# Development and application of *ex vivo* models for estimation of fetal exposure

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Radboudumc

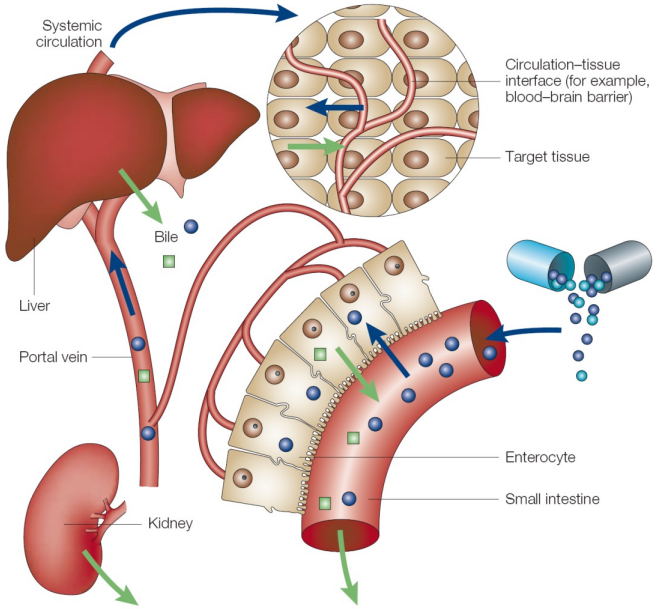
# Disclosure slide






# Mechanistic knowledge on drug disposition

Exposure drives effects, yet the mechanisms determining systemic and intracellular drug exposure levels are often neglected in toxicity/efficacy testing




Human tissues and cells

Physiology-based pharmacokinetic modeling

# Pregnancy and placenta

Improve drug safety and outcomes of pharmacotherapy during pregnancy by translating molecular-based knowledge of drug exposure and action to a clinical or risk assessment setting

# Problem from a pharmaceutical perspective

Pregnant women are not readily enrolled in clinical pharmacology trials

Data on drug efficacy and safety are scarce and/or become available only at a late stage after market introduction


Time to first available clinical PK and safety data after market introduction

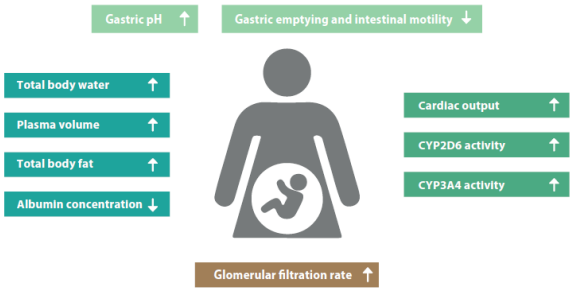
Explore alternative strategies based on studies in human tissues and cells

WHO toolkit, 2018

# Determinants of exposure

Determinants of exposure

Maternal pharmacokinetics



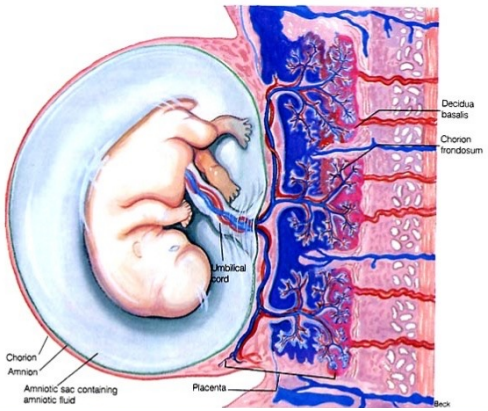

Modified from Colbers et al.

