

PBK modeling for beginners

Modeling Toxicokinetic and Toxicodynamics

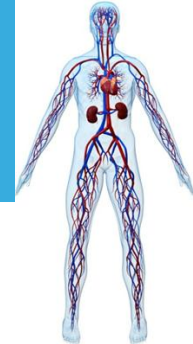
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**IHCP, ST Unit
Predictive Toxicology**

www.jrc.ec.europa.eu



11 June 2014



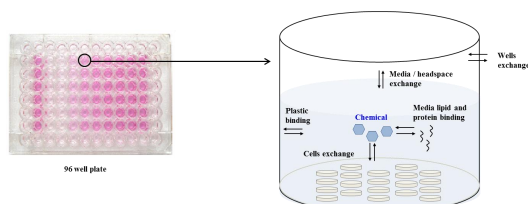
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Content

PBK modeling for beginners: Modelling Toxicokinetics & Toxicodynamics

1. Introduction to Physiologically Based Kinetic (PBK) modelling
2. Steps to build a PBK model
3. Examples of model Scripts
4. Example of human bioaccumulation model in KNIME workflow
5. Introduction to the Virtual Cell Based model
6. Show the KNIME workflow for the VCB model

Questions? Always

TOXICOKINETIC

Toxicokinetics (TK) is essentially the study of "how a substance gets into the body and what happens to it in the body". Four processes are involved in toxicokinetics:

Absorption: is the process of a substance entering the body.

Distribution: is the dispersion of substances throughout the fluids and tissues of the body.

Metabolism: is the irreversible transformation of substances and its daughter metabolism.

Excretion: is the elimination of substances from the body.

TOXICODYNAMICS

Toxicodynamics (TD): The actions and interactions of an exogenous compound within an organism, including the compound's affects on processes at the organ, cellular, and molecular levels

In order to use in vitro and in vivo kinetic & dynamic data and reduce uncertainties related to interspecies, intraspecies, high to low dose, route to route, and exposure scenarios

PBK models can be used

PBK models, in general, are developed to:

1. Integrate diverse sets of kinetics data on a particular chemical;
2. Investigate the kinetics basis of toxicity of a chemical that appears complex at the administered dose;
3. Predict tissue dosimetry for situations other than what has been or could be tested experimentally

PBK/D : physiologically based kinetic/dynamic

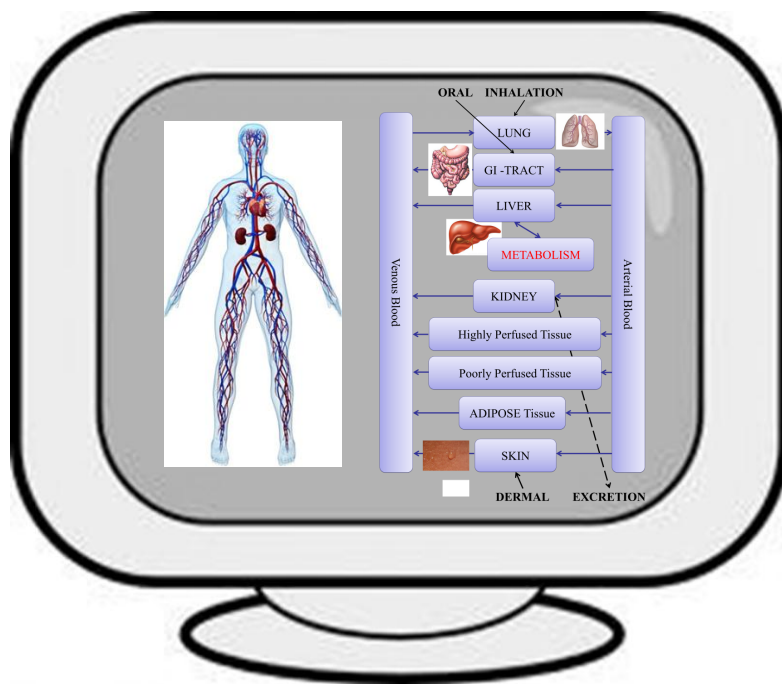
**PBTK/D : physiologically based
toxicokinetic/toxicodynamic**

**PBBK/D : physiologically based
biokinetic/biodynamic**

**PBPK/D : physiologically based
pharmacokinetic/pharmacodynamic**

DEFINITION OF PBK model

A physiologically based kinetic (PBK) model is represented as a set of mathematical equations that together describe the absorption, distribution, metabolism and excretion of a compound of interest within an organism.



Under the SEURAT 1 – Project
- within the COSMOS cluster -
we are developing
mathematical models:

Steps to build a PBK model

1. definition of the conceptual model,
2. translation into a mathematical model,
3. defining parameter values,
4. solving the equations,
5. evaluation of model performance, and
6. making predictions.

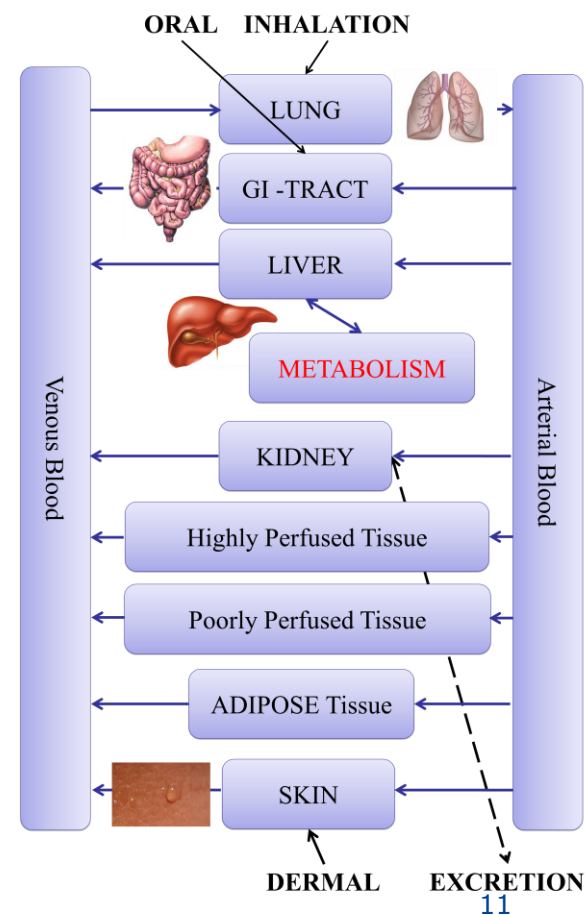
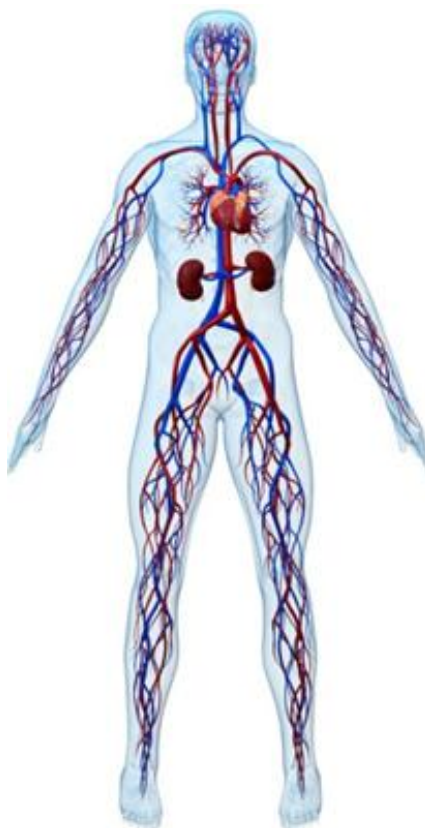
As Reported in Rietjens et al (2011)

1. definition of the conceptual model

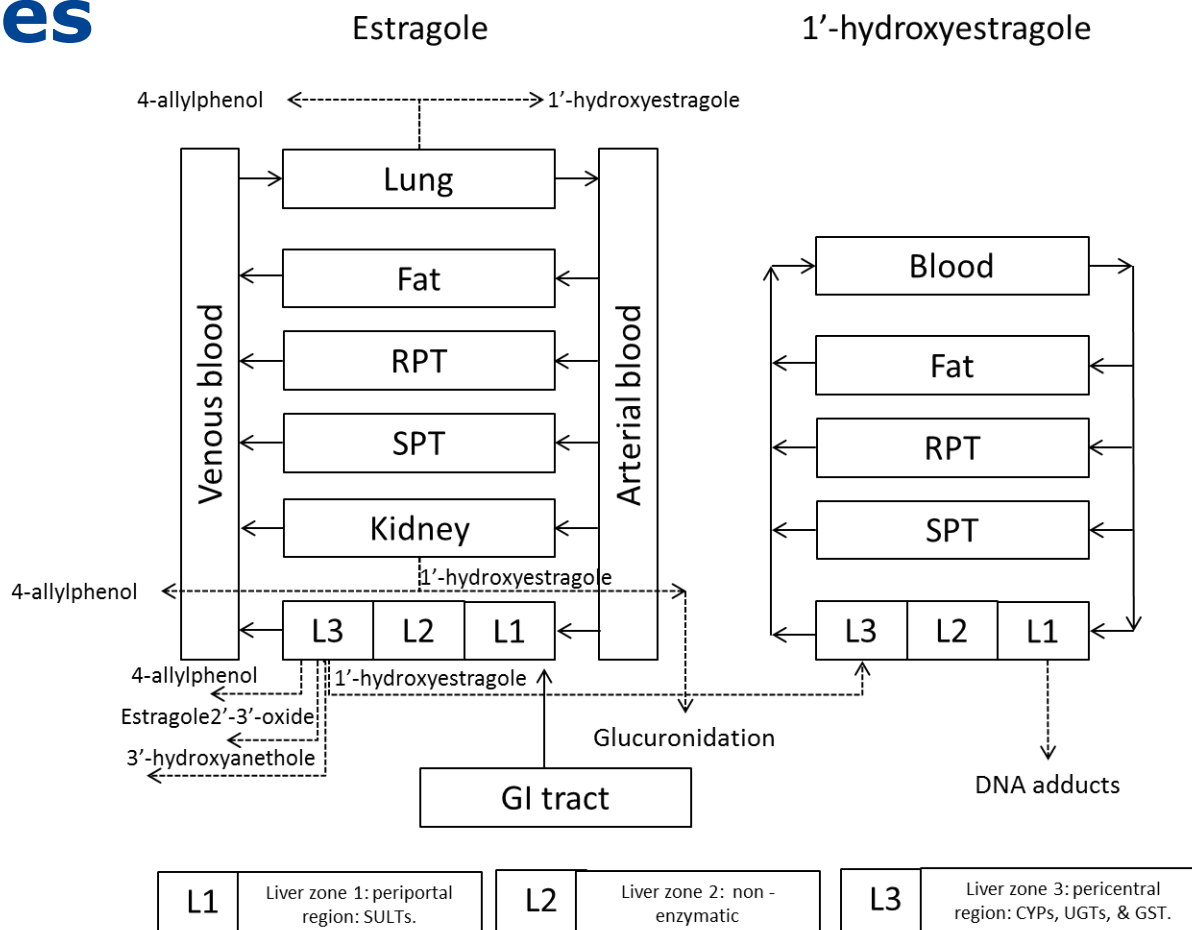
Example Input: Dermal dose

PBK model
calculates

Example Output: Concentration at
which the toxic metabolite is formed
in relevant tissue



