esqLABS
WE EMPOWER HEALTH CARE

# Towards an OSP IVIVE toolbox

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OSP community conference 2024

# Why *in vitro* ?

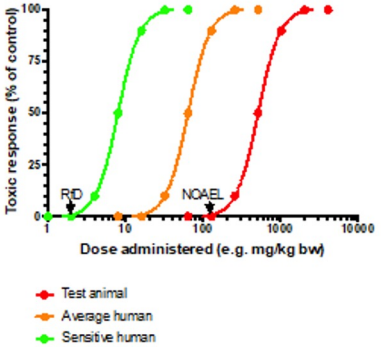
Only using animals experiments in drug development and chemical risk assesment leads to several issues...

## Costs

-Time and Money
- Waste

## Ethics


## Science




- Black-box models
- Species extrapolation
- Precision/variability
- Mixtures

# What can in vitro models be used for?

**A**bsorption

**D**istribution

**M**etabolism

**E**limination

- *Caco-2 assays*
- *PAMPA assays*
- *Rapid dialysis assays*
- *SP(M)E*
- *Membrane transporter studies*
- *Hepatocytes clearance*
- *Biliary excretion*
- *Placental transfer (e.g. BeWo cells)*

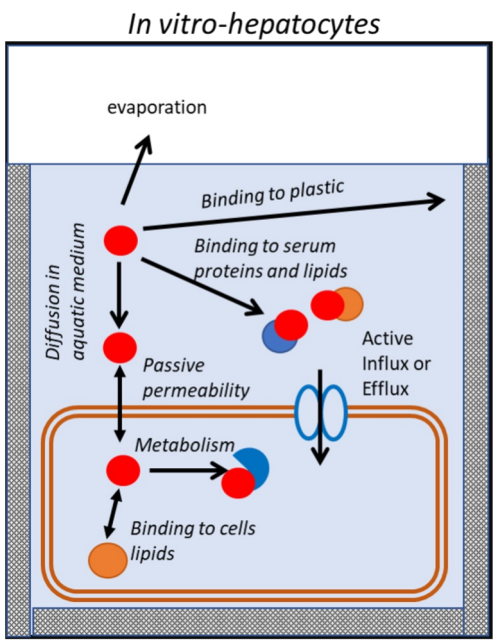
… but also for characterizing the dynamics/effect of the chemical

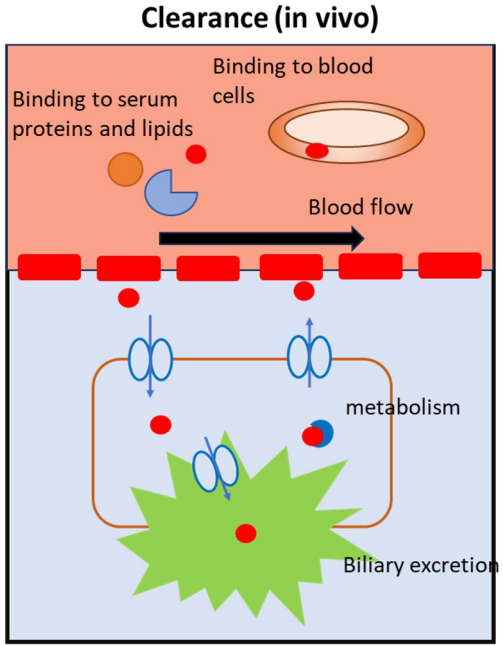
# In vitro-in vivo extrapolation

## Results from in vitro assays cannot be used directly for in vivo predictions!

Clearance measured *in vitro* is not the same as in vivo:

- Differences in serum concentration
- Differences in cell-microsomes number/surface area
- Difference expression/activity or certain transporters or enzymes
- Possibility do bind to plastic or evaporate



# In vitro-in vivo extrapolation

Dependently on the *in vitro* assay used and its characteristics, in vitro clearance can be quite different…

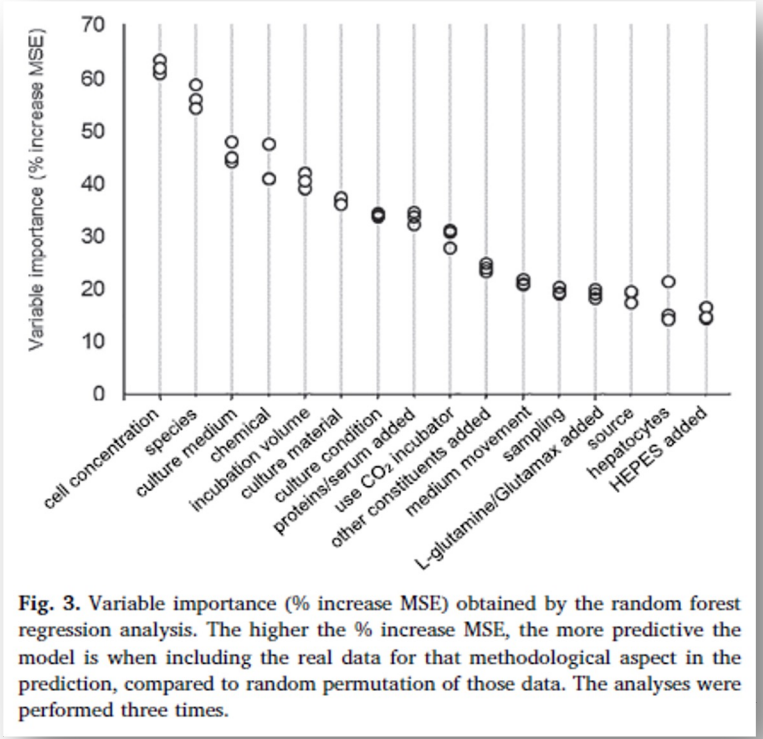



**Fig. 3.** Variable importance (% increase MSE) obtained by the random forest regression analysis. The higher the % increase MSE, the more predictive the model is when including the real data for that methodological aspect in the prediction, compared to random permutation of those data. The analyses were performed three times.

