Exercise – PBPK/PD Modeling of antibody-drug-conjugates (ADC)

Background

Example: Effects of Ab Pre-Treatment

aims to extend the therapeutic window by reducing the uptake in non-tumor tissues while preserving tumor uptake and efficacy due to a large overexpression of target in the tumor tissue.

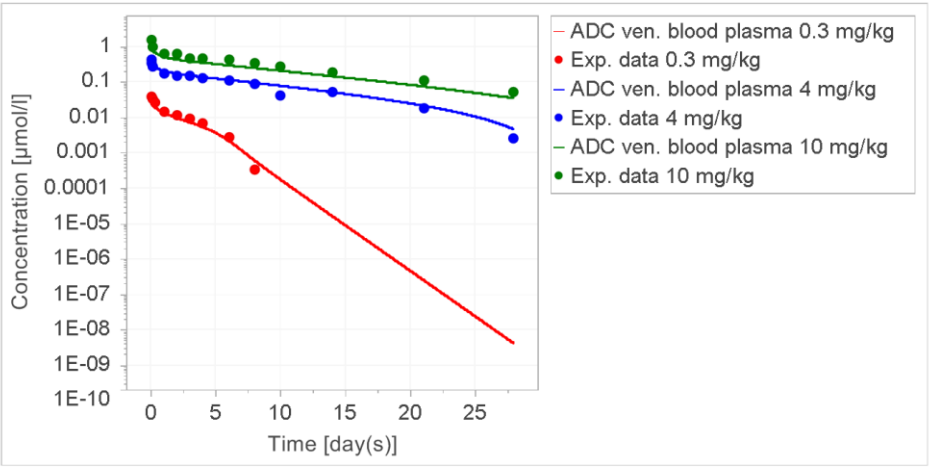
nAb predosing to saturate off-target receptors → ADC dosing to reach target receptors → Reduce toxicity and still get enough efficacy

Example: TENB2 ADC and anti-TENB2 pre-treatment

- published by Boswell *et al.*, Genentech[1,2]
- Toxophore: MMAE
- Linker: cleavable MC-vc-PAB
- Mouse, non-tumor-bearing mice and prostate cancer explant model (LuCaP 77)
- Intestines were identified to contribute to the target-mediated clearance of the anti-TENB2 antibody and its drug conjugate in rodents

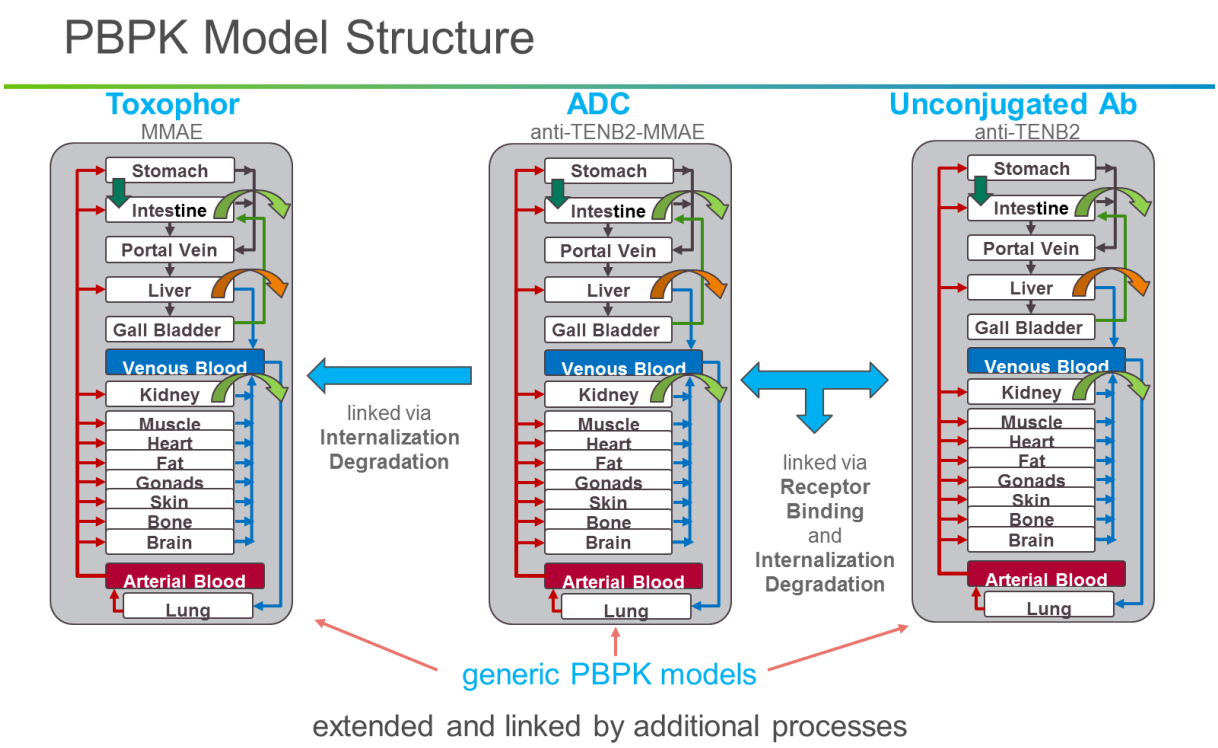
[1] Boswell et al., Br J Pharmacol. 2013; 168(2): 445–457.
[2] Boswell et al., J Nucl Med. 2012; 53(9):1454-61.

Plasma Concentration of ADC in Non-Tumor Bearing Mice



➔ **PK including target mediated clearance is represented well for different dosages**

Experimental data:
Boswell et al., Br J Pharmacol. 2013; 168(2): 445–457.



General Objectives

Learn to set up a simulation for an ADC and compare the simulation to observed data
Examine the reason why the simulation does not fit the experimental data
What processes are missing to describe the data?
Think about how the toxophore should be modeled to describe toxophor release mechanistically

Part 1: ADCI set up a (mouse model) simulation for TENB2 ADC in PK-Sim and compare the result to observed data

Objectives

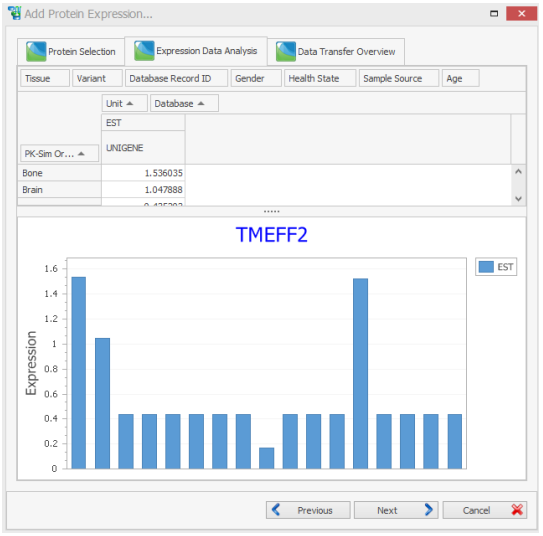
1. Set up the building blocks for Individual, Compound and Administration Protocol
2. Set up the simulation and analyze the result
3. Import observed data ("Exp_data_Boswell_2012.xlsx") sheet "0.3 mg/kg" and drag & drop it to the figure panel
4. Compare the observed data with your simulation results

Open a new PK Sim Project

Create the individual

1. Click "**Individual**" in the "**Create**" group of the "**Modeling**" tab or right click on "**Individuals**" in the "**Building Blocks**" Explorer and select "**Add Individual**".
2. Initialize the Individual by giving it a name (*here*: "**Mouse**").

3. Select “**Mouse**” as Species and click “**Next**”.
4. Click “**Next**” to get to the “**Expression**” tab.
5. Add “**TENB2 (also called TMEFF2)”** as a protein binding partner:
    a. “**Right click**” on “**Protein Binding Partners**”, choose “**Add Protein Binding Partner… (Database Query)**”
    b. Search for “**TENB2**”
    c. “**Double click**” on “**TENB2”**
    d. Check the available expression profile and click “**Next**”



    e. Click “**OK**”. The relative expression profile is added to your individual “**Mouse**”
    f. Set “**Reference concentration**” to “**0.0005 µmol/l**”.
    g. Set “**Location in tissue**” to “**Extracellular membrane**”
    h. Set “**Location on vasc. endothelium**” to “**basolateral**”
6. Click “OK”. Your individual is created.

In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_1.pksim5**.

Create the compound

- Click “**Compound**” in the “**Create**” group of the “**Modeling**” tab or right click on “**Compounds**” in the “**Building Blocks**” Explorer and select “**Add Compound**”.
- Initialize the Compound by giving it a name (*here*: “**ADC**”).
- Uncheck the box “**Is small molecule**”.
- Define the compound data as depicted in the following table:

Properties of anti-TENB2		
	Value	**Unit**
Lipophilicity (log P)	-5	Log Units
Plasma fu	1	
Molecular Weight	148000	g/mol

aqueous solubility pH 7.0	999	mg/l
Radius (solute)*	5	nm
Kd (FcRn) Endosomal*	1.5	µmol/l

*The parameters "**Radius (solute)**" and "**Kd (FcRn)**" can be found under "**Advanced Parameters**"

- Go to the "**ADME**" tab (or click "**Previous**" when you have been in the "**Advanced Parameters**" tab)
- "**Right click**" on "**Protein Binding Partners**". Select "**Add Protein Binding Partner**".
    - Choose the target "**TENB2**" (it is named "**TMEFF2"** here, which is another synonym for the target) from the drop-down menu.
    - Type "**Boswell et al.**" as "**Data source**".
    - Set "**koff**" to "**0.9 1/min**" and "**Kd**" to "**0.01 µmol/l**".
    - Click "**OK**". Your compound is created.

In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_2.pksim5**.