# Determinants of exposure

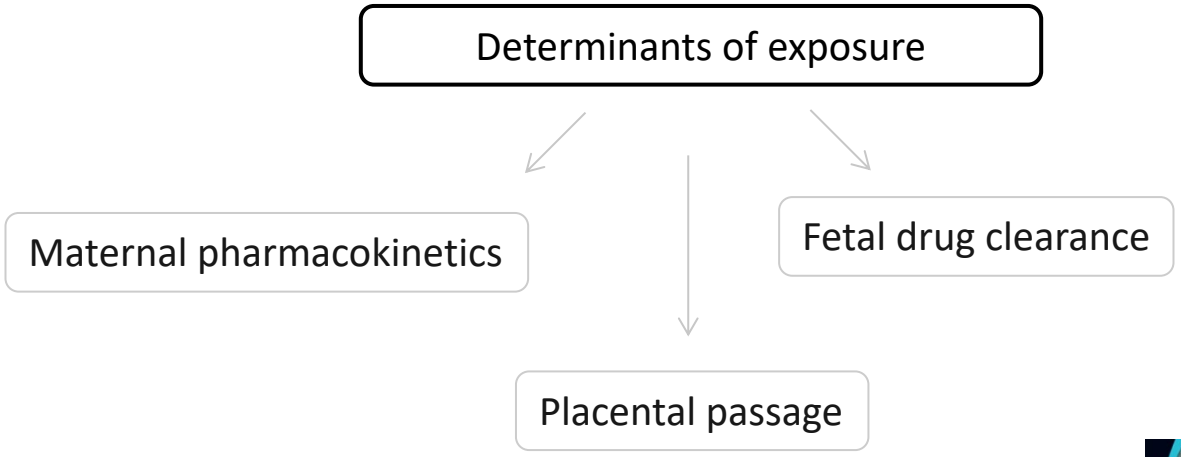
Determinants of exposure

Maternal pharmacokinetics

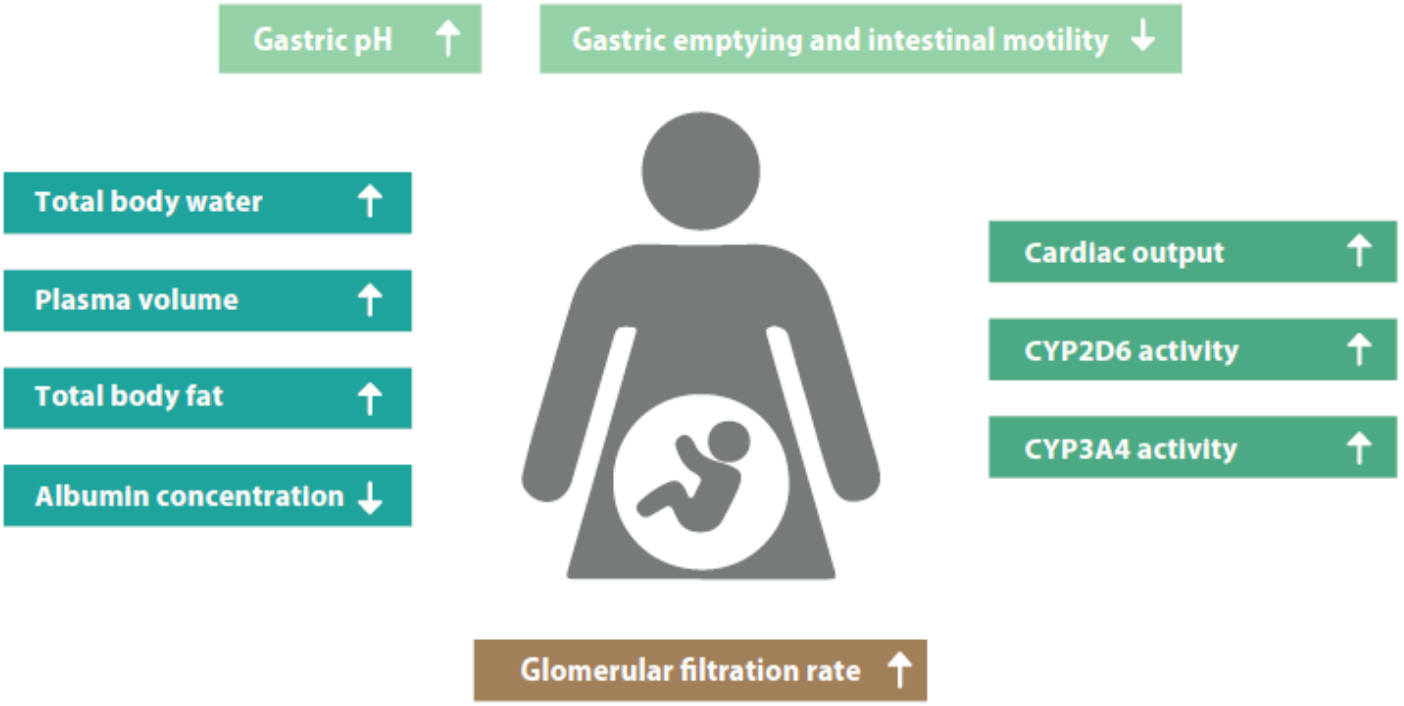
Placental passage



# Determinants of exposure



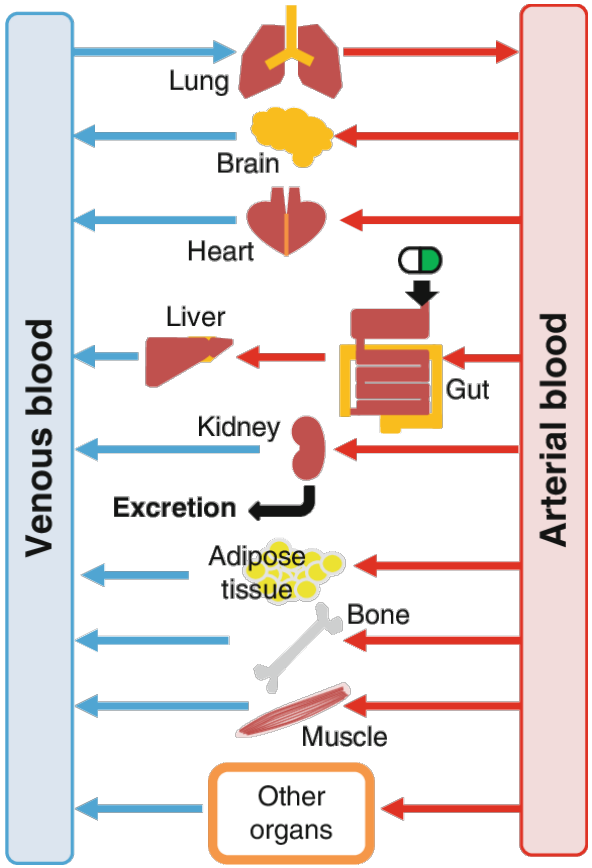

# Maternal pharmacokinetics



# Maternal pharmacokinetics - modeling

Physiologically-based pharmacokinetic (PBPK) model

- Is a mathematical model
- Is a multi-compartmental model
- Reflects human physiology, including maternal gestational changes
- Describes drug pharmacokinetics (ADME)