Towards harmonization of test methods for *in vitro* hepatic clearance studies

Jochem Louisse[a,*], Martin Alewijn[a], Ad A.C.M. Peijnenburg[a], Nicole H.P. Cnubben[b], Minne B. Heringa[b], Sandra Coecke[c], Ans Punt[a]

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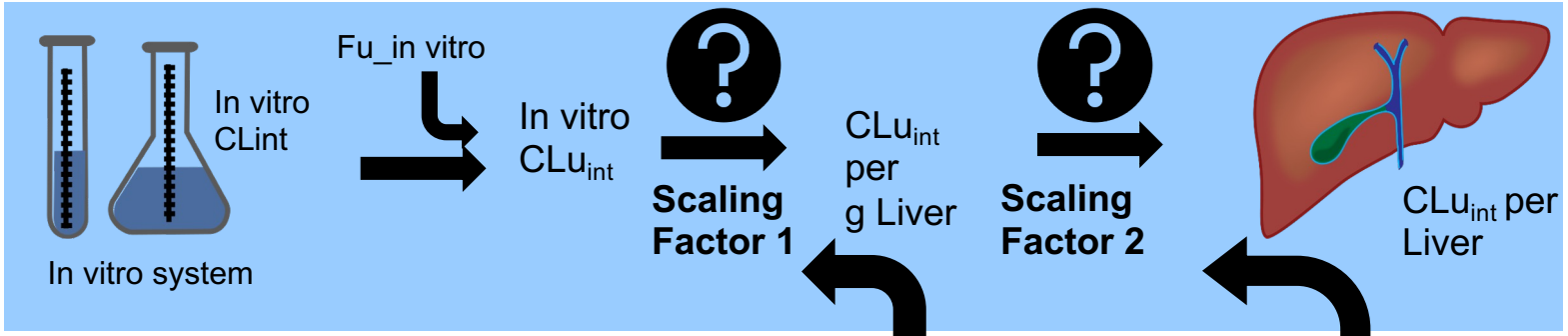
[c] European Commission Joint Research Centre (JRC), Ispra, Italy

# Standard IVIVE framework

IVIVE for Hepatic Clearance

This is different in PK-Sim




HLM $\frac{\mu L * min^{-1}}{mg\ mic\ protein}$ X Microsomal Protein per g Liver (MPPGL)

HHEP $\frac{\mu L * min^{-1}}{10^6\ cells}$ X Hepatocytes per g Liver (HPGL)

rhCYP $\frac{\mu L * min^{-1}}{pmol\ P450\ isoform}$ X $\frac{pmol\ p450\ isoform}{mg\ mic\ protein} * MPPGL$

X Liver Weight

# Standard IVIVE framework

To parameterize hepatic clearance PK-Sim assumes that you have the half-life :
tHalf (min)=Ln2/$K_{elim}$(min-1)
Or that you have the Specific clearance in min-1*
**despite being in the same units Kelim and Specific clearance are not the same*

| Parameters used in simulations | |
|---|---|
| Specific clearance | 0 1/min |

**Description**

Intrinsic clearance normalized to the volume where the process occurs

**Formula**

0.693 / tHalf * N_Liver / N_cells / fu_mic / f_cell

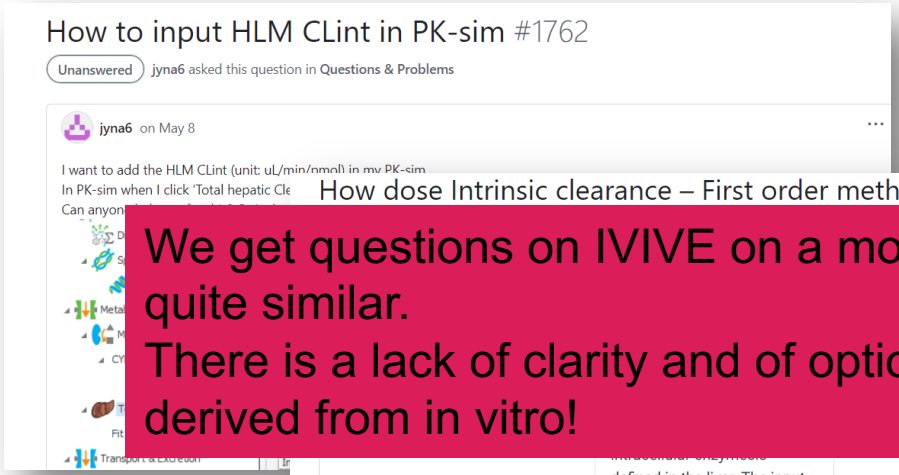
**References**

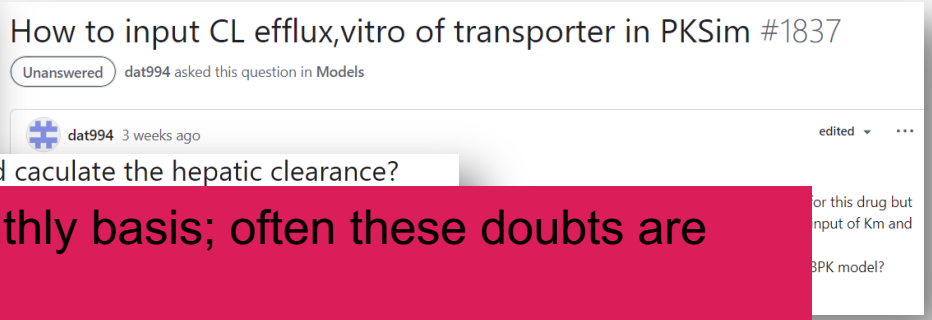
*f_cell* is defined as: ..|Fraction intracellular (liver)
*fu_mic* is defined as: ..|Fraction unbound (assay)
*N_Liver* is defined as: ..|Number of cells/g liver tissue
*N_cells* is defined as: ..|Number of cells/incubation
*tHalf* is defined as: ..|t1/2 (hepatocyte assay)