1. Definition of the conceptual model, examples



2. Translation into a mathematical model

Absorption (is the process of a substance entering the body)

Oral

ORAL UPTAKE IS DESCRIBED AS A FIRST ORDER PROCESS:

$$\frac{dA_o}{dt} = K_o * A(stom)$$

Dermal

DERMAL UPTAKE IS DESCRIBED BY A MASS BALANCE DIFFERENTIAL

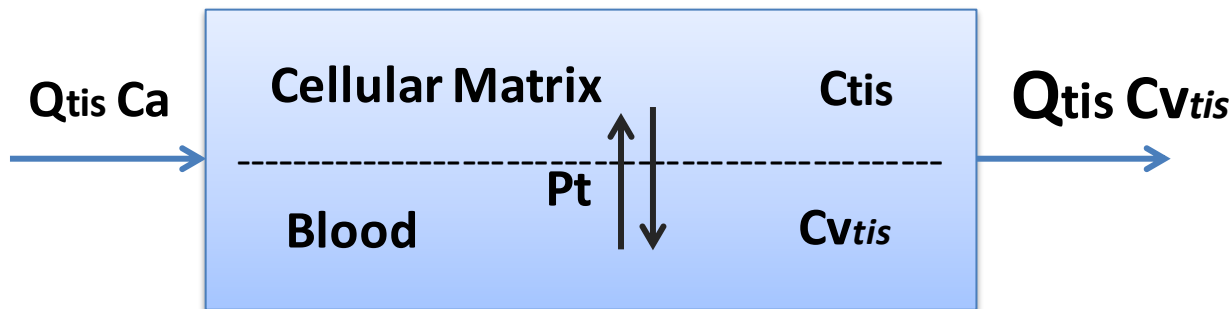
EQUATION:
$$\frac{dC_{skin}}{dt} = \frac{(Q_{skin} (C_a - C_{vSkin}) + K_p * A [C_{water} - (\frac{C_{skin}}{P_{skin:enc}})])}{V_{skin}}$$

Inhalation

INHALATION UPTAKE:
$$C_a = \frac{Q_p C_{inh} + Q_c C_v}{Q_c + \frac{Q_p}{P_b}}$$

2. Translation into a mathematical model

Distribution (is the dispersion of substances throughout the fluids and tissues of the body)



$$V_{tis} \frac{dC_{tis}}{dt} = Q_{tis} * (C_a - C_v)$$

2. Translation into a mathematical model

Metabolism: (is the irreversible biotransformation of substances and its daughter metabolism in the biota)

This process can occur in three different ways, as:

First order:
$$\frac{dA_{met}}{dt} = K_f C_v V_{tis}$$

Second order:
$$\frac{dA_{met}}{dt} = K_s C_v V_{tis} C_{cof}$$

or Saturable:
$$\frac{dA_{met}}{dt} = \frac{V_{max} C_{vt}}{K_m + C_{vt}}$$

K_f = first order metabolism constant (h⁻¹);

C_{cf} = is the concentration of the cofactor in tissue, tis;

V_{tis} = is the volume in of the tissue;

K_s = is the second order metabolism constant (L/mg/h);

V_{max} = is the maximum velocity of enzymatic reaction (mg/h);

and K_m is the Michaelis – Menten affinity constant (mg/L)

2. Translation into a mathematical model

Excretion: is the elimination of substances from the body

Urinary excretion is modelled as function of the rates of filtration, reabsorption, and secretion.

This amount of chemical filtered (dF/dt) equals the glomerular filtration rate (GFR) and the blood concentration of unbound chemical (C_u):

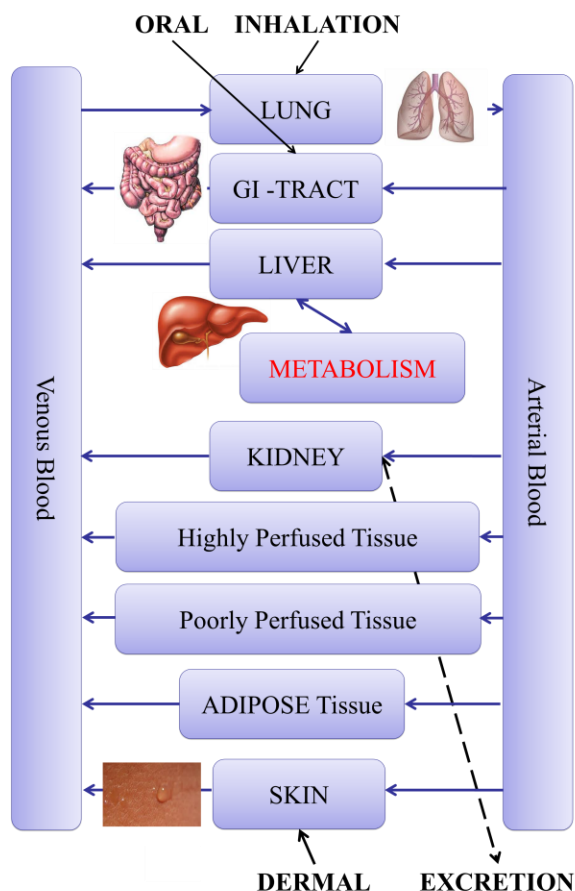
$$dF/dt = GFR * C_u$$

The rate of change in the concentration of chemicals or its metabolite in the urine (dU/dt) equals the following:

$$dU/dt = U_o * C_u$$

where U_o is the urinary output (mL/min) and C_u is the chemical concentration in urine (mg/mL).

3. Defining parameter values



Parameters needed for the model:

- 1. Physiological parameters (cardiac output, tissue blood flow rate, tissue volumes)**
- 2. Physicochemical parameters (partition coefficients)**
- 3. Kinetic and Biochemical parameters (describing metabolic processes, rates of absorption, biotransformation) - Metabolism in a realistic biological environment**
- 4. Data on fate of chemical (accumulation, binding, side metabolism, subsequent reactions)**
- 5. Effect of vehicle**

These Parameters are obtained via literature, experimentally (in vitro or in vivo), using other in silico tools QSARs

4. Solving the equations

R: <http://www.r-project.org/>

Berkeley Madonna: <http://www.berkeleymadonna.com/>

MatLab: <http://www.mathworks.com/>

For the examples presented today we implemented the PBK and VCB Models as an open source platform using Knime, MySQL and R programs (which are all freely available).

Knime (<http://www.knime.org/>);

MySQL (<http://www.mysql.com/products/>);

R (<http://www.r-project.org/>).

5. Evaluation of model performance

Model evaluation is done by comparing the model predictions against experimental in vivo data (if possible).

A sensitivity analysis is important to perform and provides a quantitative evaluation of how the input parameters of the model influence the model output.

One input parameter is changed (1 or 5 %) the other parameters are kept at initial value.

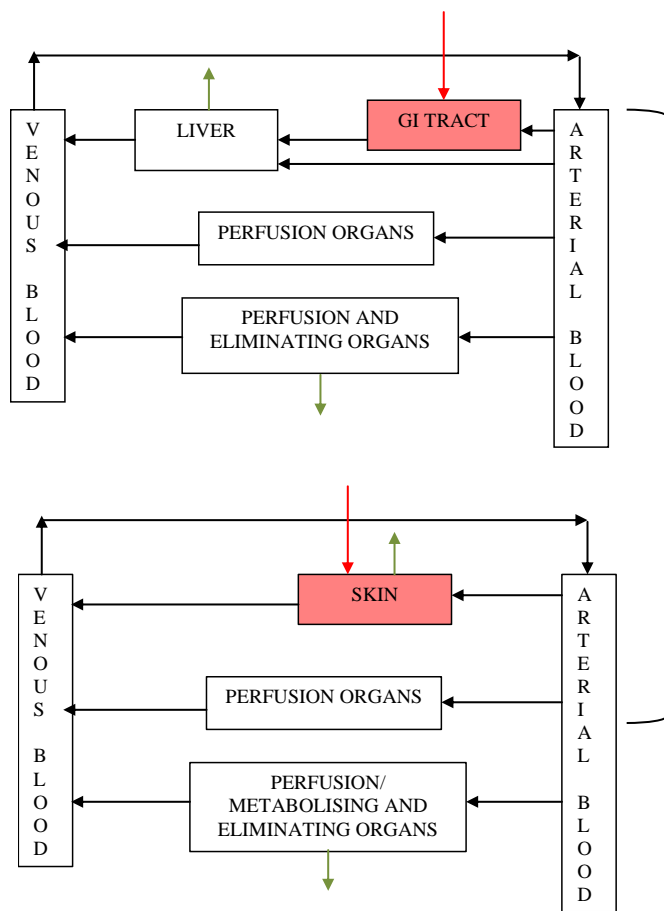
Example input parameter is changed (1%) and this generates a sensitivity coeff. of 0.8 = 0.8% change in the input parameter.