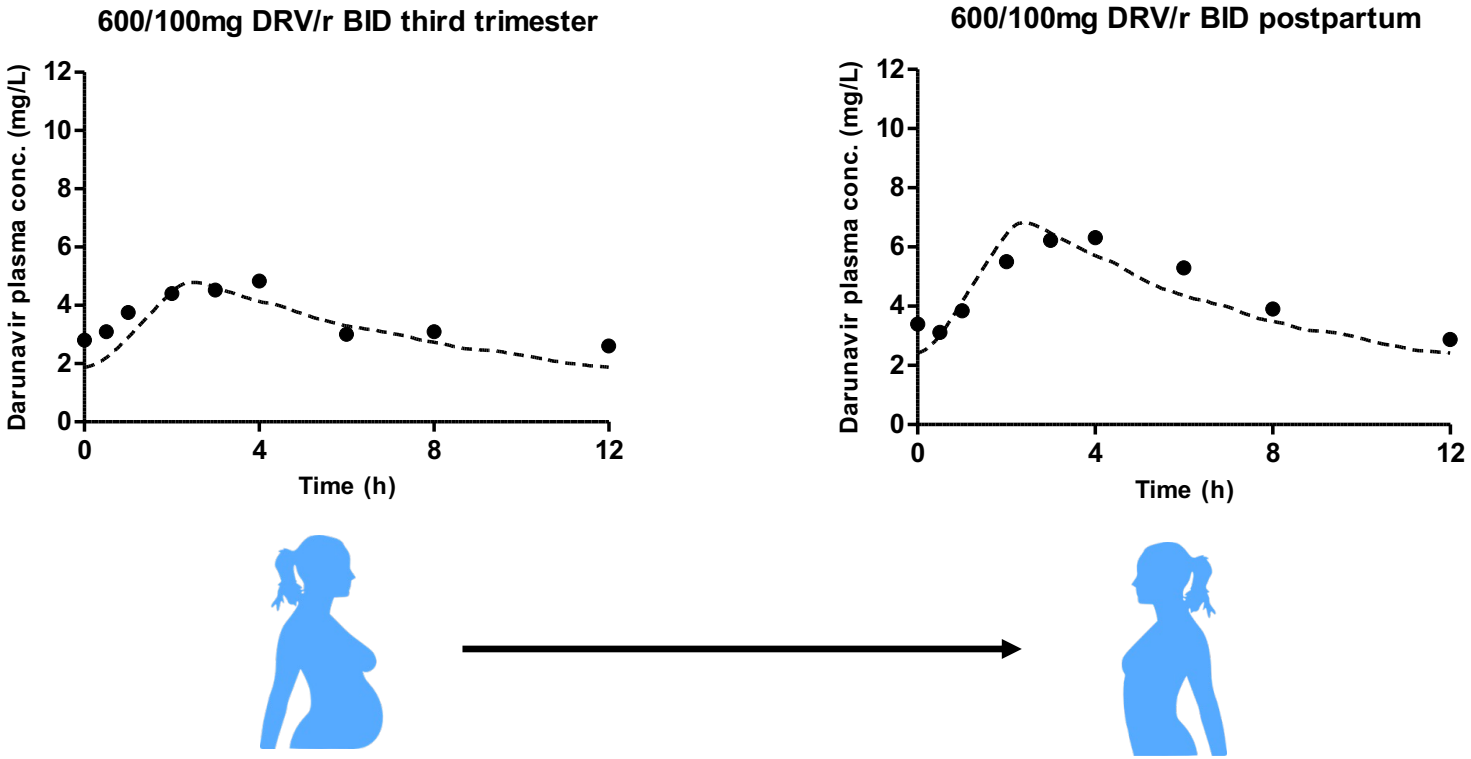
# Maternal pharmacokinetics - modeling

Clin Pharmacokinet (2016) 55:381–396
DOI 10.1007/s40262-015-0325-8

ORIGINAL RESEARCH ARTICLE

## Physiologically Based Modelling of Darunavir/Ritonavir Pharmacokinetics During Pregnancy

Angela Colbers[1] · Rick Greupink[2] · Carlijn Litjens[1,2] · David Burger[1] · Frans G. M. Russel[2]



# But what about fetal exposure

At the time no commercial PBPK modeling platforms were available that allowed for placental transfer modeling