Create the Administration Protocol

1. Click "**Administration Protocol**" in the "**Create**" group of the "**Modeling**" tab or right click on "**Administration Protocols**" in the "**Building Blocks**" Explorer and select "**Add Administration Protocol**".
2. Initialize the Administration Protocol by giving it a name (*here*: "**i.v. 0.3 mg/kg**").
3. Set "**Dose**" to "**0.3 mg/kg**".
4. Click "**OK**". Your Administration protocol is created.

In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_3.pksim5**.

Create the Simulation

1. Click "**Simulation**" in the "**Create**" group of the "**Modeling**" tab or right click on "**Simulations**" in the "**Simulations**" Explorer and select "**Add Simulation**".
2. Initialize the Simulation by giving it a name (*here*: "**Mouse ADC i.v. 0.3 mg/kg**").
3. Under "**Model Settings**", choose the "**Model for proteins and large molecules**" in the drop-down menu
4. Click "**Next**"
5. Click "**Next**"
6. In the "**Processes**" tab, check that the defined "**Protein in individual**" is linked with the correct "**Binding process**"
7. Click "**Next**"
8. Choose the defined "**Administration**"
9. Click "**Next**" and then "**OK**"

10. The simulation is created

In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_4.pksim5**.

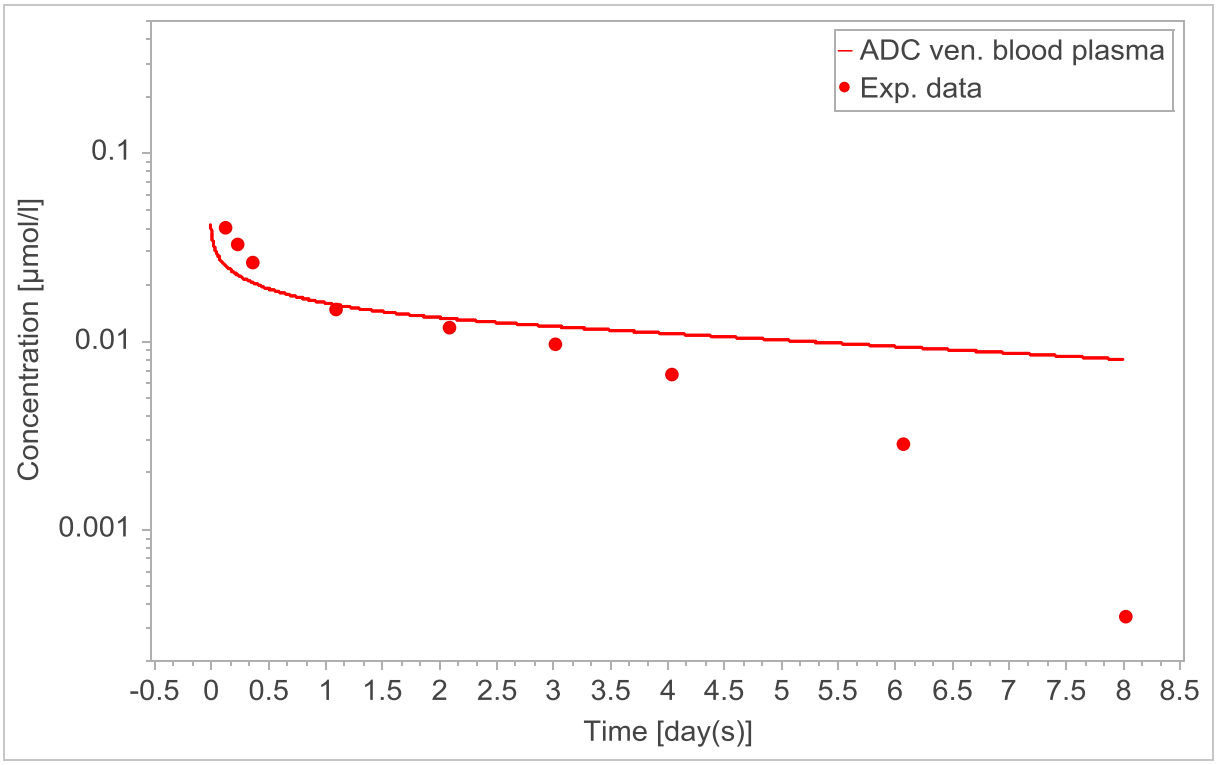
Run the Simulation

1. "**Double click**" on the selected simulation.
2. Click "**Run**" in the "**Simulation**" group of the "**Run & Analyze**" ribbon tab.
3. Select the predefined "**Venous Blood Plasma ADC Concentration**" and click "**OK**".
4. The simulation is processed.

In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_5.pksim5**.

Compare the simulation result with experimental data

1. Click "**Observed Data**" in the "**Import**" group of the "**Import/Export**" tab or right click on "**Observed Data**" in the "**Building Blocks**" Explorer and select "**Add Observed Data**".
2. Choose "**Exp_data_Boswell2012.xlsx**" as experimental data set and click "**Open**".
3. Choose the data for "**0.3 mg_kg"** and click "**Import (1)**".
4. Choose "**ADC**" as "**Molecule**", "**Mouse**" as "**Species**" and "**Venous Blood"** as "**Organ**". Finally, choose "**Plasma**" as "**Compartment**" and click "**OK**".
5. The observed data are imported.
6. Open the "**Analysis**" tab in the "**Simulation: Mouse ADC i.v. 0.3 mg/kg**" window and add the experimental data via drag and drop.
7. Open the "**Parameters**" tab in the "**Simulation: Mouse ADC i.v. 0.3 mg/kg**" window and click on "**Settings**".
8. Change the "**End Time**" of the simulation to 8 days.
9. Click "**Run**" in the "**Simulation**" group of the "**Run & Analyze**" ribbon tab.
10. Go back to the "**Analysis**" tab in the "**Simulation: Mouse ADC i.v. 0.3 mg/kg**" window and compare the simulation with the data. Why the simulation does not fit the experimental data?



In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_6.pksim5**.

Part 2: – ADC II -TMDD and Model Coupling-

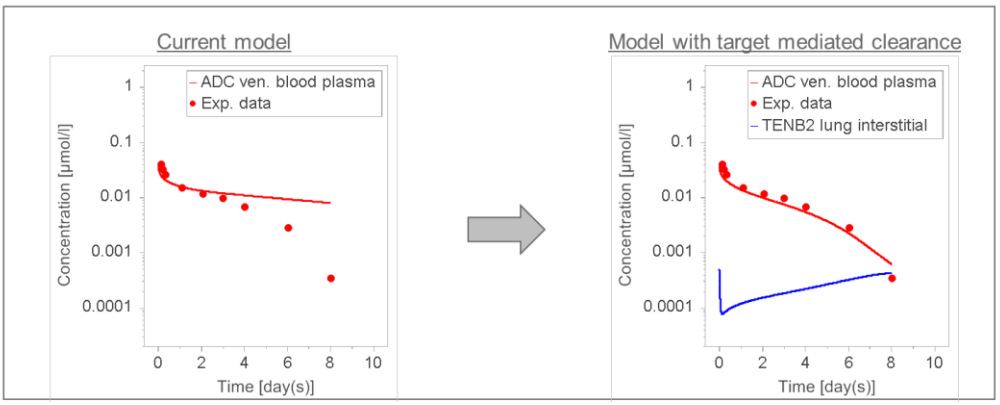
Background

Modeling Target Mediated Drug Clearance

What processes are missing to describe target mediated drug clearance?

- Receptor binding alone is insufficient
- Modeling of receptor synthesis and degradation required

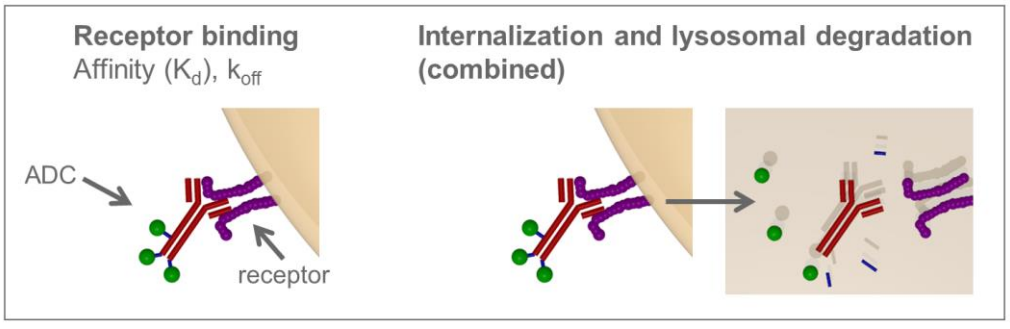
Re-synthesis of TENB2 receptor enables the target mediated clearance