> 0.1 in absolute value = parameter that influences the model output.

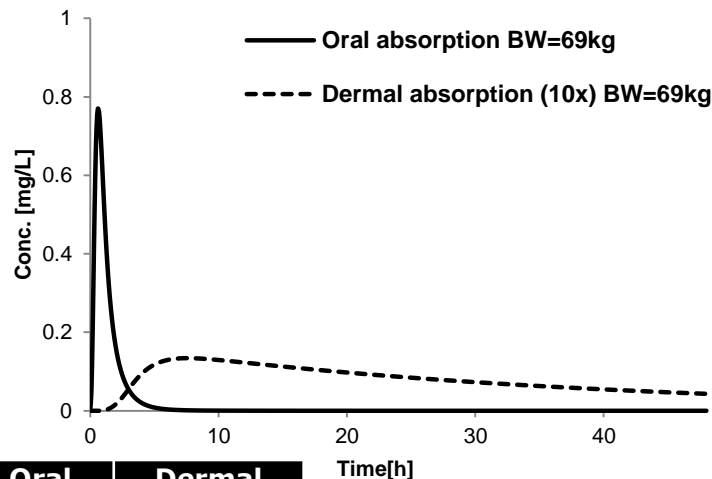
6. Making predictions



Hydroquinone – oral to dermal extrapolation



Oral NOEL dose



	Oral	Dermal
Simulation time	24h	24h
Exposure time	-	2h
Administration type	Drinking rate	-
Vehicle type	Water	Cream
Conc. of formulation	-	16mg/mL
Occlusion	-	Yes
Skin Area	-	2*448cm ²

AUC liver
oral 1.322
 dermal 0.022
AUC blood
oral 1.004
 dermal 0.463

Cmax liver
oral 1.579
 dermal 7e-04
Cmax blood
oral 0.769
 dermal 0.0134

Example & Exercises

```

Berkeley Madonna - Equations - [modelrat_final_ap_230609 - Equations]
File Edit Flowchart Model Compute Graph Parameters Window Help

Run

=====
;Biochemical parameters
=====

;Linear uptake rate (hr-1)
Ka = 1

;-----
;Metabolism liver phase I
;maximum 1hydroxyestragole rate estragole in liver = nmol min-1 mg mic. protein-1
VmaxLHEc=1.48
;maximum O-demethylation rate estragole in liver = nmol min-1 mg mic. protein-1
VmaxLAPc=0.85
;maximum epoxidation rate estragole in liver = nmol min-1 mg mic. protein-1
VmaxLEE=2.16
;maximum 3hydroxylation rate estragole in liver = nmol min-1 mg mic. protein-1
VmaxLHAc=1.05

VmaxLHE=VmaxLHEc/1000*60*MPL*L*BW
VmaxLAP=VmaxLAPc/1000*60*MPL*L*BW
VmaxLEE=VmaxLEE/1000*60*MPL*L*BW
VmaxLHA=VmaxLHAc/1000*60*MPL*L*BW

KmLHE=116 ;km 1hydroxylation estragole (umol/L)
KmLAP=458 ;km o-demethylation estragole (umol/L) leading to 4-allylphenol (AP)
KmLEE=154 ;km epoxidation estragole (umol/L) leading to estragole 2'-3'-oxide (EE)
KmLHA=93 ;km 3hydroxylation estragole (umol/L) leading to 3-hydroxyestragole (HA)

;-----
;Metabolism liver phase II
VmaxLHESc=0.019 ; nmol min-1 mg s9
VmaxLHEGc=7 ; nmol min-1 mg s9

VmaxLHES=VmaxLHESc/1000*60*S9PL*L*BW
VmaxLHEG=VmaxLHEGc/1000*60*S9PL*L*BW

KmHES=63 ;(umol/L)
KmHEG=137

;-----
;Metabolism Kidney phase I
VmaxKHEc=0.26 ;maximum 1hydroxyestragole rate estragole in Kidney = nmol min-1 mg s9 kidney
VmaxKAPc=0.54 ;maximum O-demethylation rate estragole in Kidney = nmol min-1 mg s9 kidney

VmaxKHE=VmaxKHEc/1000*60*MPK*K*BW
VmaxKAP=VmaxKAPc/1000*60*MPK*K*BW

KmKHE=22 ;km 1hydroxylation estragole (umol/L)
KmKAP=0.5 ;km o-demethylation estragole (umol/L) leading to 4-allylphenol (AP)

;-----
;Metabolism Lung phase I
VmaxLuHEc=0.44 ;maximum 1hydroxyestragole rate estragole in Lung = nmol min-1 mg s9 Lung
VmaxLuAPc=0.67 ;maximum O-demethylation rate estragole in Lung = nmol min-1 mg s9 Lung

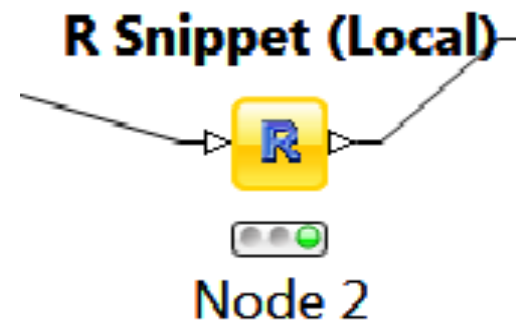
VmaxLuHE=VmaxLuHEc/1000*60*MPLu*Lu*BW
VmaxLuAP=VmaxLuAPc/1000*60*MPLu*Lu*BW

KmLuHE=25 ;km 1hydroxylation estragole (umol/L)

```

Example of human bioaccumulation model in KNIME workflow





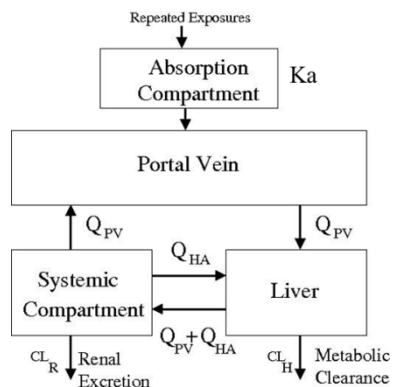
**KNIME is a user-friendly graphical
workbench for analysis process**

Screening of chemicals for human bioaccumulative potential with a physiologically based toxicokinetic model

Arnaud Tonnelier,^{1,2} Sandra Coecke,¹ and José-Manuel Zaldivar^{3,1}

2. PBTK model

A generic model!
Hepatic metabolic clearance,
protein binding and renal
excretion!



$$V_{PV} \frac{dC_{PV}}{dt} = Q_{PV} (C_{sys} - C_{PV}) + D/T$$

$$V_{liv} \frac{dC_{liv}}{dt} = Q_{PV} (C_{PV} - C_{liv}) + Q_{HA} (C_{sys} - C_{liv}) - CL_H C_{liv}$$

$$V_{sys} \frac{dC_{sys}}{dt} = Q_{PV} (C_{liv} - C_{sys}) + Q_{HA} (C_{liv} - C_{sys}) - CL_R C_{sys}$$

$$C_{sys}^* = \left(\frac{D}{T} \right) / \left(CL_H + Fu * QF * \left(1 + \frac{CL_H}{Q_{HA}} \right) \right)$$

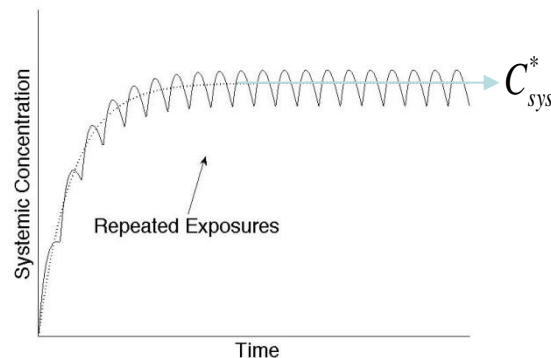
$$hBCF = \frac{C_{sys}^*}{D/T} V_{PV} / t$$

1. Background Information- Aim

To develop a predictive tool for human bioaccumulation risk assessment that incorporates not only the chemical properties of the compounds, but also metabolism.

3. Bioaccumulation

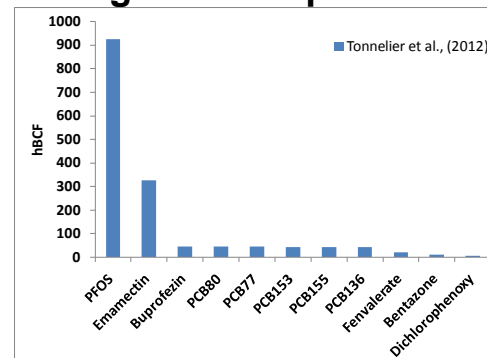
Steady-state blood concentration of the chemical in the systemic circulation!
The ratio Dose/Time mimics a constant flow and may be seen as the result of a constant exposure scenario!



4. Results

Simulations results
94 compounds selected for simulation

Highest 10 reported!!!