CERTARA

Simcyp

Clin Pharmacokinet (2018) 57:705–716
https://doi.org/10.1007/s40262-017-0583-8

CrossMark

ORIGINAL RESEARCH ARTICLE

**Prediction of Fetal Darunavir Exposure by Integrating Human Ex-Vivo Placental Transfer and Physiologically Based Pharmacokinetic Modeling**

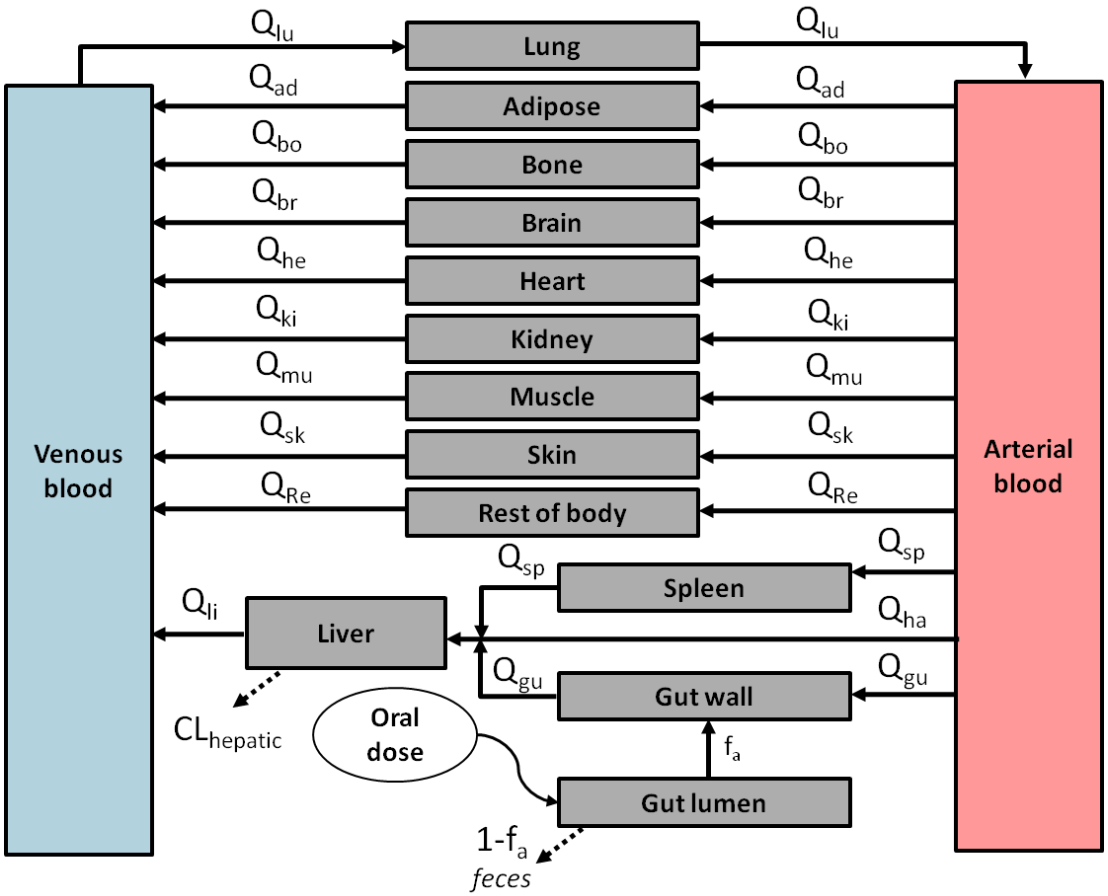
Stein Schalkwijk[1,2] · Aaron O. Buaben[1] · Jolien J. M. Freriksen[2] · Angela P. Colbers[1] · David M. Burger[1] · Rick Greupink[2] · Frans G. M. Russel[2]