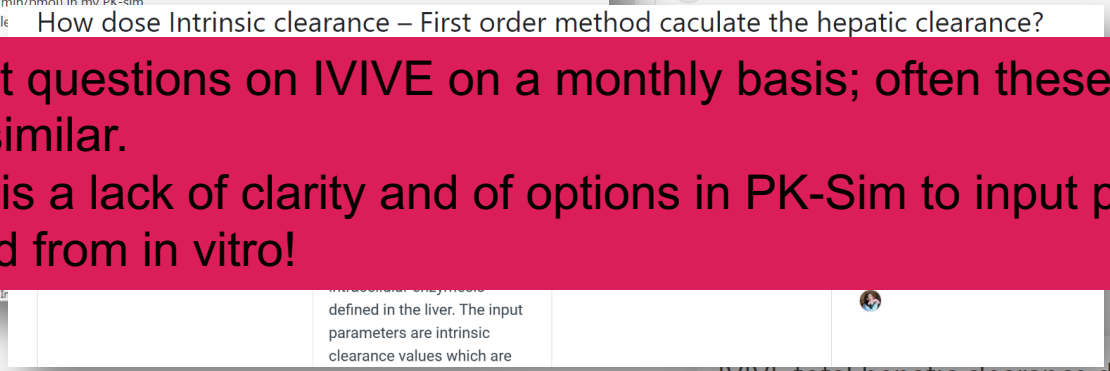
But often the value we get from the laboratory or literature is neither half-life neither specific clearance. Frequently it is uL/min/million hepatocytes. How do we use this value? Besides what is the fraction unbound in the in vitro assay?

# IVIVE in PK-Sim

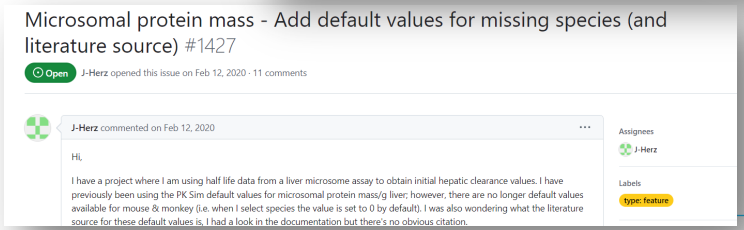
## Questions on the OSP Forum

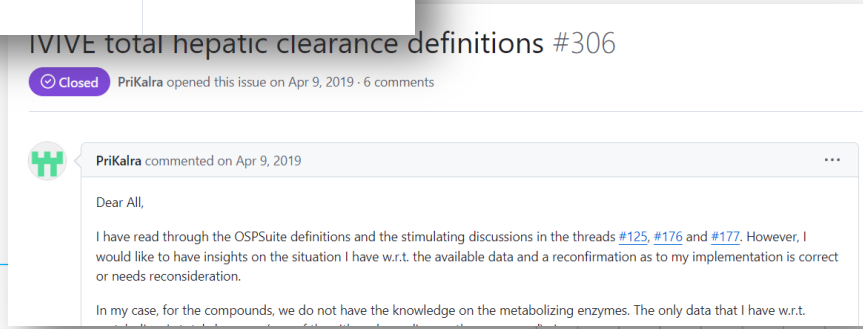


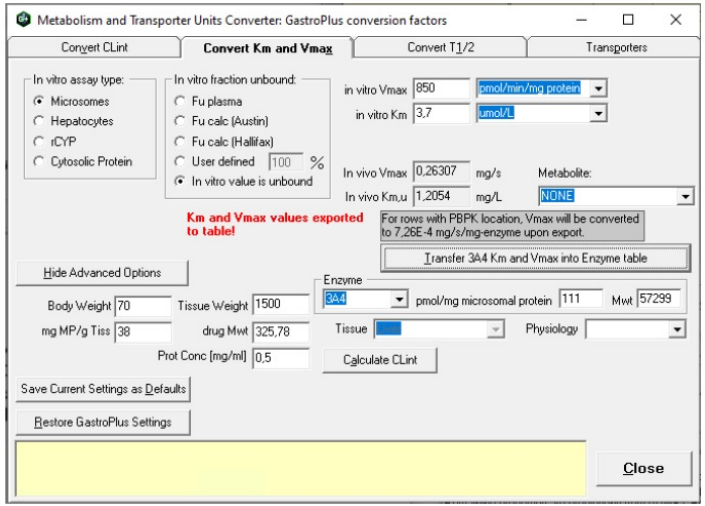

We get questions on IVIVE on a monthly basis; often these doubts are quite similar.
There is a lack of clarity and of options in PK-Sim to input parameters derived from in vitro!



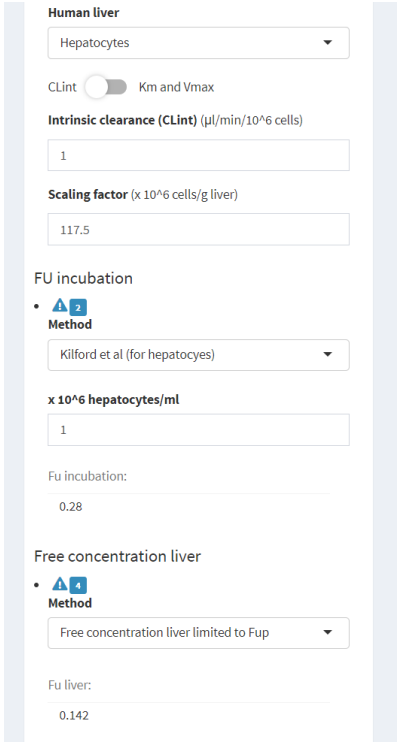



# IVIVE in other softwares

## Gastroplus metabolism and transporter module




## qivive.tools.wur.nl




## The Simcyp™ In Vitro Data Analysis (SIVA) Toolkit

- Metabolic Intrinsic Clearance
- Mechanistic Permeability and Transporter Substrate/Inhibitor
- Mechanistic Enzyme Inhibition
- Dissolution and Precipitation
- Drug Solubility
- Virtual In Vitro Distribution
- Surface pH (Dissolution Models)

# OSP IVIVE toolbox

## Motivation

We need a toolbox that seamlessly integrates different types of *in vitro* parameters in the OSP tools.