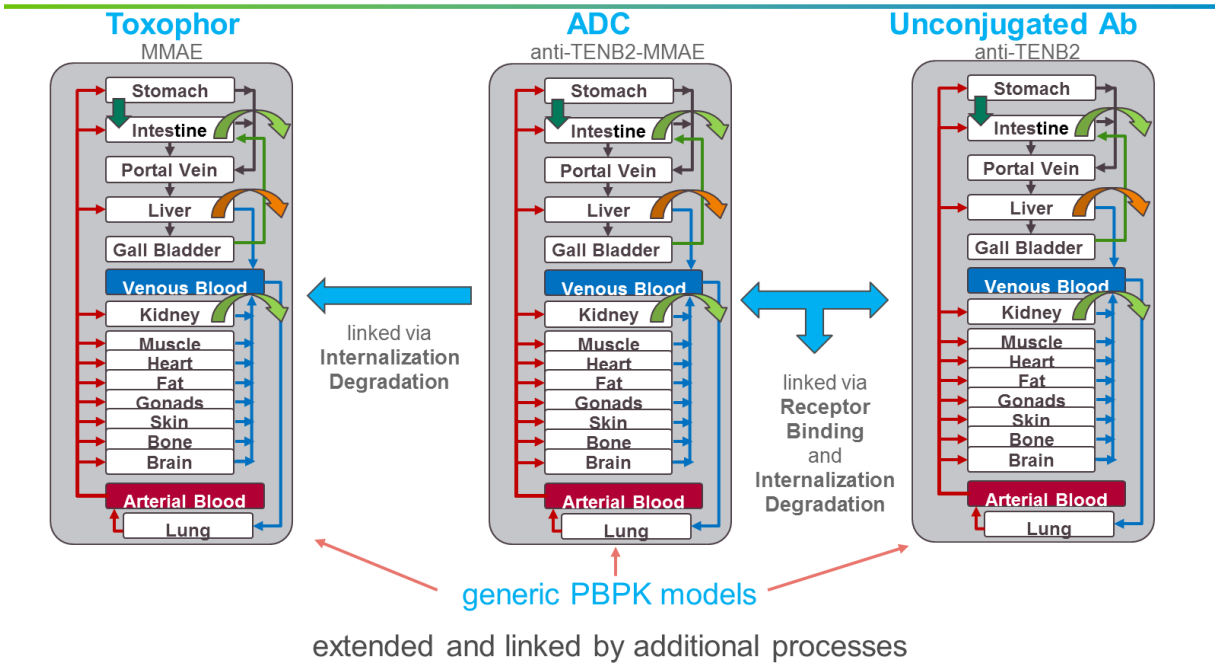
How to Model the Release of Toxophore From the ADC?

What processes are missing to describe the release of the toxophore?

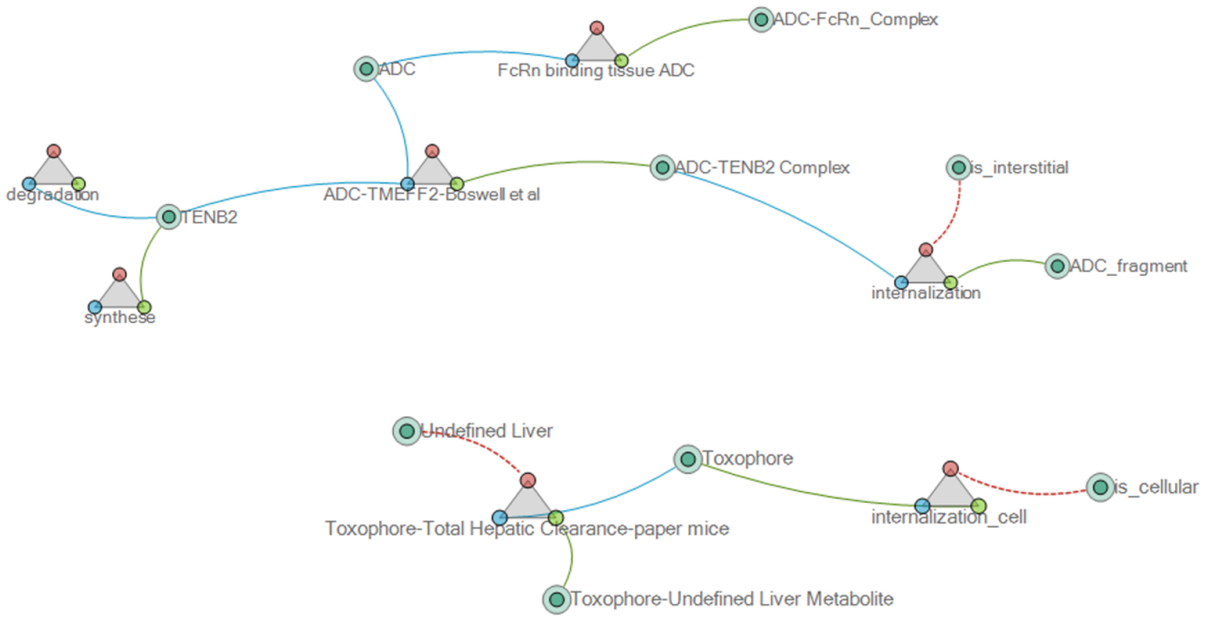
- Toxophore release is initiated after receptor binding of the ADC
- ADC-receptor complex is internalized and degraded in the lysosome
- Toxophore is released in the intracellular space



PBPK Model Structure



Reaction Network in MoBi



Objectives

Set up a combined model for the simulation of the ADC and the Toxophore including receptor dynamics and internalization of the ADC-receptor complex

1. Export established model for ADC and Toxophore to MoBi
2. Integrate the reactions for receptor dynamics and internalization of ADC-TMEFF2 complex (import “ReactionsADCModel.pkml”)

3. Simulate the model for several dosings and compare the results to the experimental data for 0.3 mg/kg, 4 mg/kg and 10 mg/kg
4. Simulate and analyze the different behavior of the toxophore concentration in venous blood and the TMEFF2 concentration in the interstitial of the lung for different doses
5. Which is the critical dose, where target mediated clearance needs to be taken into account?

Add the toxophore and create a combined simulation of ADC and toxophore

1. Open the file: **Ex_1_ADC_Tox_Modeling_6.pksim5** or continue with your file of the first exercise.
2. Click "**Compound**" in the "**Create**" group of the "**Modeling**" ribbon tab or right click on "**Compounds**" in the "**Building Blocks**" Explorer and select "**Add Compound**".
3. Initialize the Compound by giving it a name (*here*: "**Toxophore**") and define the compound data as depicted in the following table:

Properties of Toxophore		
	Value	**Unit**
Lipophilicity (log P)	3.2	Log Units
Plasma fu	0.49	
Molecular Weight	780	g/mol
aqueous solubility pH 7.0	10000	mg/l

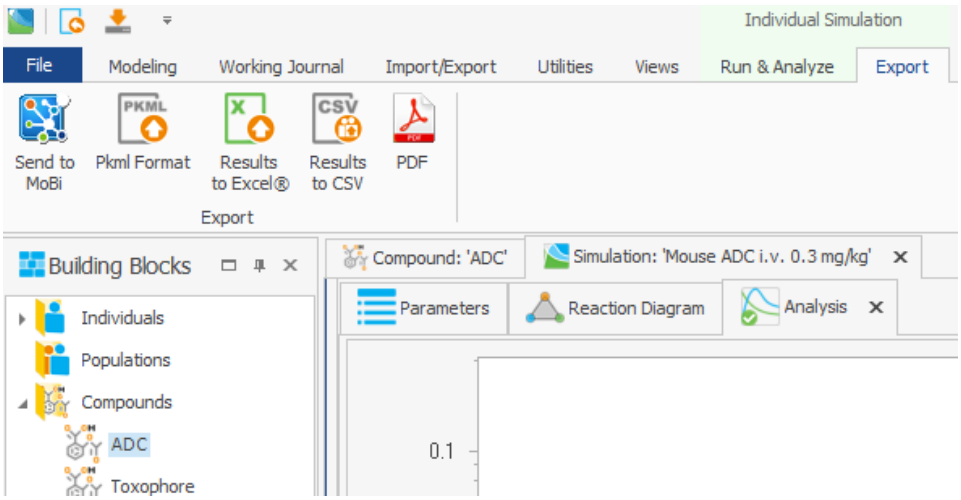
4. Click "**Next**" to go to the "**ADME**" tab.
5. "**Right click**" on "**Total Hepatic Clearance**". Select "**Add Total Hepatic Clearance Process**".
    a. Type "**Literature**" as "**Data source**".
    b. Choose "**Mouse**" as "**Species**" from the drop down menu.
    c. Choose **"Liver Plasma Clearance"** as the "**Process type"**
    d. Set "**Plasma clearance**" to "**8 ml/min/kg**". Click "**OK**".
    e. Click "**OK**". Your compound is created.
6. Click "**Simulation**" in the "**Create**" group of the "**Modeling**" tab or right click on "**Simulations**" in the "**Simulations**" Explorer and select "**Add Simulation**".
7. Initialize the Simulation by giving it a name (*here*: "**Mouse ADC i.v. 0.3 mg/kg with Tox**").
8. Select "**ADC**" and "**Toxophore**" as the compounds in the "**Compounds Selection**"
9. Under "**Model Settings**", choose the "**Model for proteins and large molecules**" in the drop-down menu.
10. Click "**Next**".
11. Click "**Next**".
12. Click "**Next**".

13. Choose the defined administration "**i.v. 0.3 mg/kg**" for ADC. Choose "**none**" as administration for Toxophore.
14. Click "**Next**" and then "**OK**".

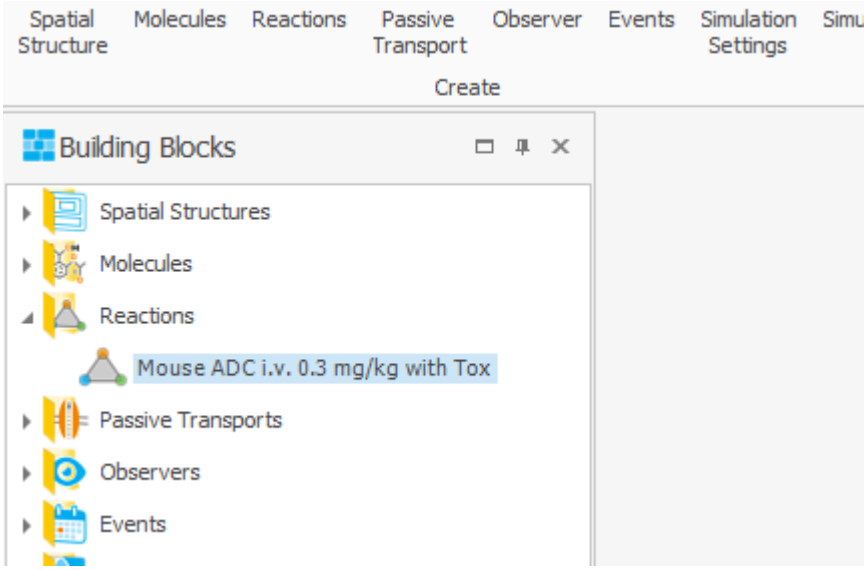
In case you wish to enter the exercise after this step and you did not perform the exercise described above, please open file **Ex_1_ADC_Tox_Modeling_7.pksim5**.