Assessment of Maternal and Fetal Dolutegravir Exposure by Integrating *Ex Vivo* Placental Perfusion Data and Physiologically-Based Pharmacokinetic Modeling

Jolien J.M. Freriksen[1,2,*], Stein Schalkwijk[2], Angela P. Colbers[2], Khaled Abduljalil[3], Frans G.M. Russel[1], David M. Burger[2] and Rick Greupink[1]

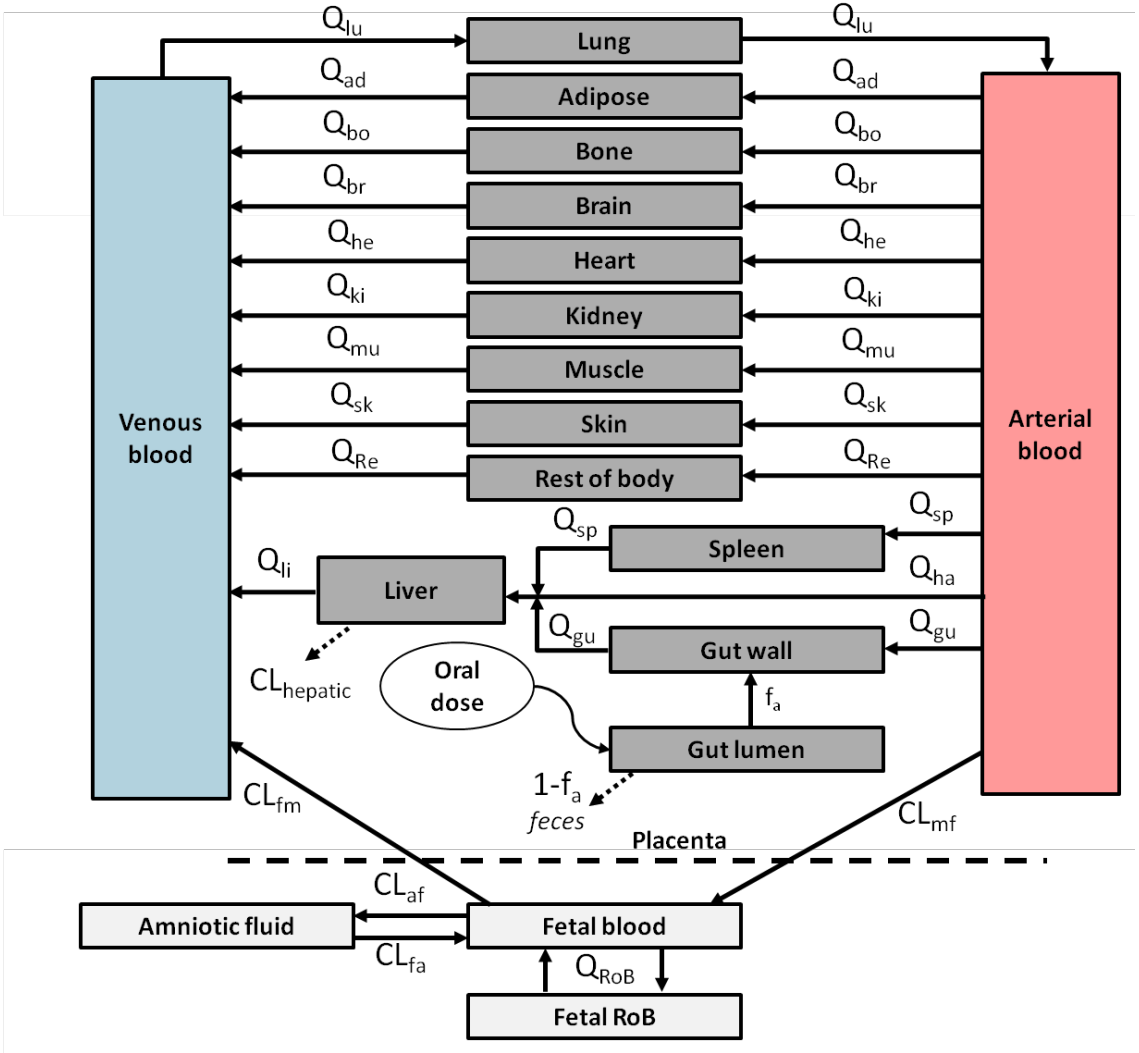
CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 107 NUMBER 6 | June 2020