## Strategy

-In a tiered approach collect up to date knowledge on IVIVE of different ADME processes and write the extrapolation module in R.

-Report the R modules in Quarto with the appropriate references and assumptions.

-Transform code in shiny app for general use.

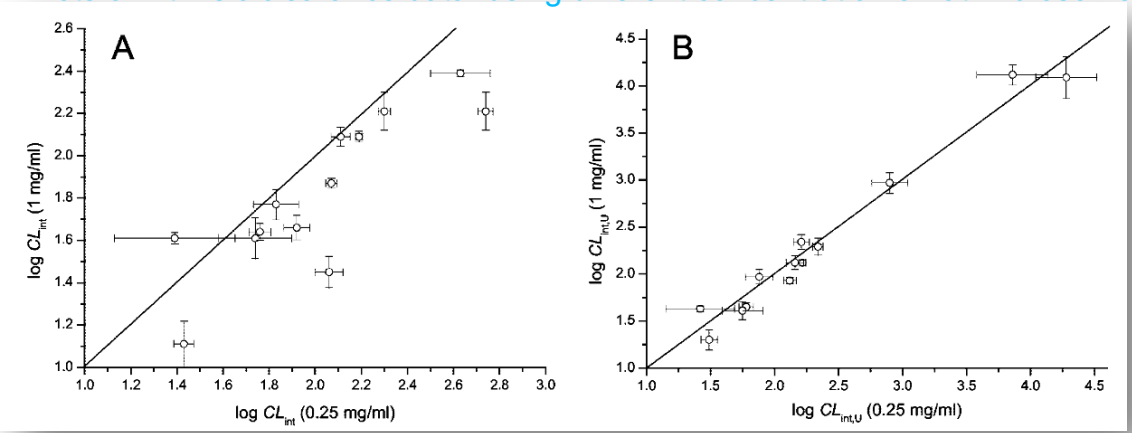
## Progress

Integrate the simple IVIVE for metabolic clearance in R .

# OSP IVIVE toolbox

## Progress: Characterizing unspecific binding

Plots of intrinsic clearance data  using different concentration of rat microsomes




*Austin et al, (2002) drug metabolism and disposition*

- The clearance derived in vitro, even after normalizing per number of cells or mg microsomal protein is still not completely agnostic to these factors.
- We need the clearance of the UNBOUND fraction.
- If it is not measured then we need to predict.

# OSP IVIVE toolbox

## Progress: Characterizing unspecific binding

There are a few QSARs to calculate the fraction unbound in hepatocytes and microsomal experiments.
Composition-based QSARs can be used for other cell system if content is known.
Ideally we have the same assumptions for binding in the in vitro assay that for the tissue partitioning!

**Table 2.** Prediction Methods Tested in This Study as Reported from the Literature[7,8]

| Models | Model Equations |
|---|---|
| **Composition-based model**[7] | **General equations:** |
| | $fu_{inc} = \frac{1}{P_{csa}}$ |
| | $P_{csa} = \frac{(1+I_m)\cdot F_{wm} + P_{nla}\cdot F_{nlm} + I_m\cdot P_{apla}\cdot F_{aplm} + (1+I_m)\cdot P_{pra}\cdot F_{prm}}{(1+I_m)}$ |
| | **Predominantly neutral compounds and ionized acids:** |
| | $P_{csa} = F_{wm} + \frac{P_{nla}\cdot F_{nlm}}{1+I_m}$ |
| | $LogP_{nla} = LogP_{ow}$ |
| | $I_m = 0$ or $I_m = 10^{pH\,-\,pKa}$ |
| | **Predominantly ionized bases (at least one $pK_a \geq 7.0$):** |
| | $P_{csa} = F_{wm} + \frac{P_{nla}\cdot F_{nlm} + I_m\cdot P_{apla}\cdot F_{aplm}}{1+I_m}$ |
| | $P_{apla} = \left[P_{ea} - \frac{(1+I_e)\cdot F_{we} + P_{ow}\cdot F_{nle}}{1+I_p}\right]\cdot\frac{1+I_p}{I_e\cdot F_{aple}}$ |
| | $I_m, I_e, I_p = 10^{pKa\,-\,pH}$ |
| **Regression equation**[8] | **Predominantly neutral compounds and ionized acids:** |
| | $fu_{inc} = \left[\frac{1}{1+C_h\times 10^{0.40\cdot \log P_{ow} - 1.38}}\right]$ |
| | **Predominantly ionized bases (at least one $pK_a \geq 7.0$):** |
| | $fu_{inc} = \left[\frac{1}{1+C_h\times 10^{0.40\cdot \log D_{ow} - 1.38}}\right]$ |

- A QSAR based on Poulin assumptions was already available
- New “QSARs” based on PK-Sim, Rodgers and Rowland and Schmitt assumptions
- Also added plastic partition and flags for volatility

# OSP IVIVE toolbox

## Progress: Characterizing unspecific binding

Also important to validate ! Not just against experimental Fu but also against in vivo measured clearance!