Export to MoBi

1. Select the simulation "**Mouse ADC i.v. 0.3 mg/kg with Tox**".
2. Click "**Send to MoBi**" in the "**Export**" group of the "**Export**" tab under "**Individual Simulation**", which appears after selection of a simulation.

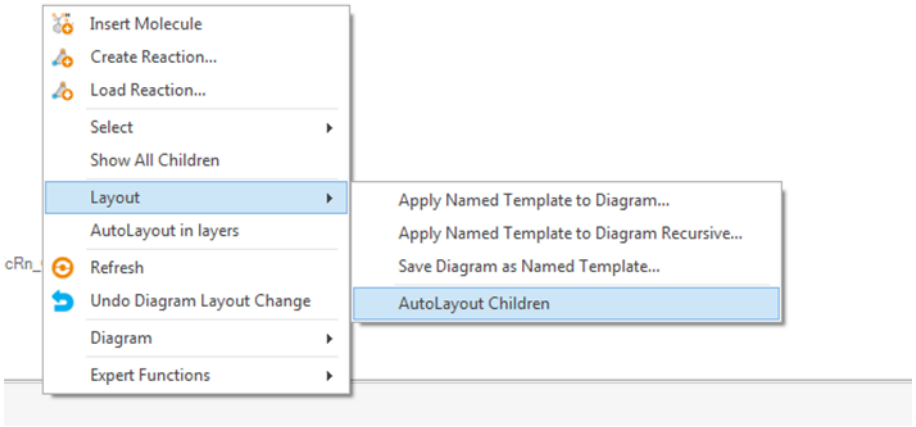


3. Close PK-Sim.
4. Open the Reactions building block "**Mouse ADC i.v. 0.3 mg/kg with Tox**" via "**double click**".

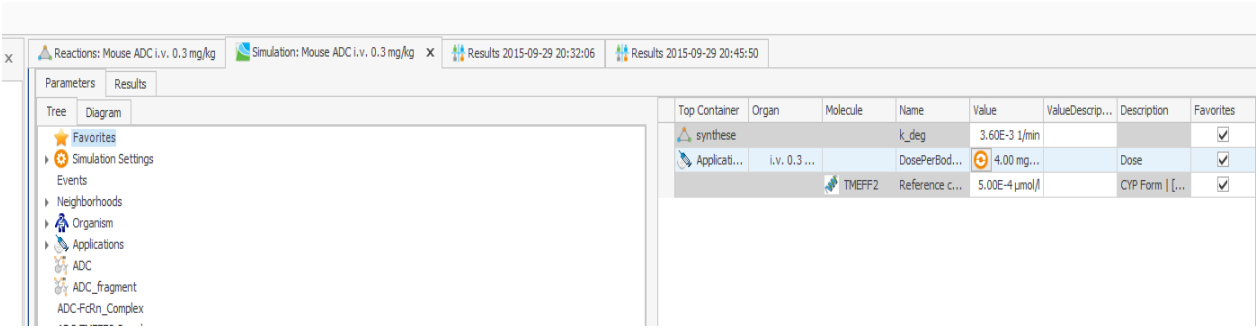


5. "**Right click**" in the reactions window. Select "**Load Reaction**" and choose the file "**ReactionsADCModel.pkml**".
6. Mark all four reactions via holding "**CTRL**" and click "**OK**".

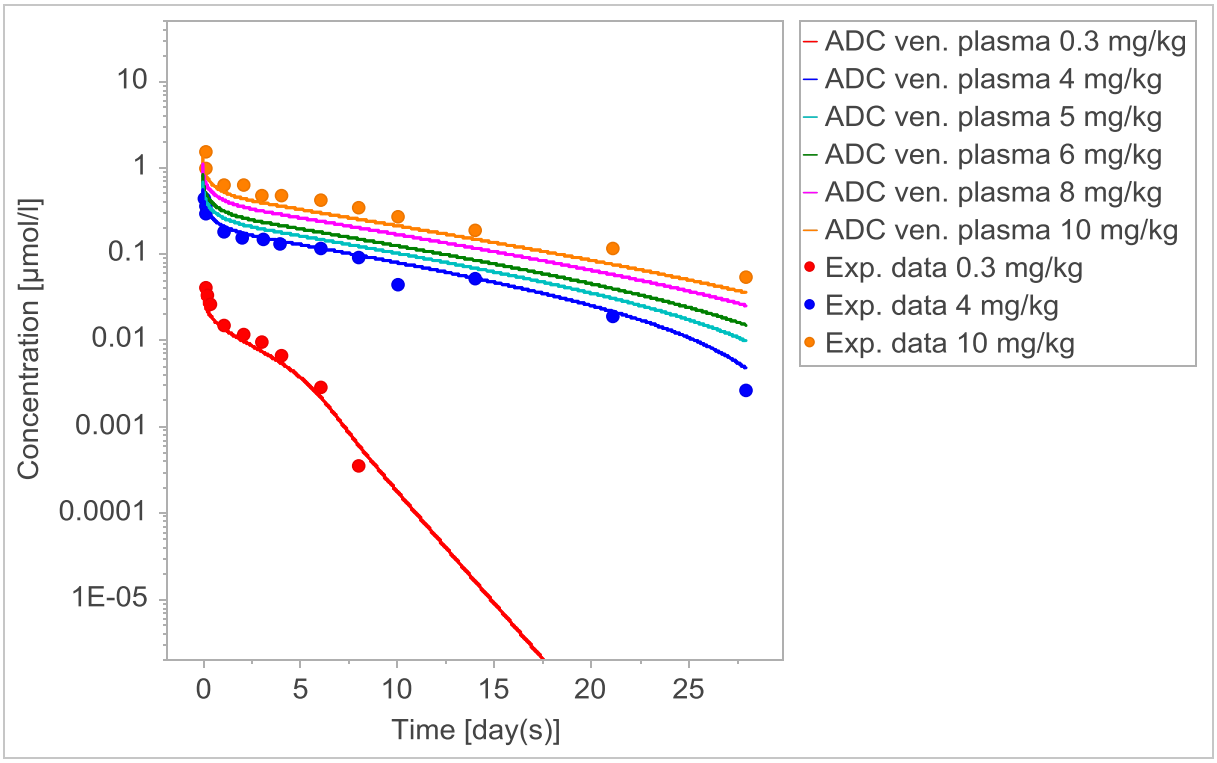
7. The reactions are integrated.
8. “**Right click**” in the reactions window and select “**Layout**”. Within the “**Layout**” menu, select “**AutloLayout children**”.



9. Check the reaction scheme. All important processes should be integrated now.
10. Close your MoBi project.
11. Open the File **Ex_1_ADC_Tox_Modeling_9.mbp3** where you find the combined model for ADC and Toxophore modeling together with experimental data. In the Favorites you find key parameters.

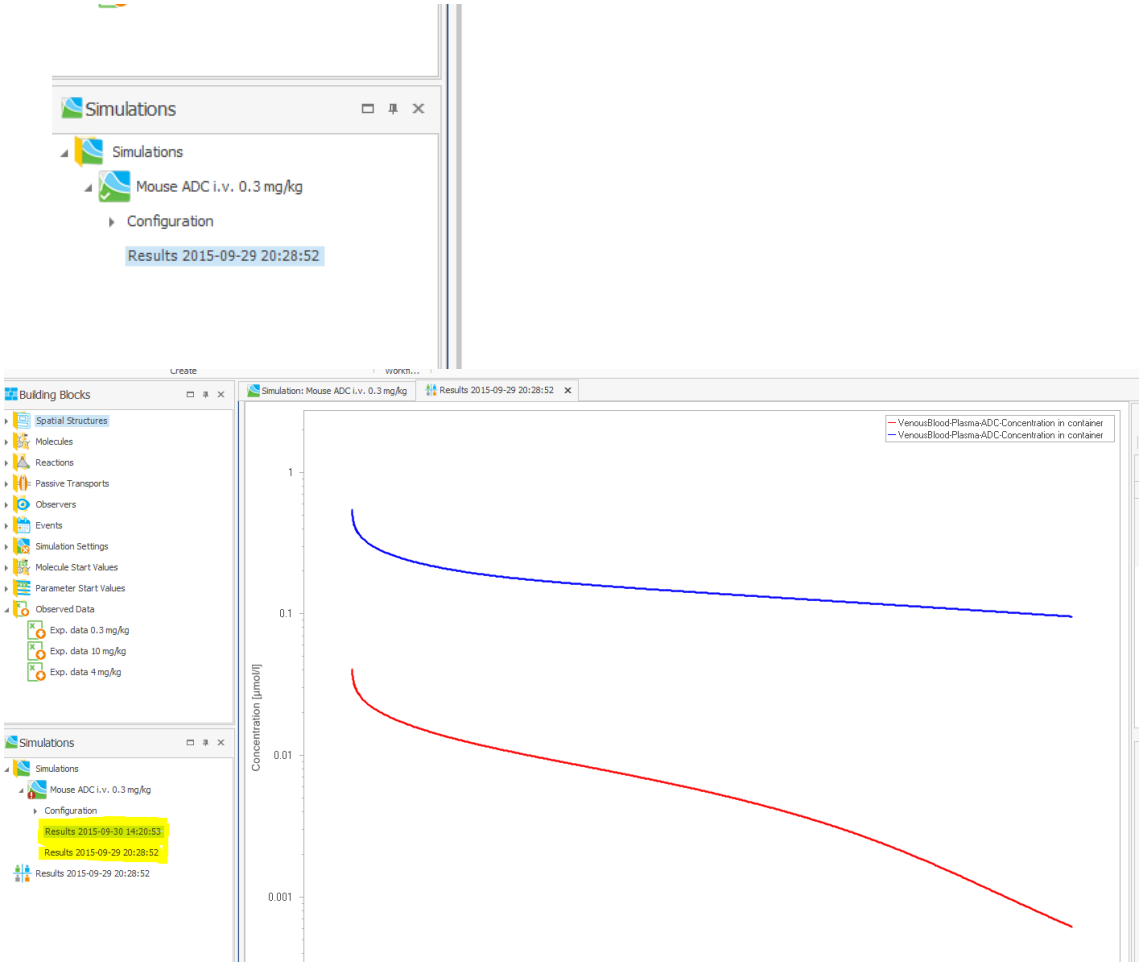


12. Simulate the model for several dosings and compare the results to the experimental data for 0.3 mg/kg, 4 mg/kg and 10 mg/kg