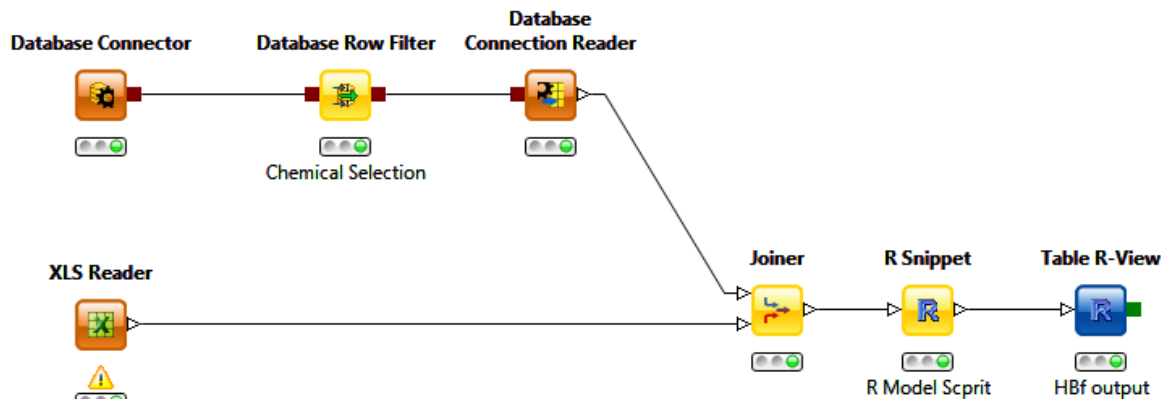
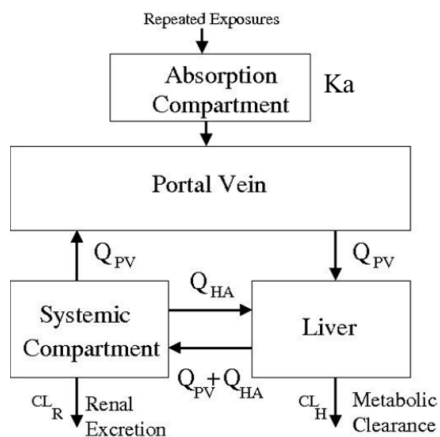
Human Bioaccumulation model

We implement the developed PBK model by Tonnelier et al., (2012) as an open source in KNIME.

R Snippet (Local)



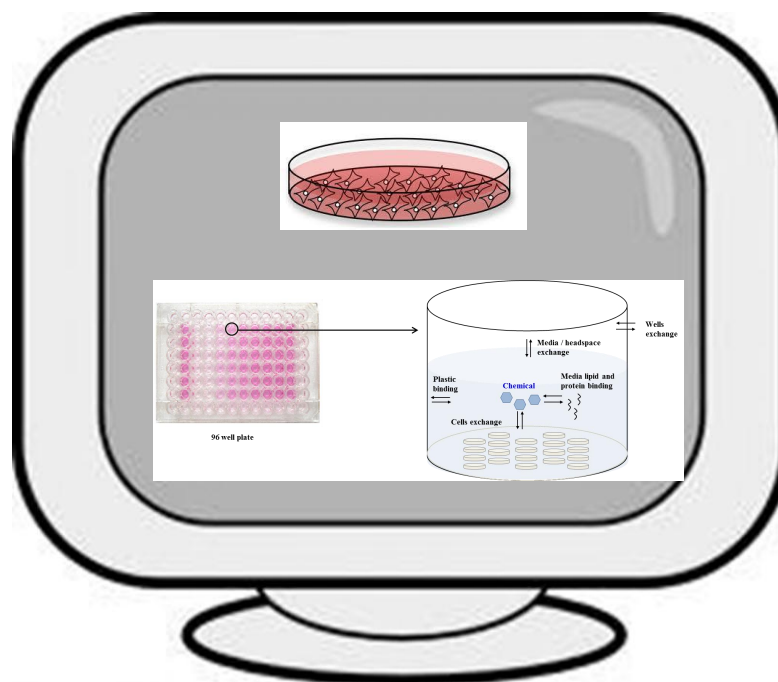
Node 2



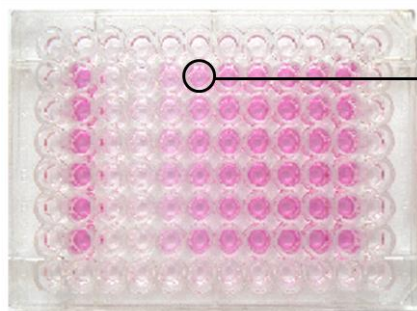
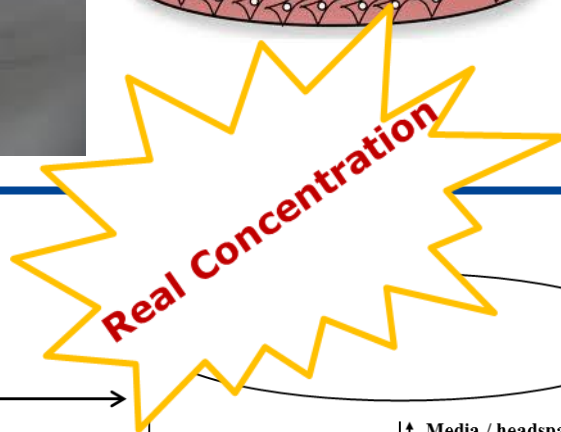
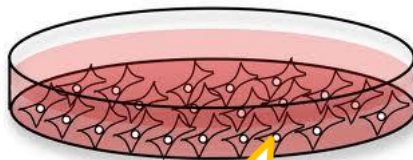
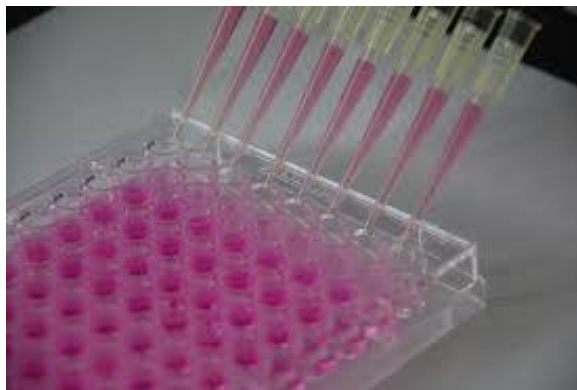
In silico tools

Under the SEURAT 1 – Project - within the COSMOS cluster - we are developing mathematical models:

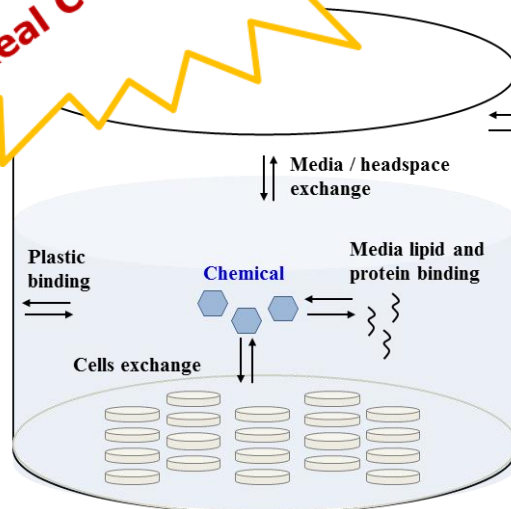
Virtual Cell Based (VCB).



In vitro experiments

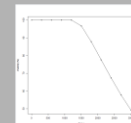


96 well plate



Wells
exchange

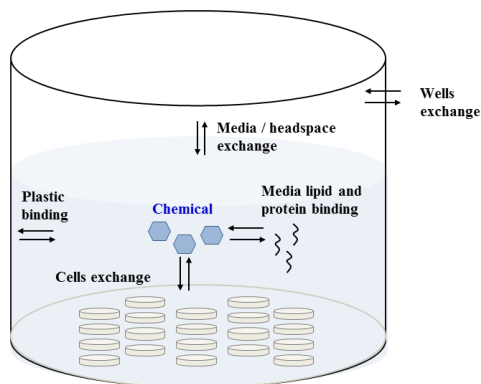
$$\frac{dC_b}{dt} = \frac{MW \cdot V^{2/3}}{W} (r_{da} \cdot C_{dis} - r_{ad} \cdot C_{aq}) - \frac{C_b dW}{W dt}$$



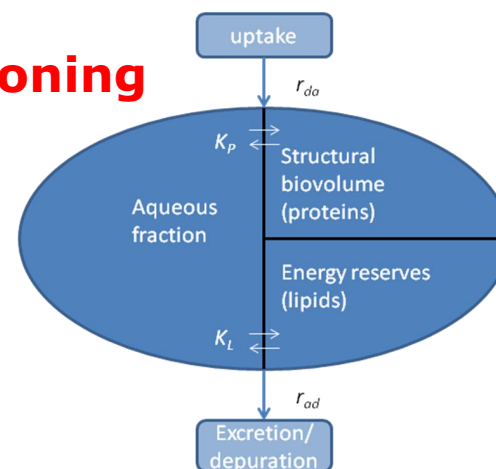
Virtual Cell Based model

The VCB model consists of ordinary differential equations representing:

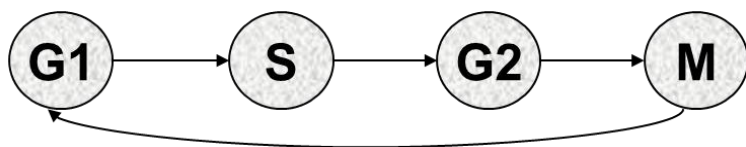
A fate model



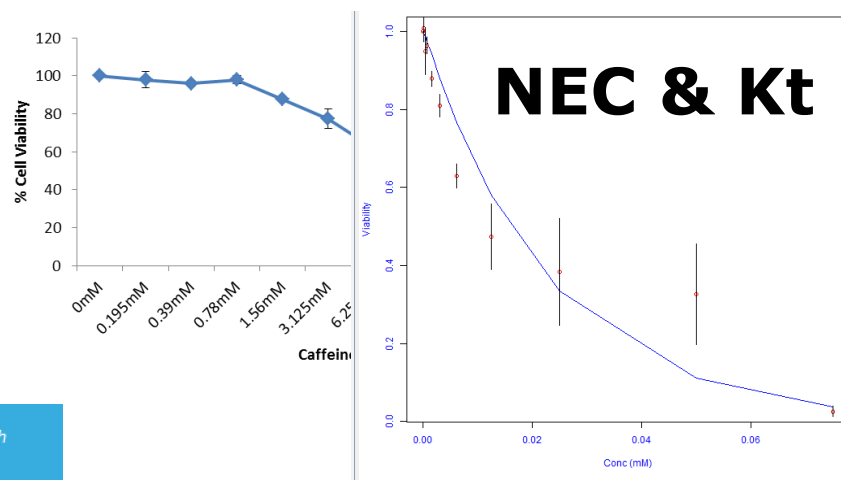
A cell partitioning model



A cell growth model

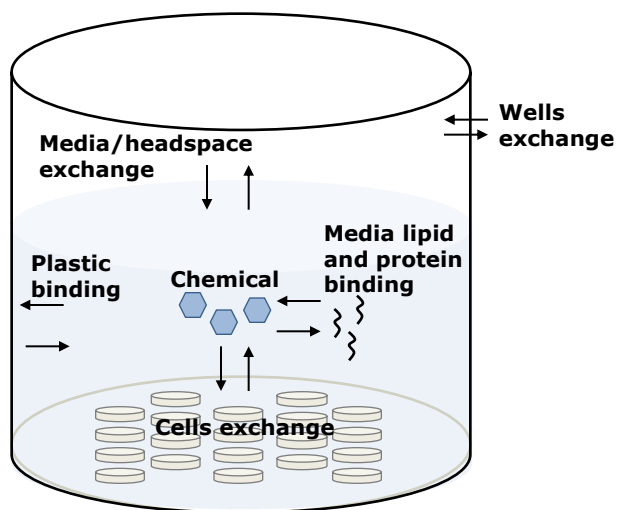


A toxic effect model



FATE OF CHEMICALS IN CELL-BASED ASSAYS

Partitioning approach:



In vitro experiments

Total concentration in the medium

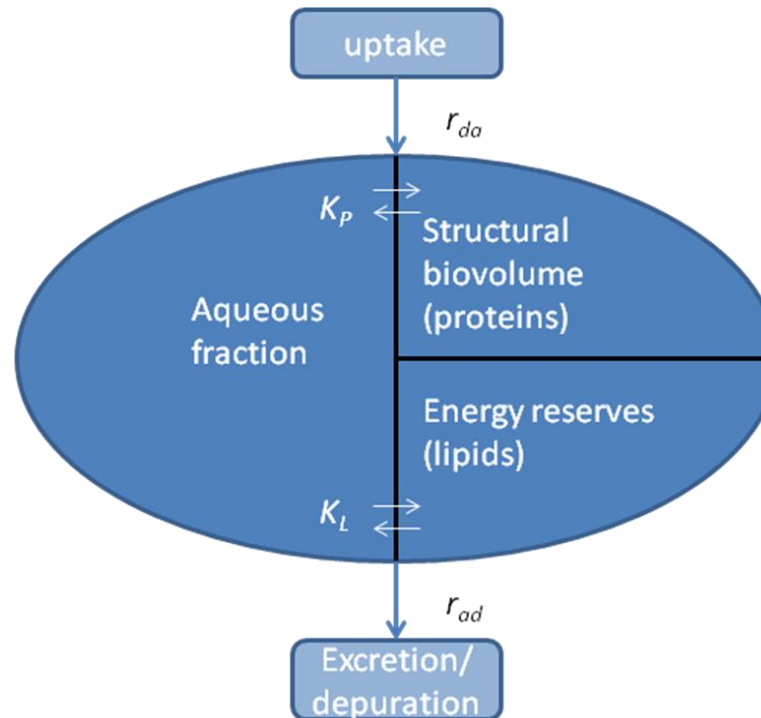
$$V_{\text{medium}} \frac{dC_{\text{Total}}}{dt} = A_s \cdot F_{\text{AW}} - R_{\text{uptake}} - K_{\text{deg}} V_M C_{\text{diss}}$$

$$C_{\text{dissolved}} = \frac{C_{\text{Total}}}{1 + K_s \cdot C_{\text{serum}} + K_p \cdot C_{\text{plastic}} + K_l \cdot C_{\text{lipid}}}$$

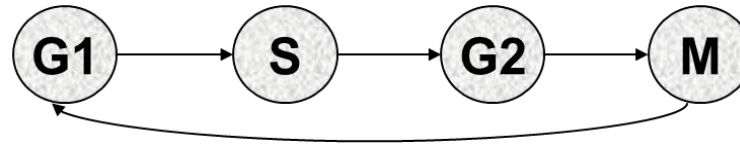
Cell Partitioning Model

Cell internal concentration:

$$\frac{dC_b}{dt} = \frac{MW \cdot V^{2/3}}{W} \left(r_{da} \cdot C_{dis} - r_{ad} \cdot C_{aq} \right) - \frac{C_b dW}{W dt}$$



Growth model



$$A = \begin{bmatrix} P_1 & 0 & 0 & F \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{bmatrix}$$

$$n_{t+1} = An_t$$

$$P_i = p_i(1 - \gamma_i)$$

$$p_i = \exp(-z_i)$$

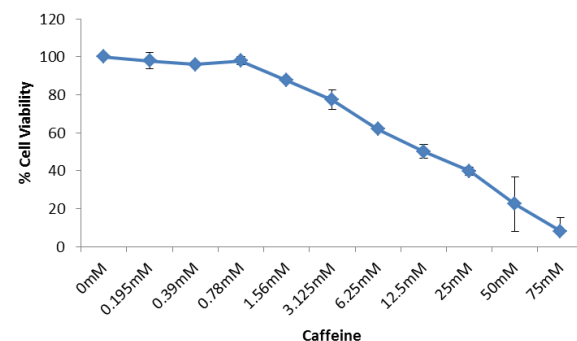
Toxicity model

NEC: No effect concentration
Kt: killing rate

$$z_i \begin{cases} = z_i + k_t (C_b - NEC) \\ = z_i \end{cases}$$

$$p_i = \exp(-z_i) \quad [2]$$

$$\frac{dC_b}{dt} = \frac{MW \cdot V^{2/3}}{W} (r_{da} \cdot C_{dis} - r_{ad} \cdot C_{aq}) - \frac{C_b dW}{W dt} \quad [1]$$



What type of data you need

- **In vitro concentration profiles (with cells selected) – cell viability and mmp**
- **In vitro cell dynamics and cell characterization (cell cycle)**
- **In vitro partition coefficients**
- **Information on Chemical: Cas #, Mw, LogKow, H, Mv, water and air degradation**






Can be used to predict

- **Single and repeat in vitro exposure**
- **Real concentration of the parent compound**

Limitation

- **Does not predict full metabolism**

Poster

The Virtual Cell Based Model – simulating the fate and effects of chemicals in multiple cell systems

Alicia Páris, Jochem Louisse, Dorella Lipska, Jose Vicente Sala Benito, Pilar Prieto Peralta, Andrew Worth
European Commission, Joint Research Centre, Institute for Health & Consumer Protection, IGC, Italy

INTRODUCTION

In the context of reducing and eventually replacing the use of animals in toxicity testing there is a need to predict human in vivo toxic doses from concentrations that cause effects in vitro. The characterization of the concentration that produces an effect (whether this is a perturbation of a molecular pathway or an apical toxic endpoint) is necessary at two levels: first, in analysing the results of vitro experiments, since "nominal" concentrations do not represent the real concentration experienced by the cell, and, second, in extrapolating to humans, since the low concentration experienced by cells within the target organ is more representative for human toxicity assessment. In order to address these issues concerning the in vitro side our group developed a Virtual Cell Based (VCB) model for liver cell lines (3T3cl8alco, HepG2 and HepaRG). The VCB model has been refined to include cell lines from different organs: lung A549 cells and from heart cardiomyocytes. Here we present an overview of the different VCB models and of the chemicals tested in each cell line.

THE GENERAL VIRTUAL CELL BASED MODEL

The VCB model consists of ordinary differential equations whose solution allows the calculation over time of the dissolved concentration of a chemical in cell culture as well as the internal concentration in the cells. The VCB model comprises four linked models:

- (1) The fate and transport model that calculates the time-dependent chemical concentration in the medium as well as in the headspace. This takes into consideration a series of processes including evaporation, partitioning of chemicals from the dissolved phase to serum proteins and lipids, adsorption onto the plastic, and also degradation and metabolism.
- (2) The cell partitioning model that was built on the assumption that once the chemical is taken up by the cell, a partitioning occurs between three compartments: one aqueous fraction and two non-aqueous fractions corresponding to structural components (proteins) and energy resources (lipids).
- (3) The cell growth and division model that is based on a four stage based approach, with each stage corresponding to one of the four cell cycle phases: G₁, S, G₂ and M.
- (4) Toxicity and effects model. The direct effects of a chemical concentration, C, on cell dynamics (survival/mortality) are expressed by using the killing rate, K, and the no effect concentration, N_{EC}.

$$\frac{dC_{ext}}{dt} = -k_{deg}C_{ext} - k_{evap}C_{ext} + k_{cond}C_{aq} + k_{diff}C_{aq}$$

$$\frac{dC_{aq}}{dt} = k_{evap}C_{ext} - k_{cond}C_{aq} - k_{diff}C_{aq} - k_{ads}C_{aq} + k_{des}C_{pl}$$

$$\frac{dC_{pl}}{dt} = k_{ads}C_{aq} - k_{des}C_{pl} - k_{deg}C_{pl}$$

$$\frac{dC_{int}}{dt} = k_{uptake}C_{aq} - k_{efflux}C_{int} - k_{met}C_{int}$$

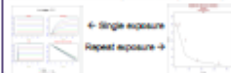
$$\frac{dN}{dt} = \mu N - K N$$

$$K = \frac{C}{N_{EC}}$$

LIVER

The HepaRG cell line is a human bipotent progenitor cell line capable of differentiating into two different cell phenotypes (i.e.,iliary-like and hepatocyte-like cells), has been established from a liver tumor associated with chronic hepatitis C. This cell line represents a valuable alternative to in vivo cultured primary human hepatocytes. We used a CHO preserved Human Cell Line HepaRG obtained from Holsinger's laboratory US22.

Simulation plot obtained with the VCB model in HepaRG cells. The plot is concentration versus time, for the partitioning of acetaminophen in the medium, in the headspace, and in the cell. Furthermore we obtain the relative number of cells (viability normalized to the number of cells at time zero) versus time (in minutes).