### Function 1-Calculate composition

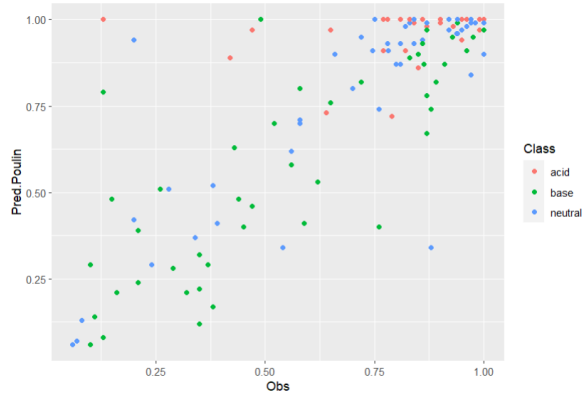
```
> getInVitroCompartment(typeSystem="microsomes",FBS=0,microplateType=24,volMedium=0.5,cMicro=1)
$cCellNL
[1] 0.0002611111

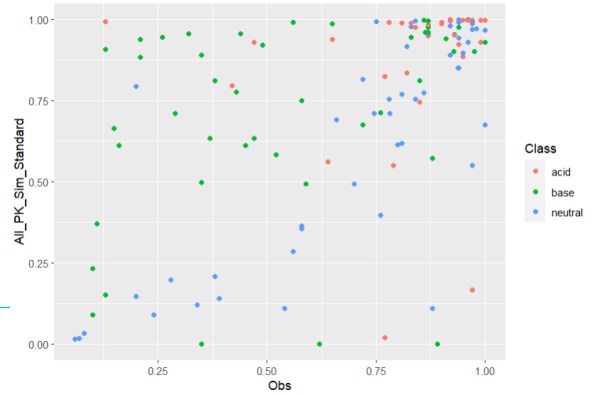
$cCellNPL
[1] 0.0007261556

$cCellAPL
[1] 0.0001594
```

### Function 2-Calculate Fu based on QSAR, chemical properties and composition

```
> FractionUnbound(partitionQSPR="All PK-Sim Standard",logLipo=3,ionization=c("acid",0,0), typeSystem="hep
atocytes",FBS=0,microplateType=96, volMedium=0.22,pKa=c(6,0,0),hlcAt=1E-6,cCells=2)
[1] 0.7058107
> FractionUnbound(partitionQSPR="All PK-Sim Standard",logLipo=3,ionization=c("acid",0,0), typeSystem="hep
atocytes",FBS=0.1,microplateType=96, volMedium=0.22,pKa=c(6,0,0),hlcAt=1E-6,cCells=2)
[1] 0.5907571
```



# OSP IVIVE toolbox

## Progress: type of clearance and units

Clspe (/min)= **Cl** (ml/min/cell) x **N_liver**(cell/g liver)/ **fu_mic**/ **f_cells**

| Units value of Clearance | Multiplying to get Cl (ml/min/nr_cells) |
|---|---|
| **When in vitro clearance was not normalized by cell concentration** : | |
| (/min) | 1/**N_cells**(n cell invitro/mL) |
| (/s) | 60 (s/min) /**N_cells**(n cell invitro/mL) |
| (/h) | 1/**N_cells**(n cell invitro/mL) / 60 (min/h) |
| **When it is normalized per volume but not per cell number** | |
| (mL/min) | 1/numbercells (n cell invitro) |
| (mL/s) | 1/ numbercells (n cell invitro) x 60 (s/min) |
| (mL/h) | 1/ numbercells (n cell invitro) / 60 (min/h) |
| **When it is normalized by cell number but not volume :** | |
| (/min/n cell invitro) | VolMedium (mL) |
| (/s/n cell invitro) | VolMedium (mL) x 60 (s/min) |
| (/h/n cell invitro) | VolMedium (mL) / 60 (min/h) |
| **Right normalization but other units:** | |
| (mL/s/n cell invitro) | 60 (s/min) |
| (mL/h/n cell invitro) | /60 (min/h) |
| (uL/min/n cell invitro) | /1000 (uL/mL) |
| uL/s/n cell invitro) | 60 (s/min) / 1000 (uL/mL) |
| (uL/h/n cell invitro) | / 60 (min/h) / 1000 (uL/mL) |
| (L/min/n cell invitro) | 1000 (mL/L) |
| (L/s/n cell invitro) | 60 (s/min) x 1000 (mL/L) |
| (L/h/n cell invitro) | 1000 (mL/L) / 60 (min/h) |

*If units of cells is (/million cells) and not per cell, just divide Cl per million

# OSP IVIVE toolbox

## Progress: IVIVE of clearance

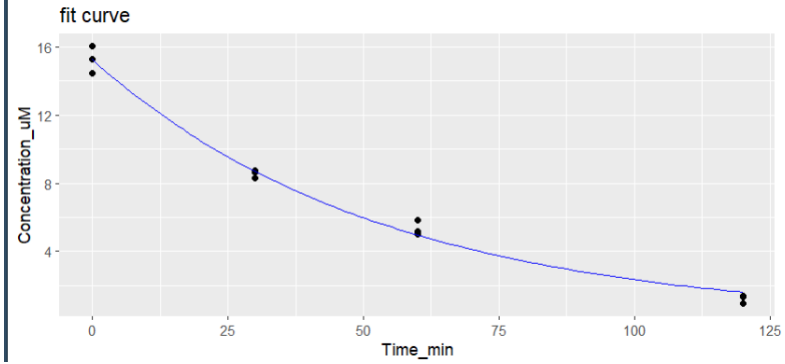
Can extrapolate different types of clearance!