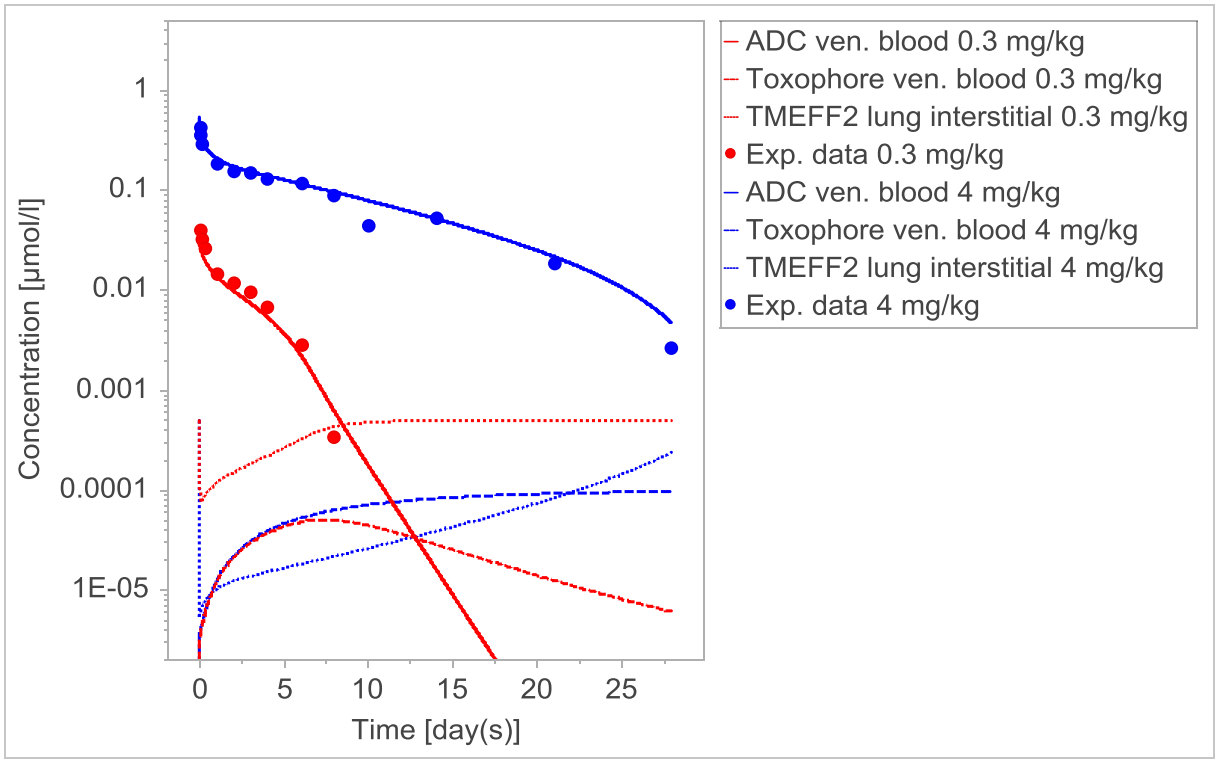

Concentration [µmol/l]
10
1
0.1
0.01
0.001
0.0001
1E-05
0
5
10
15
20
25
Time [day(s)]
ADC ven. plasma 0.3 mg/kg
ADC ven. plasma 4 mg/kg
ADC ven. plasma 5 mg/kg
ADC ven. plasma 6 mg/kg
ADC ven. plasma 8 mg/kg
ADC ven. plasma 10 mg/kg
Exp. data 0.3 mg/kg
Exp. data 4 mg/kg
Exp. data 10 mg/kg

Tip:

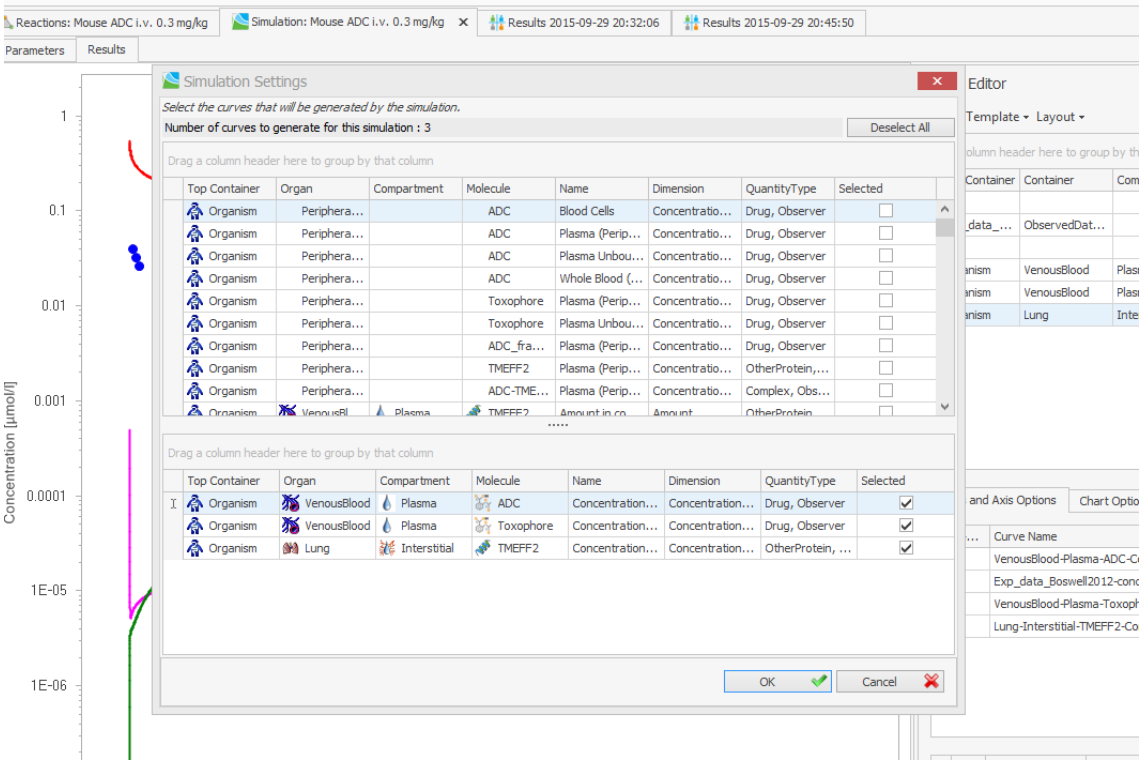
- *You can create one simulation for one dose; however, it is also possible just to change the dose in the existing simulation.*
- *To compare several simulations in one window, you can double click on the Results of your last simulation. The Results open in a new window. Drag & drop other Results into this window to be able to illustrate them in the same window.*



13. Which is the critical dose, where target mediated clearance needs to be taken into account?
14. Simulate and analyze the different behavior of the Toxophore concentration in venous blood and the TMEFF2 concentration in the interstitial of the lung for different doses.



15. *Optional: Change the degradation rate "**k_deg**" and "**reference concentration**" of TMEFF2 to analyze the effect of these parameters on the target-mediated drug clearance.*



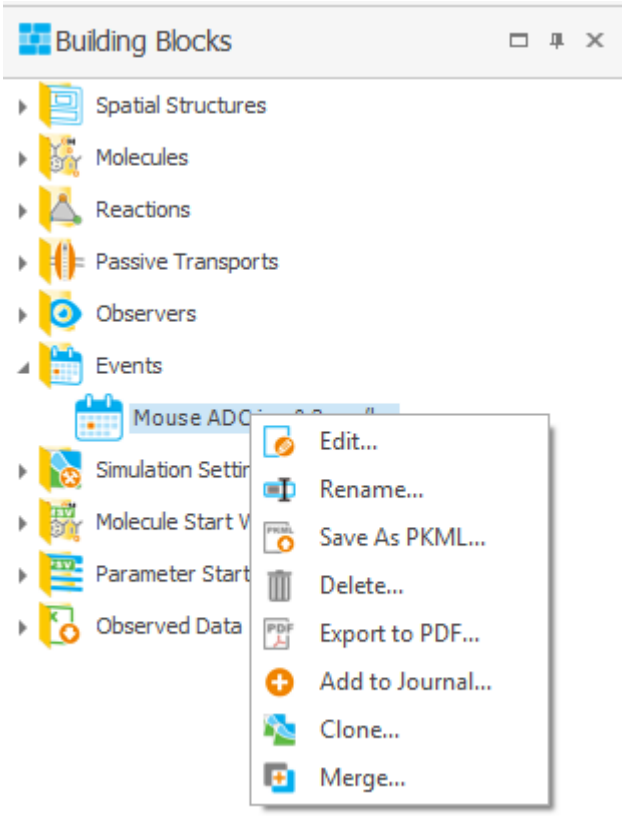
Part 3 – Parameter Identification, fitting turn over rate constant.