4- Single exposure
Repeat exposure →

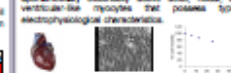
SUMMARY

In order to obtain the N_{EC} and K, for each chemical, we must obtain an experimental concentration response (cell viability) curve using the selected cell line. The results achieved can be used to optimize the VCB model. The table below summarizes the chemicals tested for cell viability on the three cell lines chosen that are used to run the virtual cell based model.

Name of Compound	Liver (HepaRG)	Lung (A549)	Heart (Cardiomyocytes)
acetaminophen	X		
amiodarone	X		X
caffeine	X	X	
clozapine	X		X
clozapine	X		X
estradiol	X		
fenofibrate + sodium lauryl sulphate		X	X
nicotine	X		
sodium lauryl sulphate	X		
succinic acid	X		
verapamil hydrochloride	X		


HEART

Cell cardiomyocytes are commercially available human induced pluripotent stem cell-derived cardiomyocytes. Cell Cardiomyocytes are a mixture of spontaneously electrically active atrial, nodal, and ventricular-like myocytes that possess typical electrophysiological characteristics.



LUNG

The human tumor alveolar epithelial cell line (A549 cells) has been characterized as a type II pulmonary epithelial cell model for drug absorption and metabolism. Therefore, A549 cells were used in the present study to assess the toxic effects of inhaled acetaminophen, clozapine products at the pulmonary epithelial level.



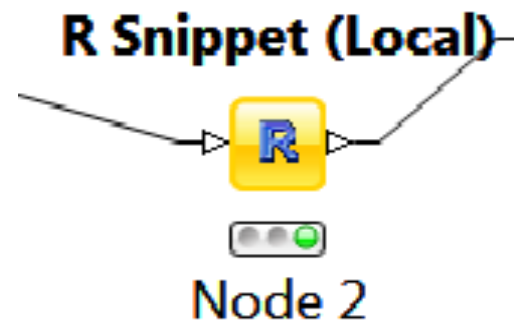
CONCLUSIONS

- Modelling in vitro experiments has the goal not only to predict experimental results but also to simulate physical and biological processes that are not easily or directly measurable but which can be of toxicological relevance, like the real concentration of chemical inside the cell.
- This VCB model for different organs can be used not only in the design of in vitro experiments, but can also be linked to PBK models to extrapolate from in vitro to in vivo exposure scenarios for risk assessment purposes when linked to PBK models.
- Combining the results from different cell lines for one specific compound is expected to give insights into the toxic effects occurring in different organs and could contribute to the total effect on the total body. The VCB model for different organs is a step towards the multi-scale approach modelling of the organisms.

Acknowledgements

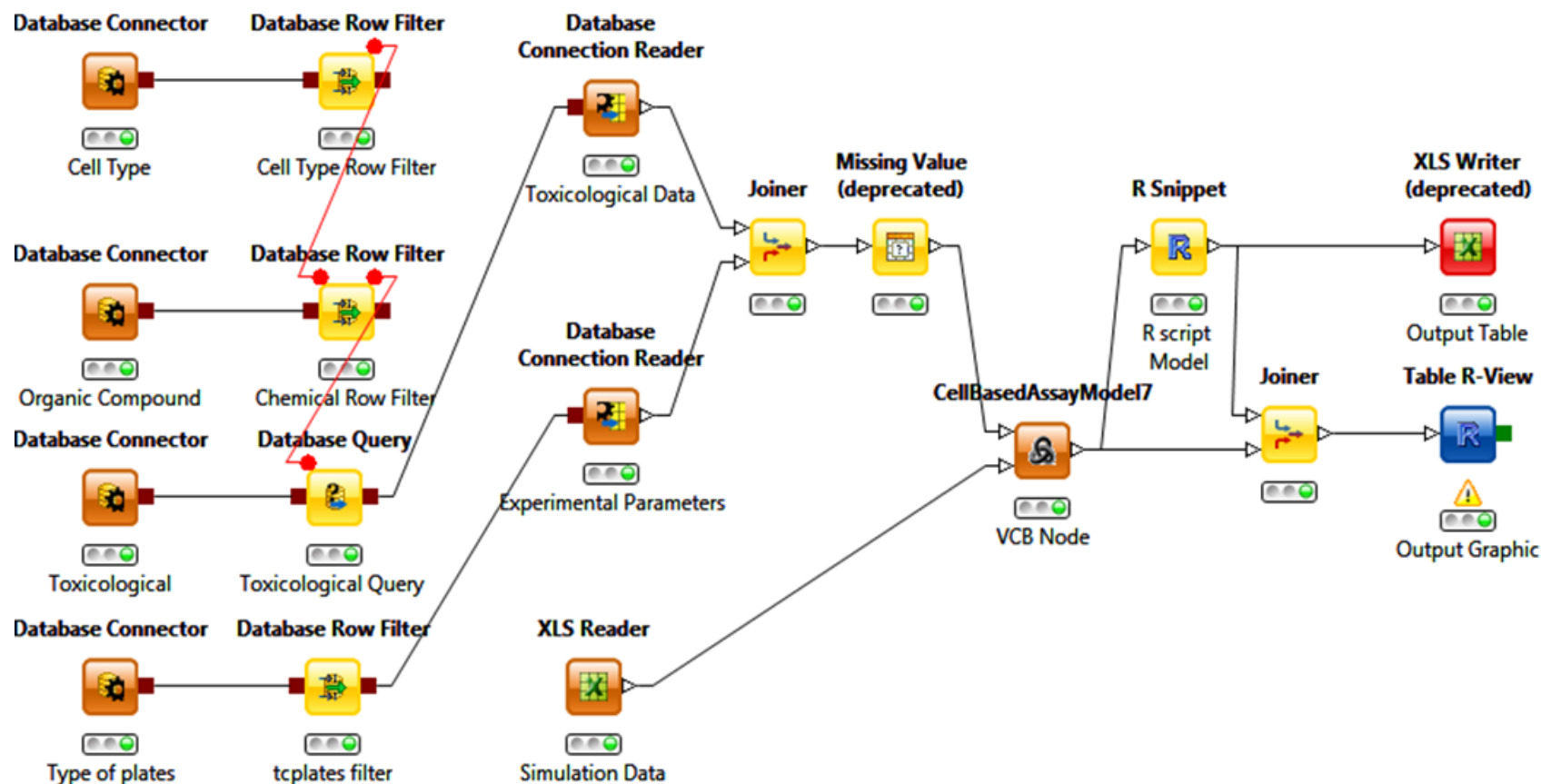
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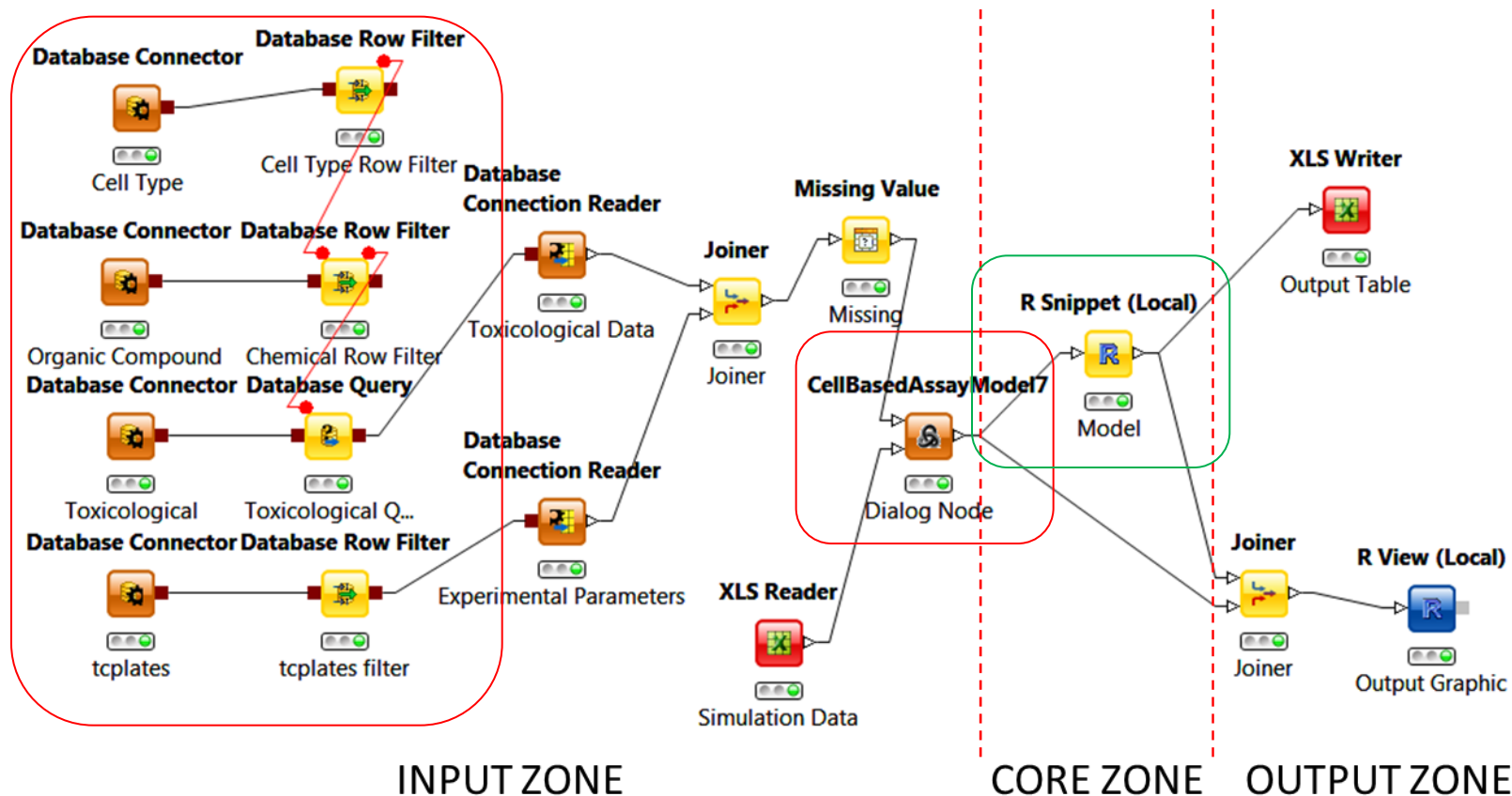