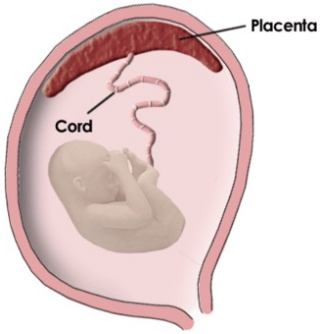
# Get from here…






# ….to there



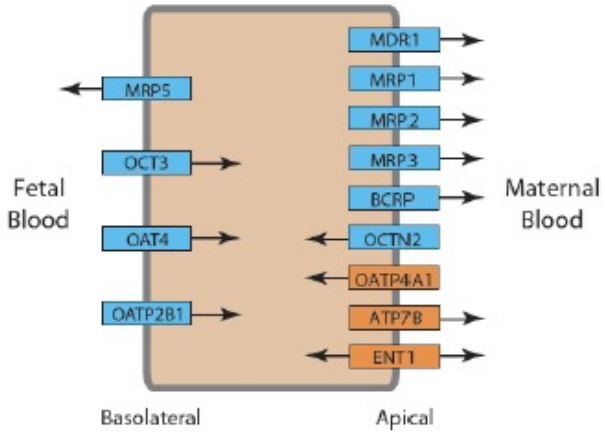




Freriksen et al. Clin Pharmacol Ther. 2020 Jun;107(6):1352-1361

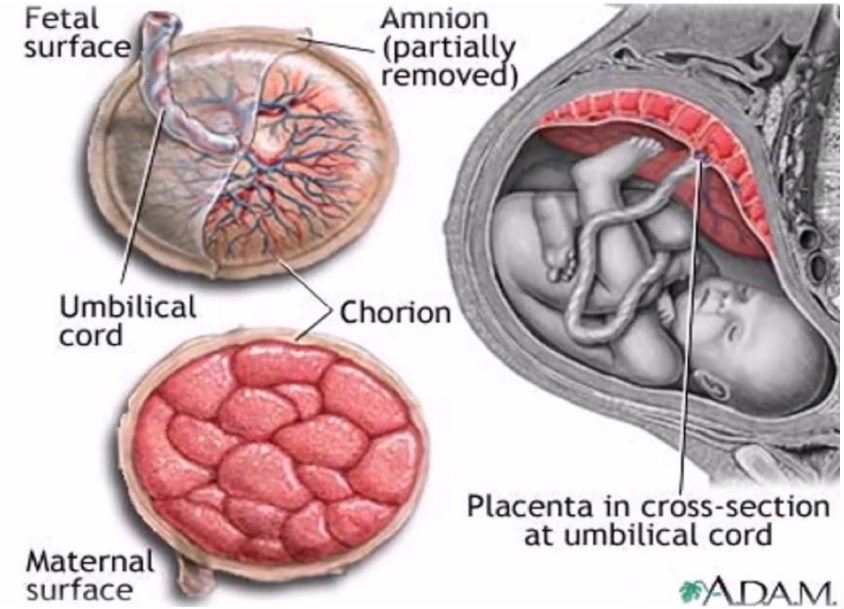
# Placental handling of pharmaceuticals

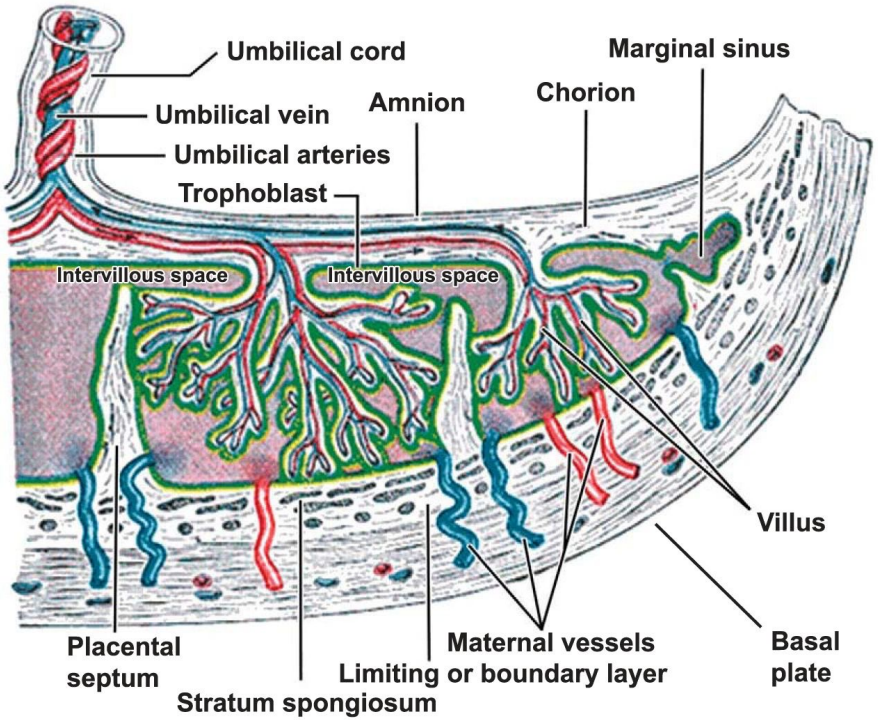