Objectives:

- set up parameter identification;
- fit TMEFF2 receptor turnover rate (k_deg);
- asses quality of the fit;

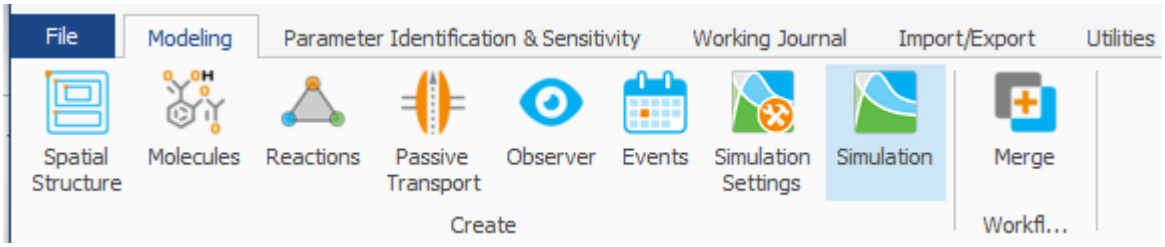
Set Up Simulations for further Parameter Identification.

1. Open file 01_PI_ADC.mbp3, it is equivalent to file build at the end of the previous chapter, however value of parameter k_deg is set to arbitrary value as goal of this exercise is to do parameter identification and find value of k_deg that best describes data.
2. Create IV administrations with doses 4 and 10 mg/kg.
    a. Clone existing IV administration: Building Blocks->Events->Clone;

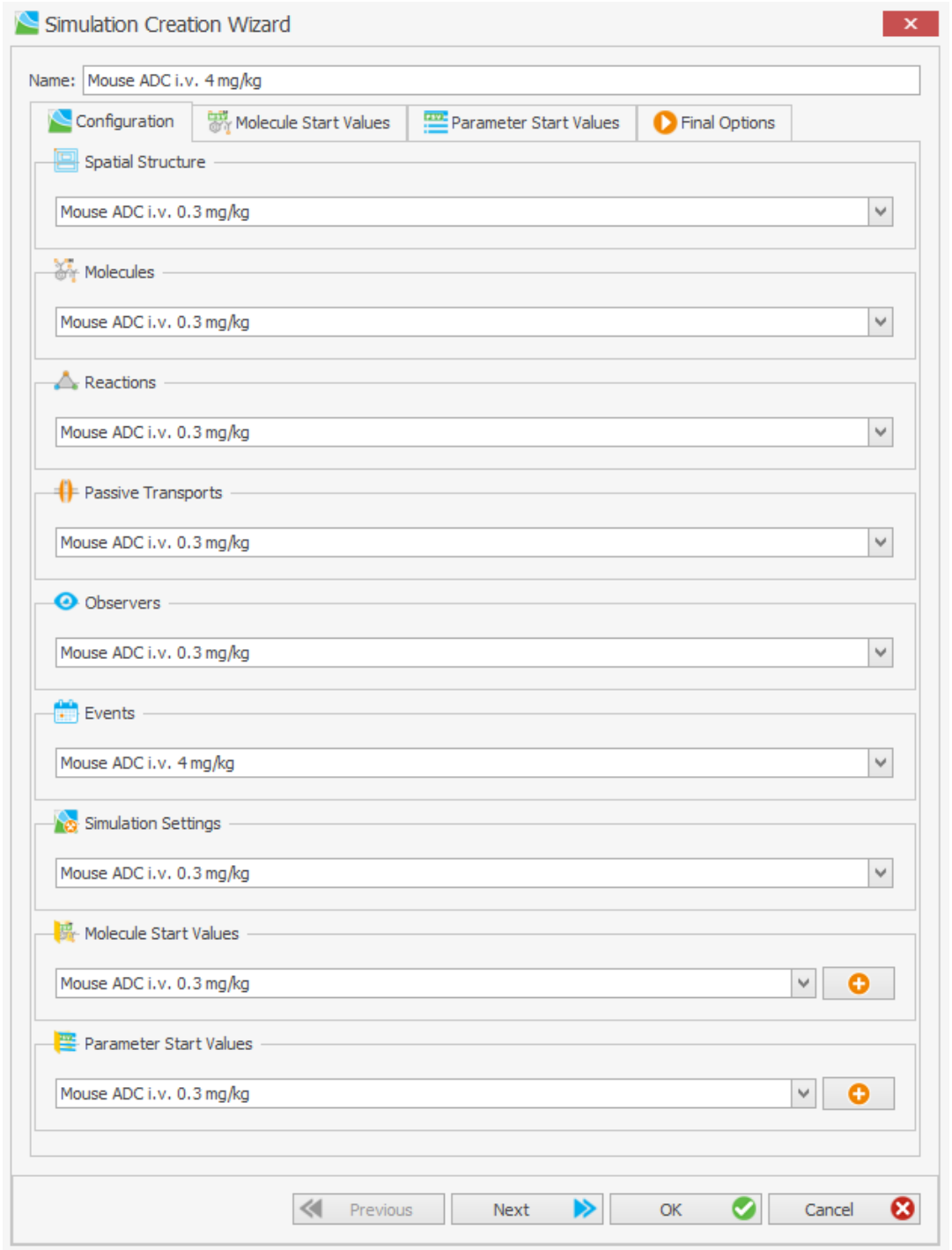


    b. Name new IV administration as ‘Mouse ADC i.v. 4 mg/kg’
    c. In the clones administration set dose to 4 mg/kg: in the Building Block double click Mouse ADC i.v. 4 mg/kg, in the Building Block Editor go to Applications -> i.v. 0.3 mg/kg -> Application_1 -> ProtocolSchemaItem, in the Properties Editor set DosePerBodyWeight to 4 mg/kg.
    d. Repeat steps a-c and create 10 mg/kg administration.
3. Create Simulations with IV doses of 4 and 10 mg

a. On the Ribbon select Modelling -> Simulation

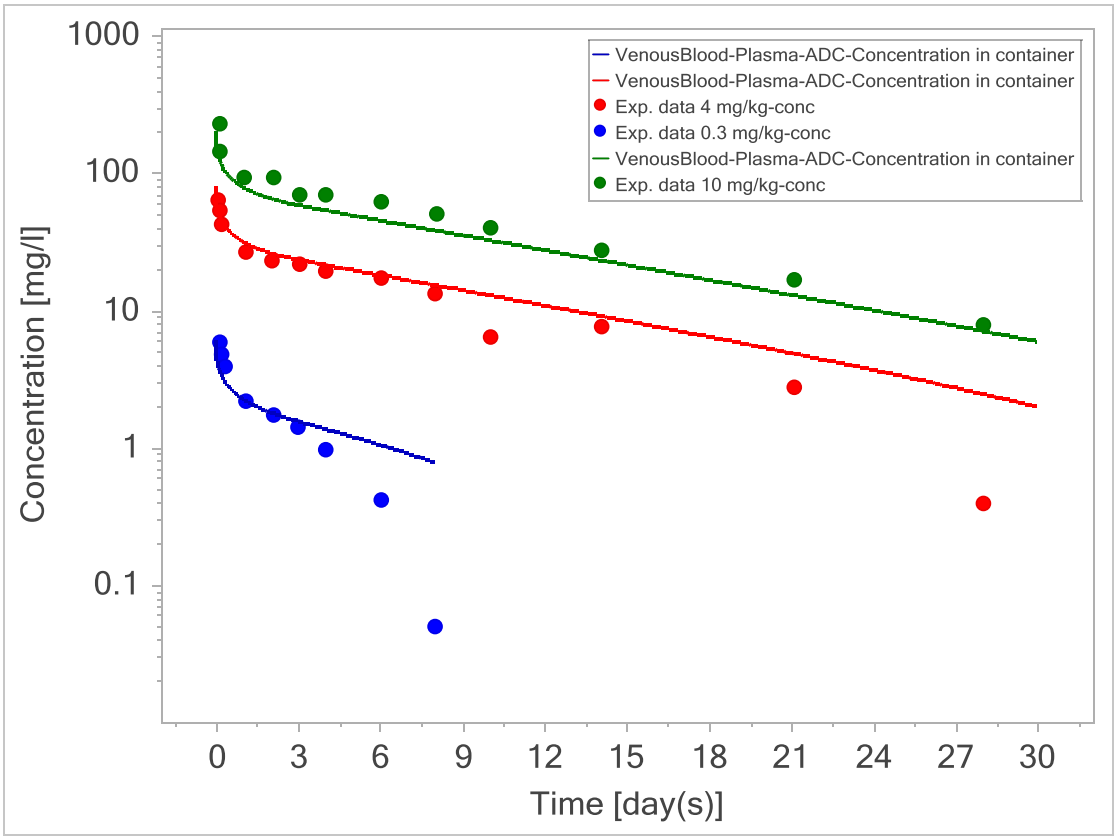


b. Name new simulation as Mouse ADC i.v. 4 mg/kg



c. Leave choice for all building blocks at their default values except for Events, in the Event drop down menu chose building block corresponding to 'Mouse ADC i.v. 4 mg/kg'.
d. Click OK
e. Repeat steps a-d and create simulation for 10 mg/kg dose.