**KNIME is a user-friendly graphical
workbench for analysis process**

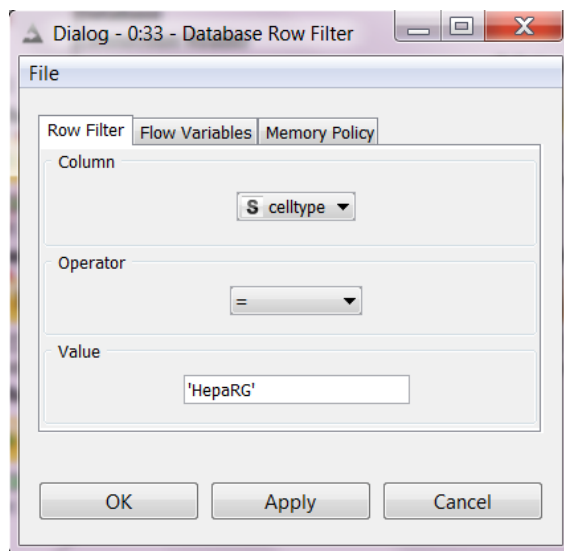
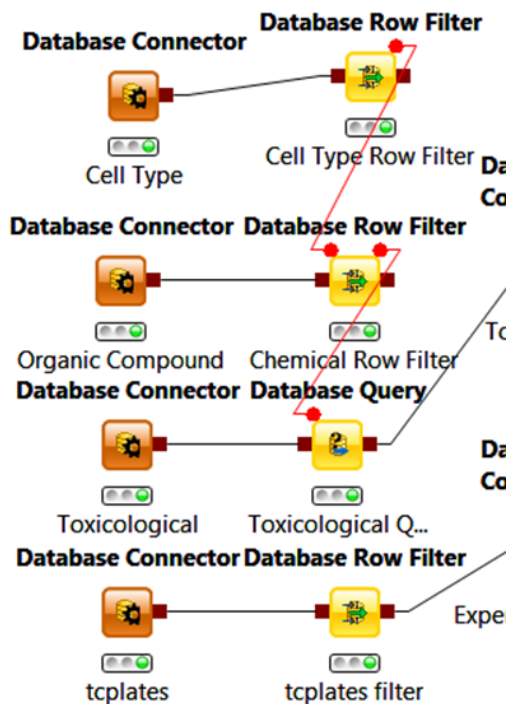
Cell-Based Integrated Model



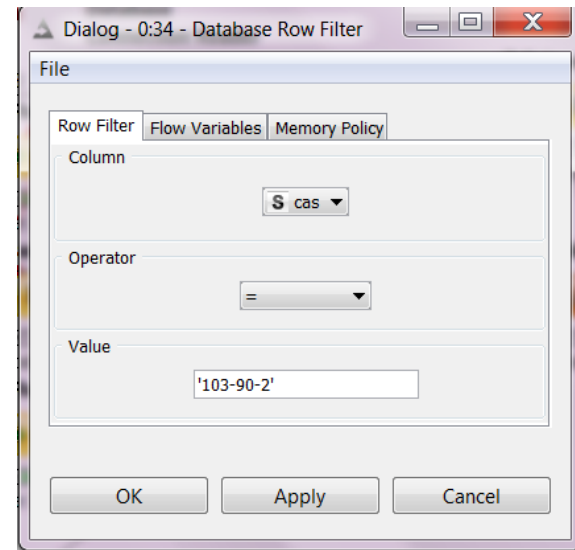
Cell-Based Integrated Model



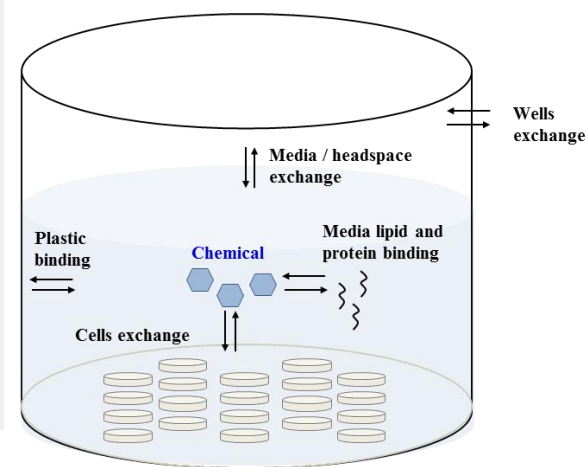
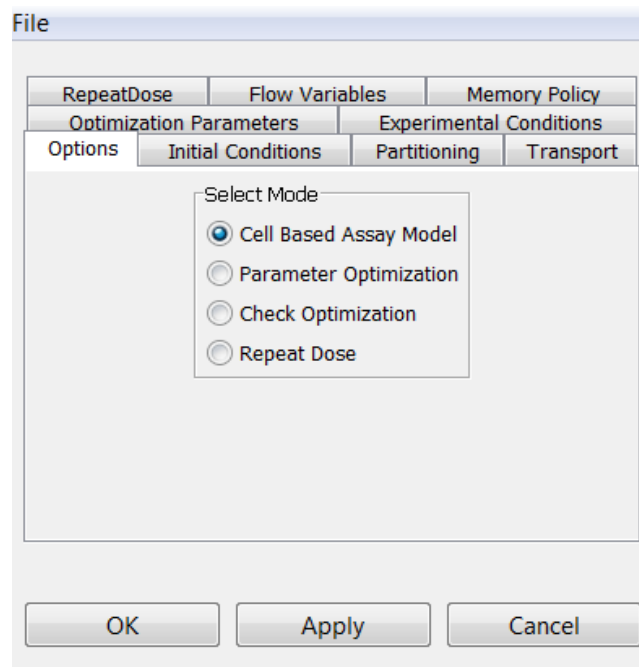
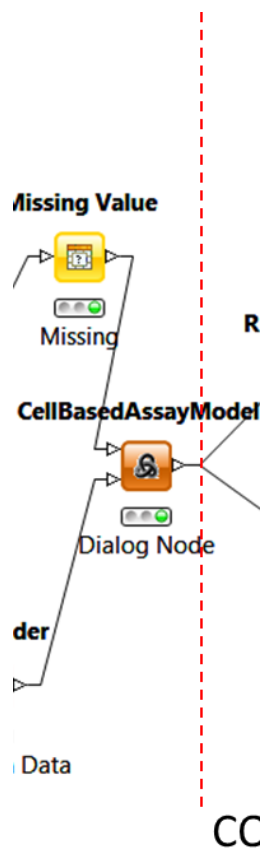
Cell-Based Integrated Model



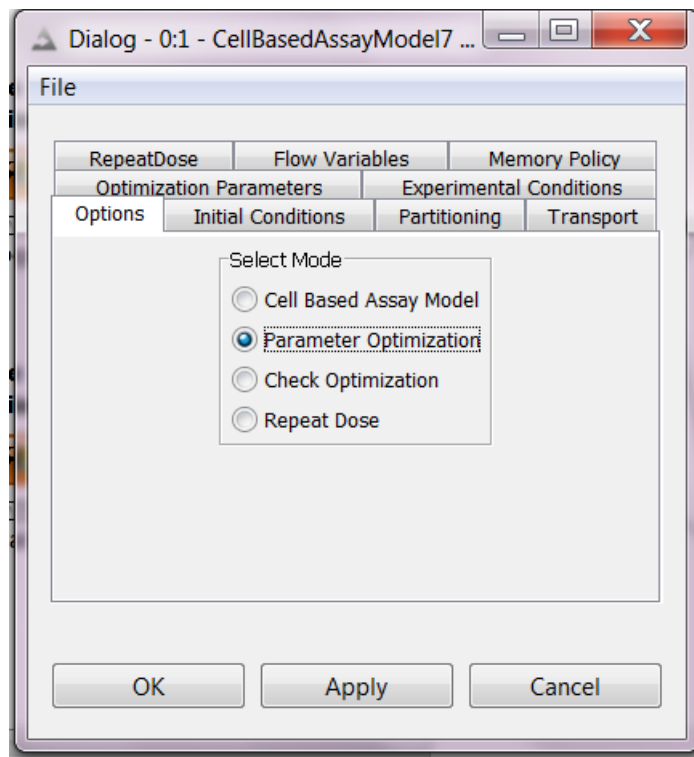
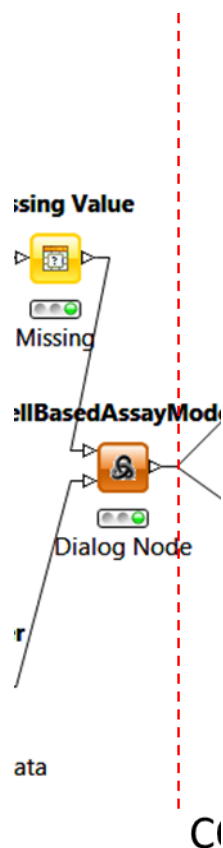
HepaRG
HepG2
3T3Balb c



