkinArea	exposed skin area – cm2
SkinFac	Skinpermeability ratio rodent/human
SkinFlux(j)	Flux of absorption of neat substance J into the blood – µMole/h
StartBolus	Time of bolus application (hour)
StartTim#	Start of observation time (= 0)
StepNum	number of time steps per hour (minimum 1000) in simulation. In case of fast enzyme reactions the time steps per hour have to be increased to 10000 or 100000
StratCorn	mass of substance in SC – mg/cm2
StratCornAdd	increase of mass in SC (by deposition) – mg/cm2/hr
StratCornDecr	decrease of mass in SC (by absorption) – mg/cm2/hr
SumBlood	sum of parent and metabolites in blood – µMole
SumCav	sum of parent and metabolites in intestinal lumen – µMole
SumDermAir	sum of parent, dermally absorbed from air µMole
SumDermNeat	sum of parent , dermally absorbed from neat substance µMole
SumExh	sum of parent and metabolites in exhaled air – µMole
SumInhal	sum of parent inhaled from air µMole
SumInt	sum of parent and metabolites in tissues – µMole
SumMetabLost	sum of metabolites , not considered in output data

SumUrin	sum of parent and metabolites in excreted urine – µMole
Temp	Temperature (K)
TimSkExp	start exposure dermal deposition in hours
TimWrExp	start exposure ambient air in hours
Tlag	Lag time (dermal exposure)
TotSkin	Body surface in cm^2
UrinConc(j)	urinary concentration of substance(j) - µMole/L
UrinExcr(j)	Amount of urinary excreted substance J µMol
UrinFlow	Litre/kgbw/day, specific for mouse, rat and human
UrinVol	Volume of produced urine – L
Vmax(i,j)	Maximum velocity of metabolism for the removal of substance J in tissue I (µMoles/kg tissue/hour)
VmaxSp(i,j)	Maximum velocity of metabolism for the production of a metabolite from substance J in tissue I (µMoles/kg tissue/hour)
VolAdip	Volume of adipose tissue - L
VolBlood	Volume of blood – L
VolBlungArt	Volume of arterial blood – L
VolBlungVen	volume of venous blood – L
VolBone	Volume of bone tissue – L
VolBrain	Volume of brain tissue – L
VolCmp#(1)	Volume of adipose tissue - L
VolCmp#(2)	Volume of bone tissue – L
VolCmp#(3)	Volume of brain tissue – L
VolCmp#(4)	Volume of heart tissue - L
VolCmp#(5)	Volume of kidney tissue - L
VolCmp#(6)	Volume of intestinal tissue - L
VolCmp#(7)	Volume of liver tissue - L
VolCmp#(8)	Volume of lung tissue - L
VolCmp#(9)	Volume of muscle - L
VolCmp#(10)	Volume of skin tissue - L
VolCmp#(11)	Volume of bone marrow tissue - L
VolHeart	Volume of heart tissue - L
VolIntest	Volume of intestinal tissue - L
VolKidney	Volume of kidney tissue - L
VolLiver	Volume of liver tissue - L
VolLungs	Volume of lung tissue - L
VolMarrow	Volume of bone marrow tissue - L
VolMuscle	Volume of muscle tissue - L
VolSkin	Volume of skin tissue - L
VpPa	Vapor pressure in Pa
Vw	velocity of air – m/h
Wsol	water solubility – mg/L