4. Run simulations as described at the end of Part 2: – ADC II -TMDD and Model Coupling.

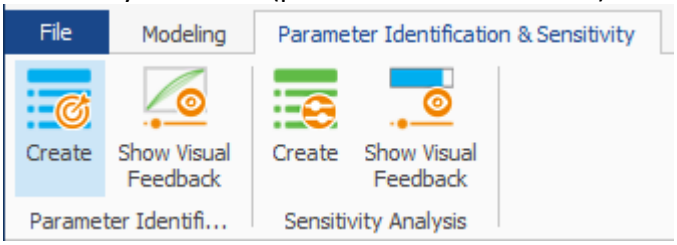


Plasma concentration of ADC should look as depicted above. This plot is very similar to that previously obtained in PK-Sim (Part 1: ADCI set up) before addition or receptor turn over model. Current model has receptor turnover model, however numerical value of turnover constant is low, so that the receptor turnover has limited effect on the plasma concentration. The next step is to set up parameter identification routine and find optimal value for k_deg.

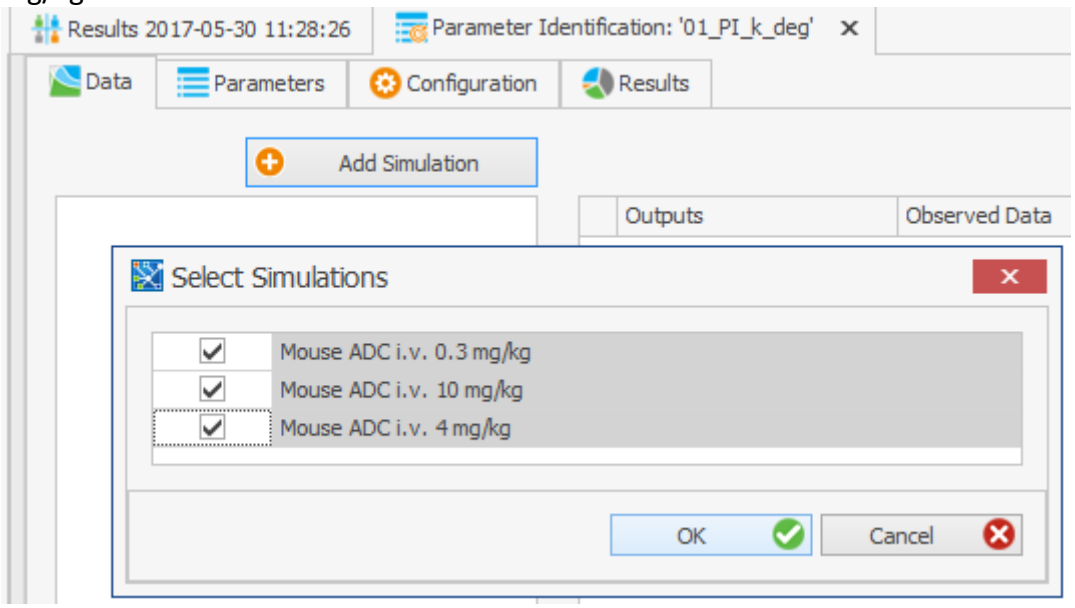
Set Up Parameter Identification.

Open file 02_PI_ADC.mbp3

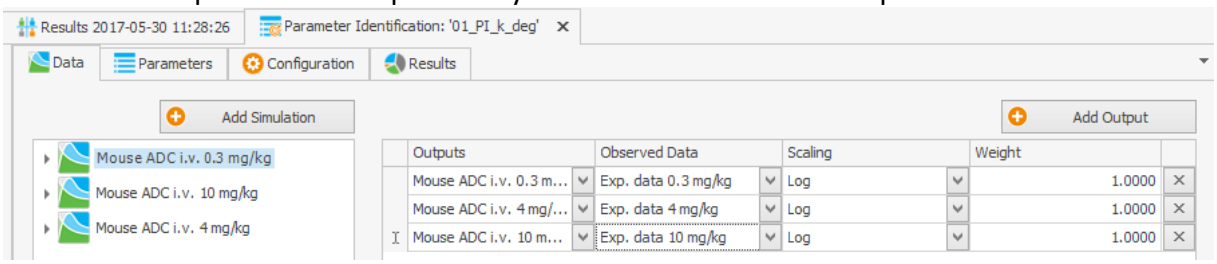
1. Create Parameter Identification: on the ribbon select Parameter Identification & Sensitivity -> Create (parameter identification).



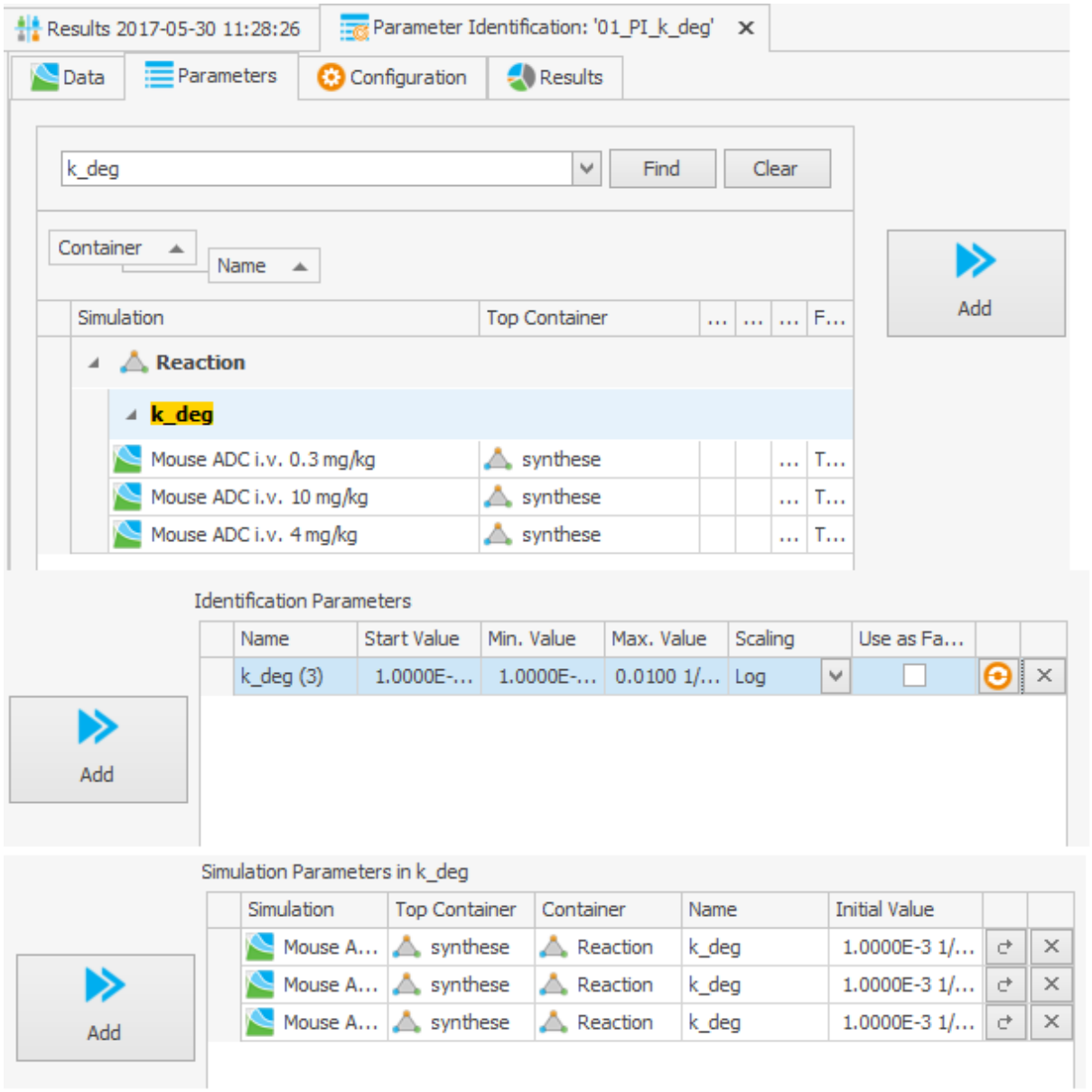
2. Rename newly created Parameter Identification 1 to 01_PI_k_deg.
3. Double click 01_PI_k_deg and in the Data click Ass Simulation and choose three models: Mouse ADC i.v. 0.3 mg/kg, Mouse ADC i.v. 4 mg/kg, Mouse ADC i.v. 10 mg/kg.



4. Match simulation output with observed data via add output interface. Note data and model output have to be previously matched in the simulation plot.



5. Add parameter for identification: switch to Parameters menu, in the Find field enter k_deg and click Find.
6. Select shown parameters and click upper button Add. Note the difference between Identification Parameters (upper) menu and Simulation Parameters (lower) menu. In this particular case Identification Parameters have single entry k_deg(3), indicating that parameter k_deg will be set equal in three models. Lower menu Simulation Parameters details on simulations related to shared parameter k_deg.



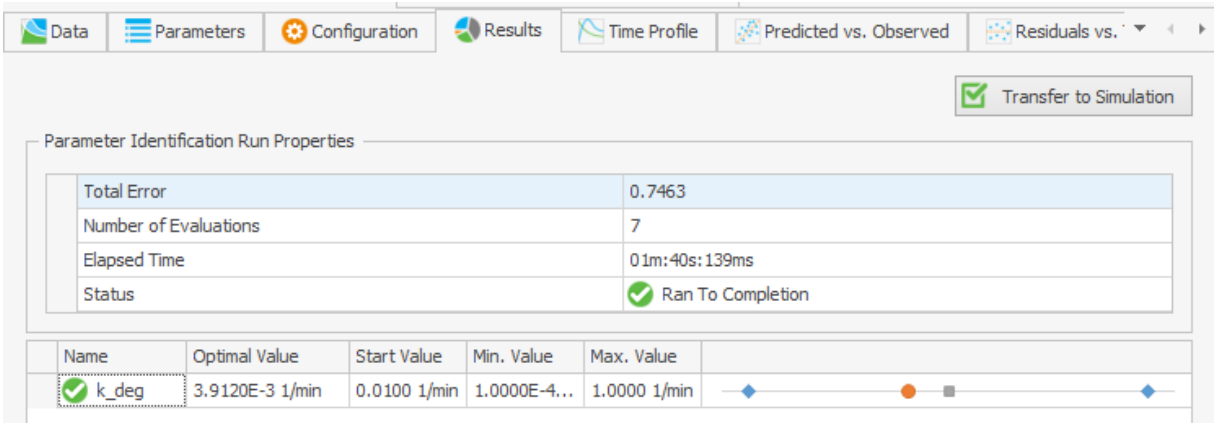
Configuration.