Cell-Based Integrated Model



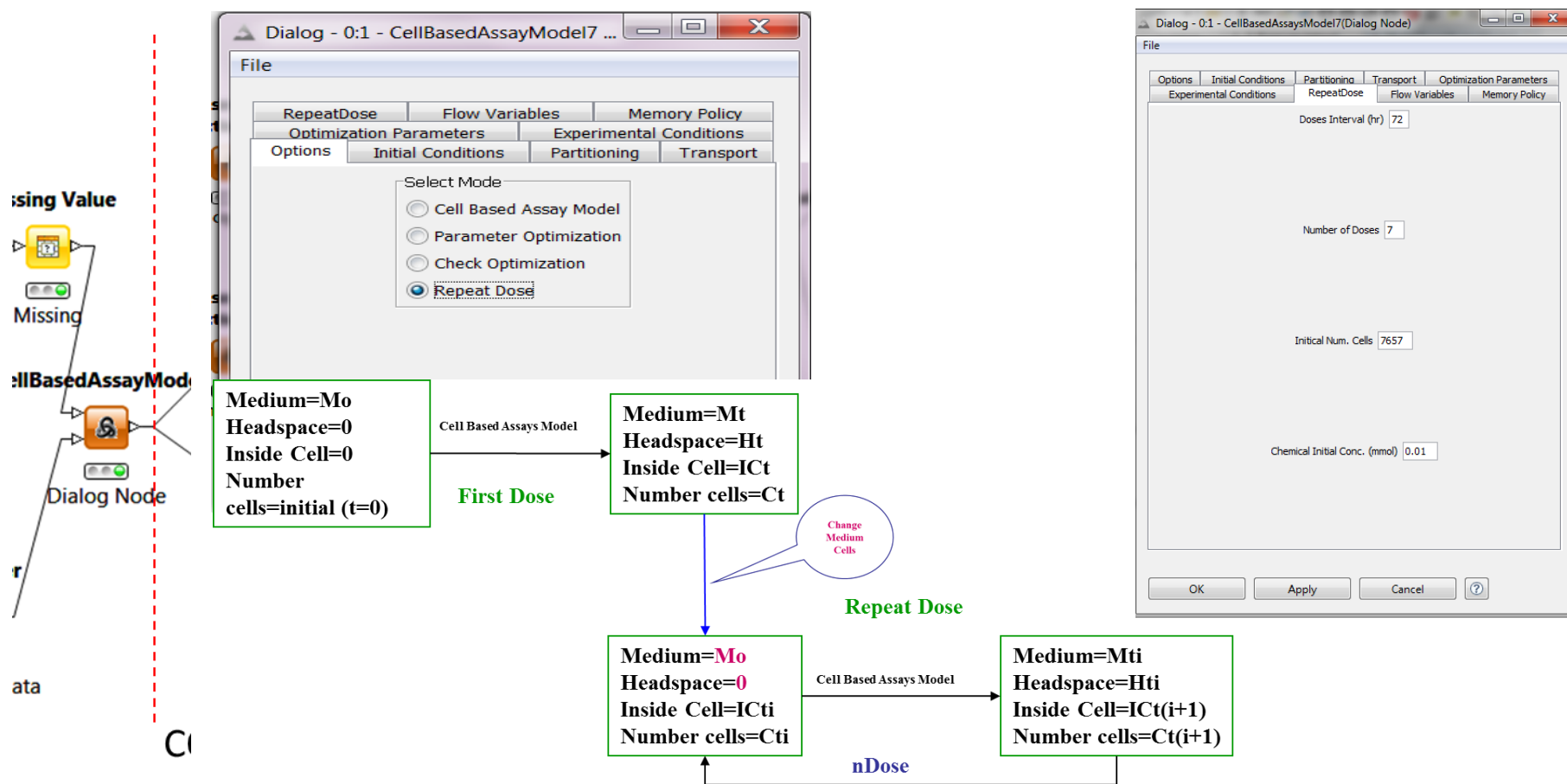
Cell-Based Assays Integrated Model



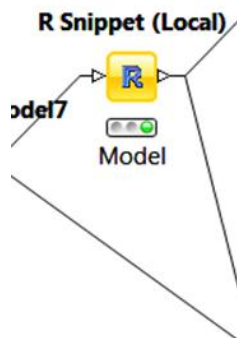
$$error = \sum_{i=1}^{n^{\circ} \text{exp}} \left(Viability_{\text{exp}} - Viability_{\text{sim}} \right)^2$$

CONC (µg/mL)	VIABILITY (%)	std
0.78	104.15	7.412476
1.56	106.40	11.24928
3.13	104.08	7.671877
6.25	108.16	10.08127
8.78	100.31	14.43041
12.5	81.37	10.16028
25	58.70	9.369224
44.44	42.82	8.410667
50	32.91	6.169209
66.67	25.37	7.814317
100	16.97	5.564936

Cell-Based Assay Integrated Model



Model Script



CORE ZONE

```

R Command | R Binary | Flow Variables | Memory Policy

Column List
S CellType
D duration1
D durations
D duration2
D durationm
D mortality1
D mortalitys
D mortality2
D mortalitym
D volume1
D volumes
D volume2
D volumem
D massg1
D masses
D massg2
D massm
D initialCellPopg1
D initialCellPops
D initialCellPopg2
D initialCellPopm
D fecundity

Flow Variable List
S chemname
S chemical
S numDoses
S IntervalDose
S SelectedMode
S cas
S cell
S knime.workspace
S hola
S primeraFila
S ultimaFila
S colTime
S colViability
S colError

R Snippet
#Differential equations
#####
xdot<-function(t,state,parameters) {
  with(as.list(c(state,parameters)), {
    #x1 total concentration in the medium
    #x2 concentration in the headspace
    #x3 concentration inside the cells
    rmax<-R$"rmax"[1]
    Ksat<-R$"Ksat"[1]
    Cdcomp<-xx1/(1+Ks*St+K1*Lt+Kp*SP/V) # (A4)
    kav<- (kgcomp*KGLcomp*klcomp)/(klcomp+kgcomp*KGLcomp) # (A16) mass transfer coefficient
    # kgcomp mass transfer coefficient on the air (m.s-1)
    # klcomp mass transfer coefficient on the water film (m.s-1)
    # KGLcomp dimensionless gas-liquid distribution coefficient.
    Fawcomp<-kav*(-Cdcomp+xx2/KGLcomp) # diffusive air-water exchange (mol.m-2.s-1) (A13)
    if(ncells>1) {
      W<-mcells/ncells #organism weight (g)
      Vcell<-Vcells*1E6/ncells
      DeltaC<- (Cdcomp-xx3)/(Wcomp*(Eaq/zhoaq+KL/zhoL+FP*KP/zhoP)))
      # Passive diffusion + mediated transport

      if(DeltaC==0.0) {
        rexchange<-0.0
      } else {
        rexchange<-Wcomp*(Vcell^(2/3)*zda*DeltaC+rmax*DeltaC/(DeltaC+Ksat))/W #im 1 mol-1 s-1
      }

      xdot3<-rexchange-kmet*xx3-weight_change*xx3 # (A17)
      cells_up<--chemdead+(rexchange-kmet*xx3)*mcells*1E-3/Wcomp/V
    } else {
      xdot3<-0
      cells_up<--chemdead
    }
  }
  Fdecomp<-kdeccomp*Cdcomp
  xdot1<-(P*Fawcomp-Fdecomp-cells_up)
  Fdecompa<-kdecompa*xx2
  Flosses<-Fexch*xx2
  xdot2<-(-Ph*Fawcomp-Fdecompa-Flosses)
  list(c(xdot1,xdot2,xdot3))
})
}
#end function
#####
#end differential equations
coreModel<-function(ci,cii,cell_i,NEC,kt) {
  library(deSolve)

  ##Initial Conditions

  if(R$"Mode"[1]=="Repeat Dose") {
    TotalTime<-R$"IntervalDoses"[1] #hours
  } else {

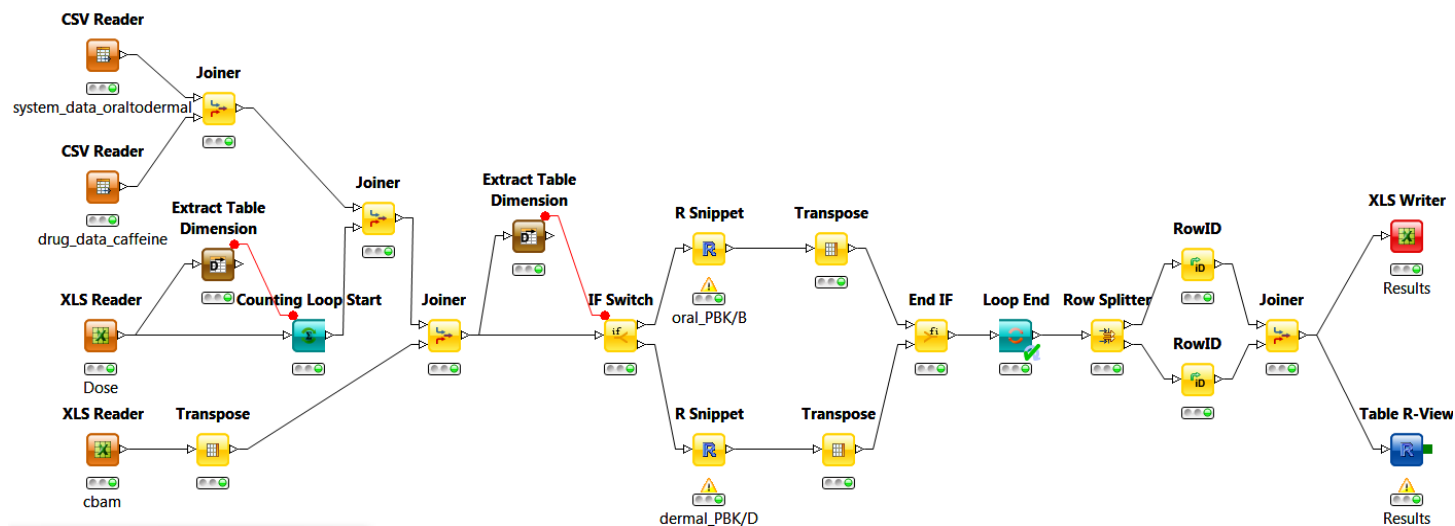
```

NO PROBLEM!!!

IVIVE model

Currently we are developing a KNIME workflow to link the PBK model (simulating organ internal concentration to the concentration inside the cell) to link to the VCB model NEC and kt (cell viability).

This allows prediction of the chemical concentration starting from a known exposure dose which could be linked to a toxicity endpoint, such as cell viability; or to perform In Vitro to In Vivo Extrapolation, starting from an experimental concentration we obtain the dose.



[illegible]