The actual parent depletion curve:

```
> clearance_IVIVE(typeValue="decay experimentalcurve",expData=expData,
+                 partitionQSPR="All Schmitt",
+                 logLipo=5,
+                 ionization=c("acid",0,0),
+                 typeSystem="hepatocytes",
+                 FBS=0.0,pKa=c(3,0,0),
+                 hlcAt=1E-6,
+                 microplateType=96,
+                 volMedium=0.2,
+                 cCells=0.02)
85.92071    (5.31e+00): par = (0.01)
6.355533    (1.16e+00): par = (0.01642129)
2.767429    (5.79e-02): par = (0.01858765)
2.758336    (8.26e-04): par = (0.01871273)
2.758334    (1.50e-05): par = (0.01871094)
2.758334    (2.71e-07): par = (0.01871097)
Waiting for profiling to be done...
[1] 283.6378
>
```

Files | Plots | Packages | Help | Viewer | Presentation

Zoom | Export | Publish



# OSP IVIVE toolbox

## Progress: IVIVE of clearance

Can extrapolate different types of clearance!

From different types of calculated clearance:

```
> clearance_IVIVE(typeValue="in vitro clearance parameter",expData=0.3,
+                 units="mL/minutes/Millioncells",
+                 partitionQSPR="All Poulin and Theil",
+                 logLipo=2,
+                 ionization=c("neutral",0,0),
+                 typeSystem="hepatocytes",
+                 FBS=0,pKa=c(0,0,0),
+                 hlcAt=0.02,
+                 microplateType=96,
+                 volMedium=0.2,
+                 cCells=0.02)
[1] 62.26361
```

```
> clearance_IVIVE(typeValue="in vitro clearance parameter",expData=0.3
+                 units="mL/seconds/mg protein",
+                 partitionQSPR="All Poulin and Theil",
+                 logLipo=2,
+                 ionization=c("neutral",0,0),
+                 typeSystem="microsomes",
+                 FBS=0,pKa=c(0,0,0),
+                 hlcAt=0.02,
+                 microplateType=96,
+                 volMedium=0.2,
+                 cMicro=0.2)
[1] 1085.03
```

**Immediate next steps:**

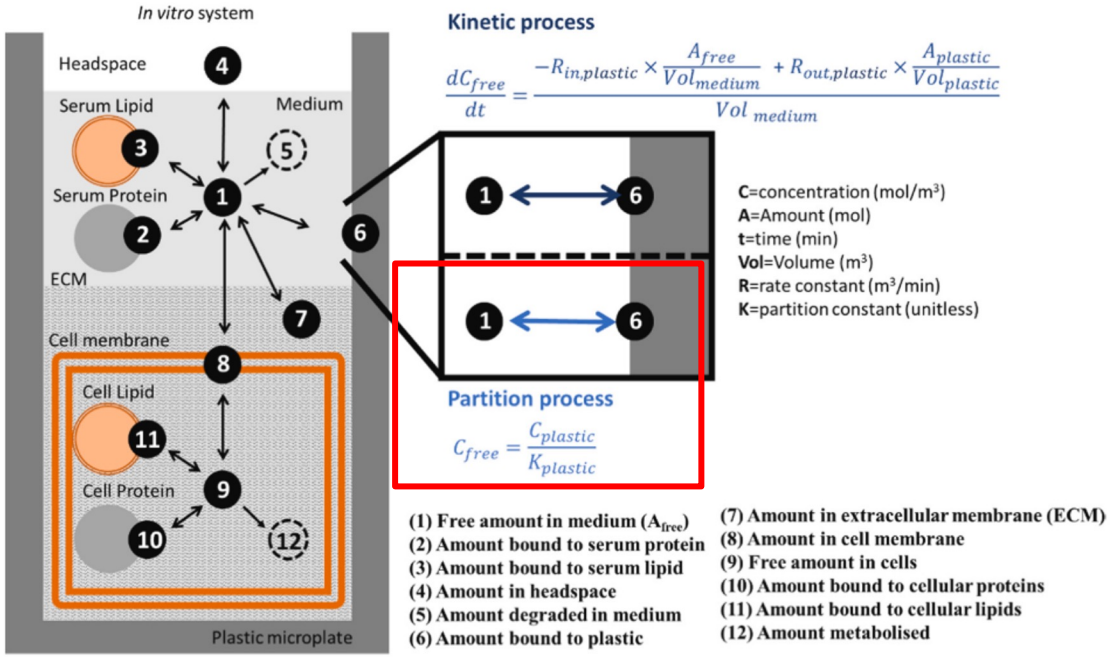
- Finish plugging Michaelis–menten kinetics (Does Km need to be correct for Fu or not?)
- Validate IVIVE of the metabolism with a dataset of chemicals
- Evaluate intersystem extrapolation factors (ISEFs) for main enzymes

# OSP IVIVE toolbox

## Simple (partition) IVIVE vs compartmental (kinetic) IVIVE

The current extrapolation protocol is assuming instant equilibrium.

But in some cases, this assumption gets further from reality with extensive impacts on the predictions
*e.g.* slow desorption from proteins, slow diffusion through the unstirred water layer, etc




Proenca et al (2021) Toxicology in vitro

# OSP IVIVE toolbox

## Simple (partition) IVIVE vs compartmental (kinetic) IVIVE

- For a few chemicals the Km values for P-gp seem to be different in different in vitro systems.
- This is at least partly because the efflux permeability was being calculated based on the total flux across the cell.
- In fact, the Km should be relative to the intracellular concentration.
- This can either be measured in tedious experiments of modelled with a compartmental model.