Configuration menu enable users to select optimization algorithm and tweak their parameters. In this example we use default Levenberg-Marquardt algorithm with defaults settings. Information regarding PI algorithms and their settings is available in Systems Biology Software Suite Manual, Chapter 34. Shared Tools – Parameter Identification.

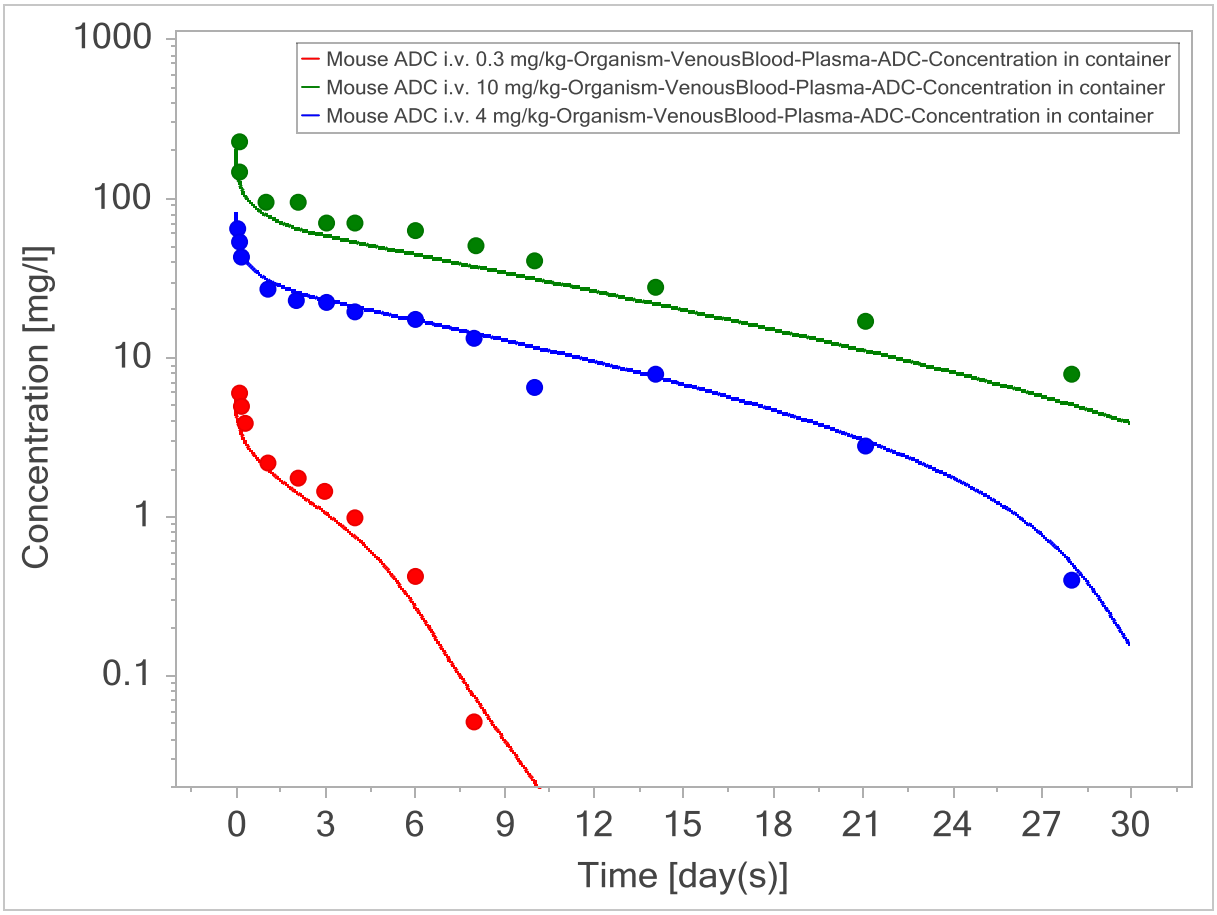
Parameter Identification.

Start Parameter Identification by clicking Run on the ribbon. Visual Feedback Provides a real time diagnostic for PI. In particular it depicts best and current value for parameters, error history, predicted vs observed and time profile fit. For this particular problem PI should take 1-2 min, depending on the PC.

Results insert contains info regarding identified parameters, in particular, Total Error and optimal parameter value, in this case particular case k_deg = 0.391 1/min.



Visual examination of fitted time profiles (accessible via Time Profile button on the ribbon) for all three doses shows that optimized model can correctly describe drop in ADC concentrations due to the target mediated clearance.



Parameter Identification Diagnostic.

Parameter identification interface provides a number of diagnostic plots to assess the quality of the fit. Some of them are described and discussed below.

Predicted vs. Observed

For each observed concentration value a point is plotted with observed value as x-Value and corresponding simulation value as y-Value. Predicted vs. Observed provides a bird view assessment for the quality of the fit. In this particular case, concentration overall are well predicted, however for doses 0.3 mg/kg (in red) points are slightly below the equality line, therefore indicating that model underpredicts plasma concentration for ADC.

Residual vs. Time

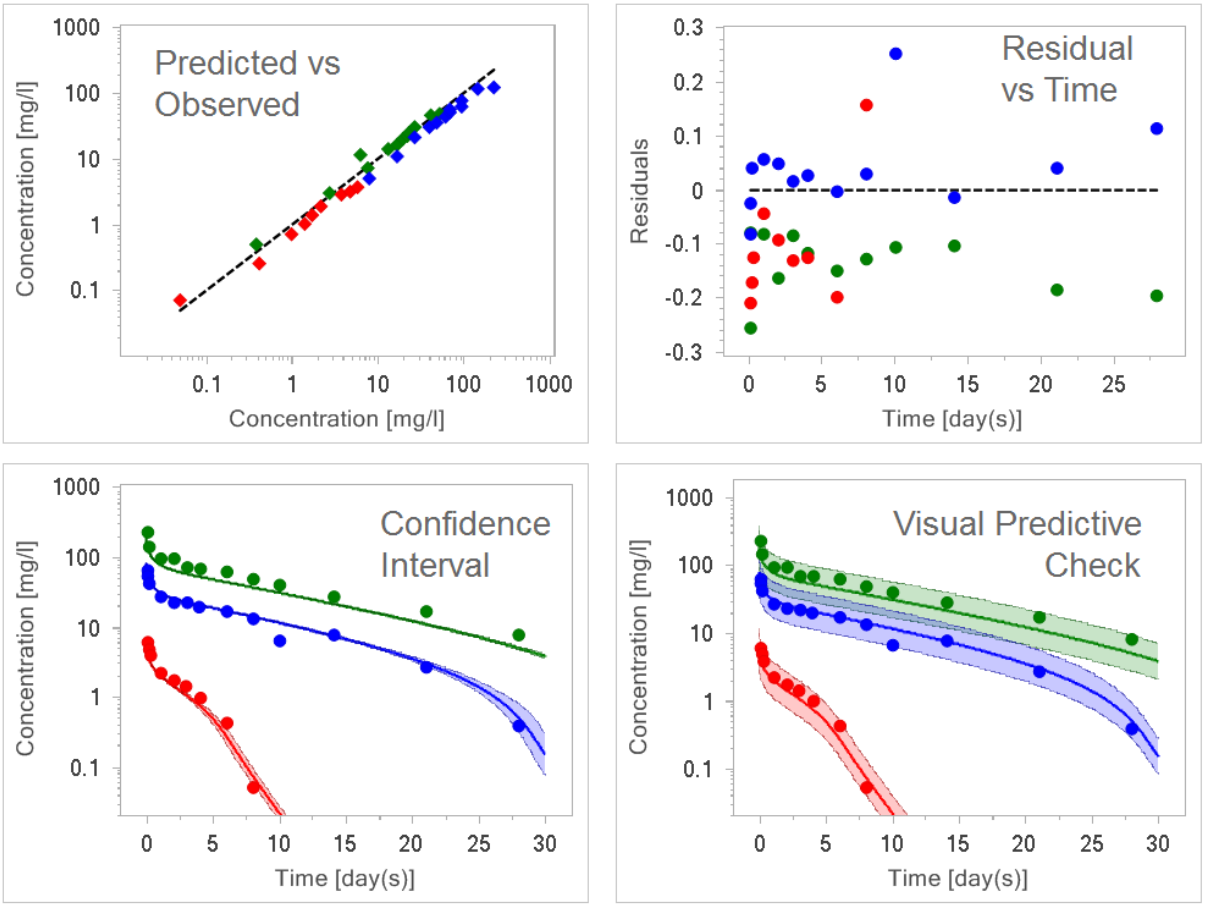
Residuals are plotted vs time. Note that residuals are as used in PI and therefore are scaled and weighted.

Confidence Interval

Confidence Interval chart displays the 95% confidence interval of the model error, which is based on the uncertainty of estimated parameters.

Visual Predictive Check Interval

This chart displays the 95% Visual Predictive Check interval, which corresponds to the uncertainty based on the data error. The data error is the standard deviation. of the distribution of the used observed data.



Optimized model is saved as 03_PI_ADC.mbp3.