**Transfer of small molecules**

- Size/molecular weight
- Lipophilicity
- Protein binding
- Target mediated uptake
- Substrate for efflux transporters



# The placenta




http://www.adameducation.com
Serov et al. J Appl Physiol 120: 17–28, 2016

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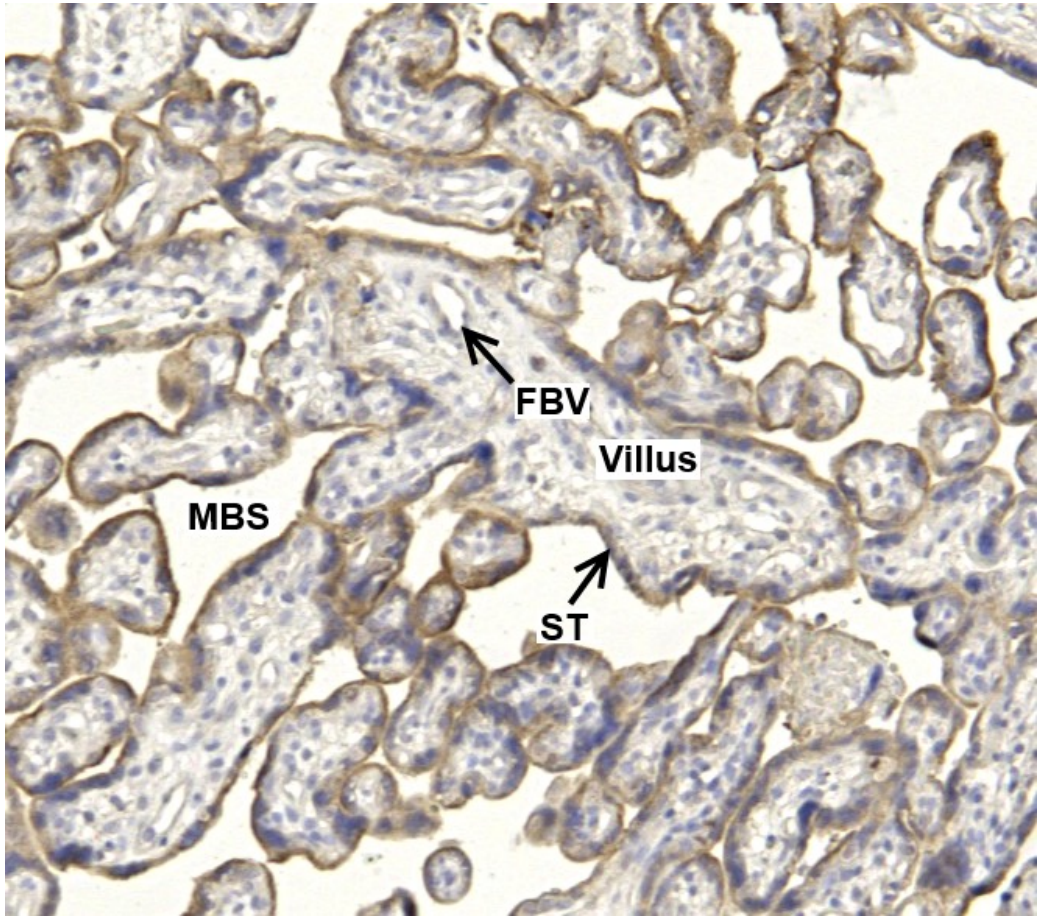
# Histology