*Pharmaceutical Research, Vol. 27, No. 3, March 2010 (© 2010)*
DOI: 10.1007/s11095-009-0026-9

*Research Paper*

**Model Analysis of the Concentration-Dependent Permeability of P-gp Substrates**

Tatsuhiko Tachibana,[1,5] Satoshi Kitamura,[2] Motohiro Kato,[1] Tetsuya Mitsui,[1] Yoshiyuki Shirasaka,[3] Shinji Yamashita,[4] and Yuichi Sugiyama[2]

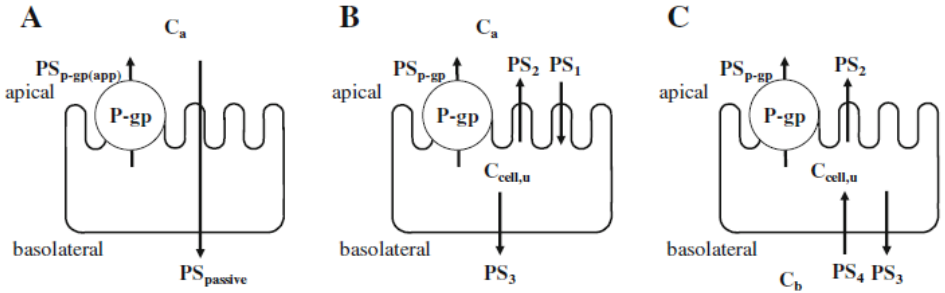



**Fig. 1.** Schematic diagram illustrating the PS products for the penetration of P-gp substrates across the cell monolayer. **A**, a previous model for apical-to-basolateral permeability considering only apical (donor) concentration; **B**, a new model for apical-to-basolateral permeability considering intracellular unbound concentration as the determiner of Km; **C**, a new model for basolateral-to-apical permeability.

# OSP IVIVE toolbox

## Oral absorption

Currently there is a internal QSAR that based on lipophilicity and Mweff calculates Pint.

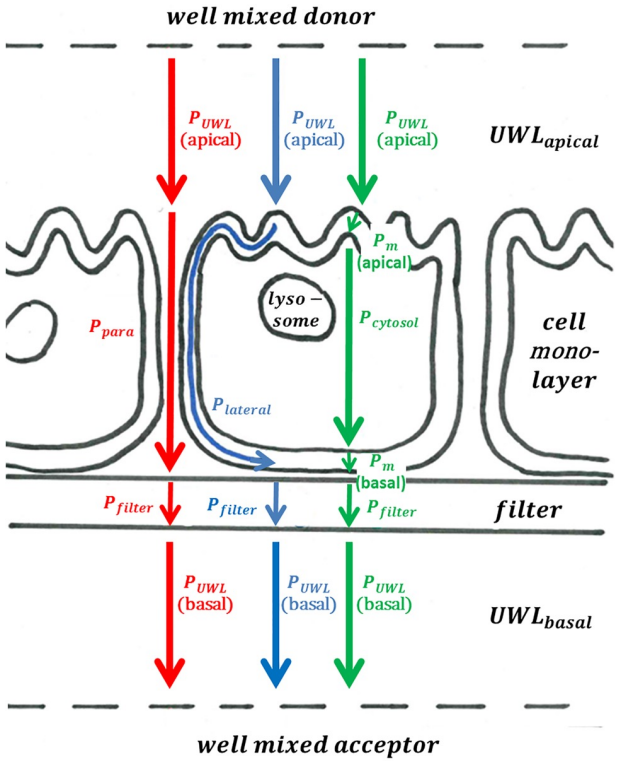
$$P_{\text{int}}(\text{MW}_{\text{eff}}, \text{MA}) = 265.796 \times \text{MW}_{\text{eff}}^{-4.49968} \times \text{MA}\,(\text{cm/s}) \quad (5)$$

And how to include Papp from Caco-2, PAMPA from in vitro or from other models?

Empirical vs Mechanistic →

↓

*Pint (cm/s)=a x Papp (cm/s) +b*



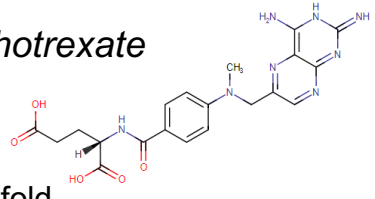

*Ref: Bitterman et al, Predicting apparent passive permeability of Caco-2 and MDCK cell-monolayers: A mechanistic model*

# OSP IVIVE toolbox

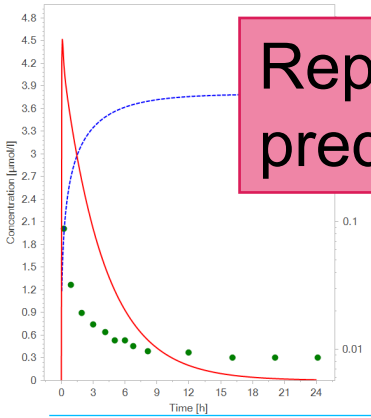
## Applicability domain

Using QSARs and other methods outside their applicability domain can lead to large mispredictions!

*Case-study of Methotrexate*

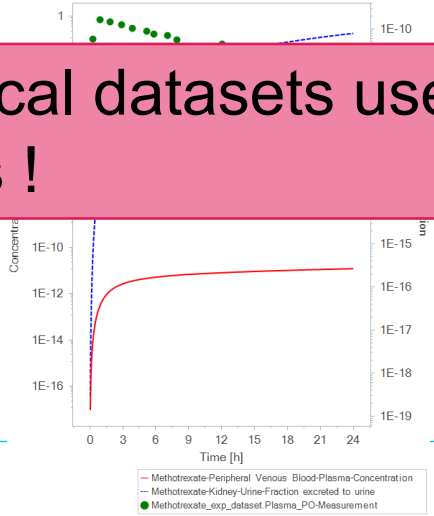


IV simulations
Simulations are within 2-fold



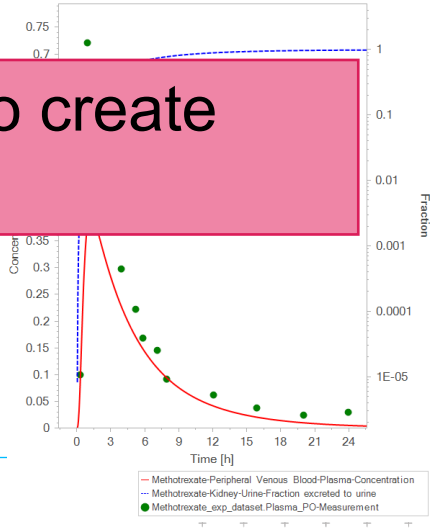

Pint =1.2e-12 cm/min

PO simulations using Pint from the system




Pint =7.6e-6 cm/min

PO simulations using Papp from a QSAR




**Report the chemical datasets used to create predictive models !**

# OSP IVIVE toolbox

## Reporting and Training

Having a toolbox that performs the extrapolation of in vitro values will improve the usability of the OSP tools !
For a matter of transparency and allowing interactive improvements, reporting of the methods will be done in Quarto

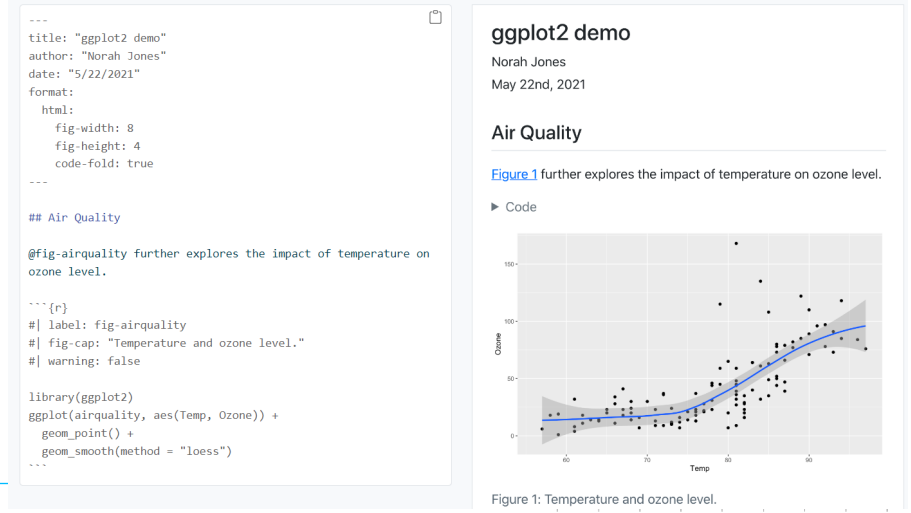
```
---
title: "ggplot2 demo"
author: "Norah Jones"
date: "5/22/2021"
format:
  html:
    fig-width: 8
    fig-height: 4
    code-fold: true
---

## Air Quality

@fig-airquality further explores the impact of temperature on
ozone level.

```{r}
#| label: fig-airquality
#| fig-cap: "Temperature and ozone level."
#| warning: false

library(ggplot2)
ggplot(airquality, aes(Temp, Ozone)) +
  geom_point() +
  geom_smooth(method = "loess")
```
```



# OSP IVIVE toolbox

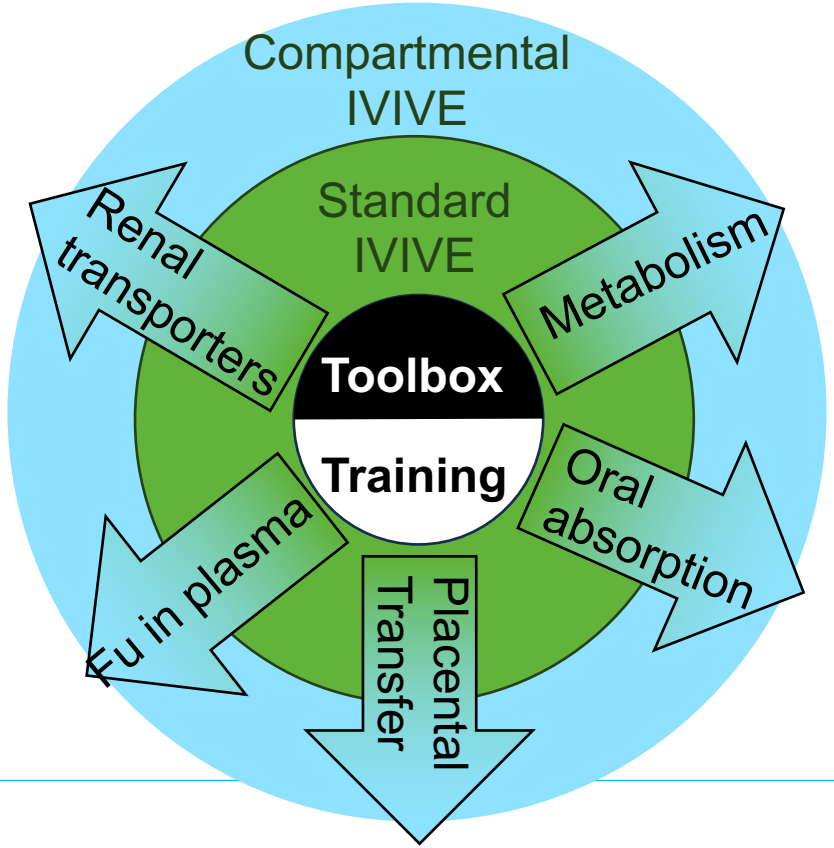
## Reporting and Training

Having a toolbox that performs the extrapolation of in vitro values will improve the usability of the OSP tools !
For a matter of transparency and allowing interactive improvements, reporting of the methods will be done in Quarto

What is the difference between plasma clearance and intrinsic/specific clearance?
What is the difference between obtaining the clearance rate from a clearance assay (1-2 concentrations used and parent depletion monitored in time) and obtaining Michaelis-Menten?
How to add a metabolizing enzyme in plasma?
How is permeability in most organs described and what QSARs are used?

# OSP IVIVE toolbox

## In summary




Other processes to be added for the future:

- Lung inhalation
- Skin absorption
- BBB permeability
- …any suggestions