**Tissue section of villous material from human placenta**



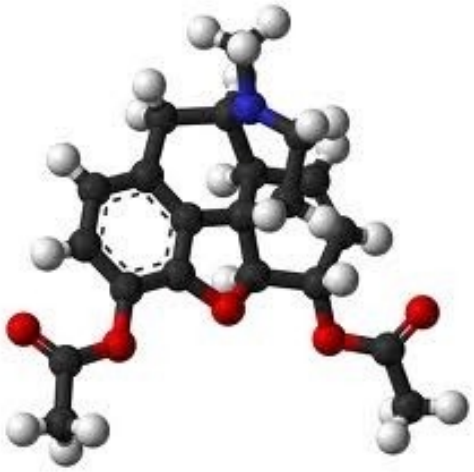

FBV: fetal blood vessel
MBS: Maternal blood space
ST: Syncytiotrophoblast layer

Brown staining: P-gp expression

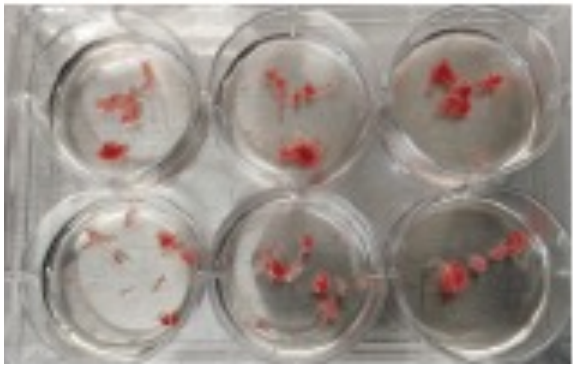
Eliesen et al. Toxicol Sci. 2017 Jun 1;157(2):500-509.

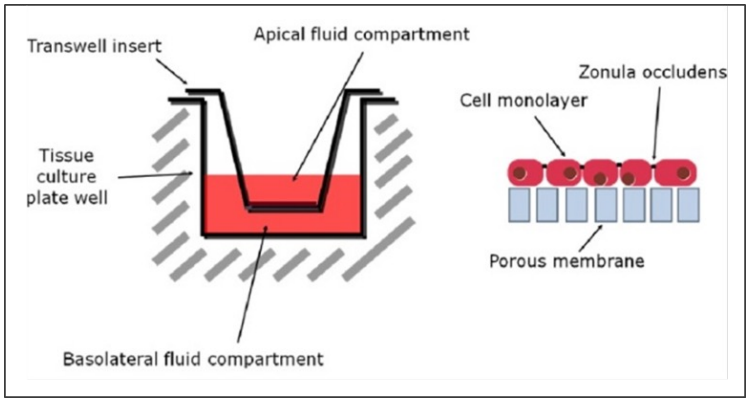
# How to parameterize for placental transfer ?

Predictions based on physicochemistry

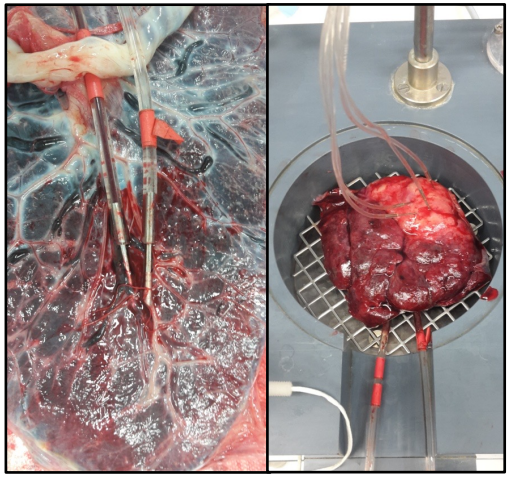


Placental villous tissue explants






Cell models, e.g. BeWo b30 cell monolayers



Ex vivo human placenta perfusion

# Isolated human placental cotyledon perfusion

Placenta 107 (2021) 8–12

Contents lists available at ScienceDirect

Placenta

journal homepage: http://www.elsevier.com/locate/placenta

## *Ex vivo* dual perfusion of an isolated cotyledon of human placenta: History and future challenges

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Placenta 122 (2022) 29–45

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

## Placental transfer and vascular effects of pharmaceutical drugs in the human placenta *ex vivo*: A review

H. van Hove [a,*], L. Mathiesen [b], J.J.M. Freriksen [a], K. Vähäkangas [c], A. Colbers [d], P. Brownbill [e,f], R. Greupink [a]

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[b] Department of Public Health, Section of Environmental Health, University of Copenhagen, Denmark
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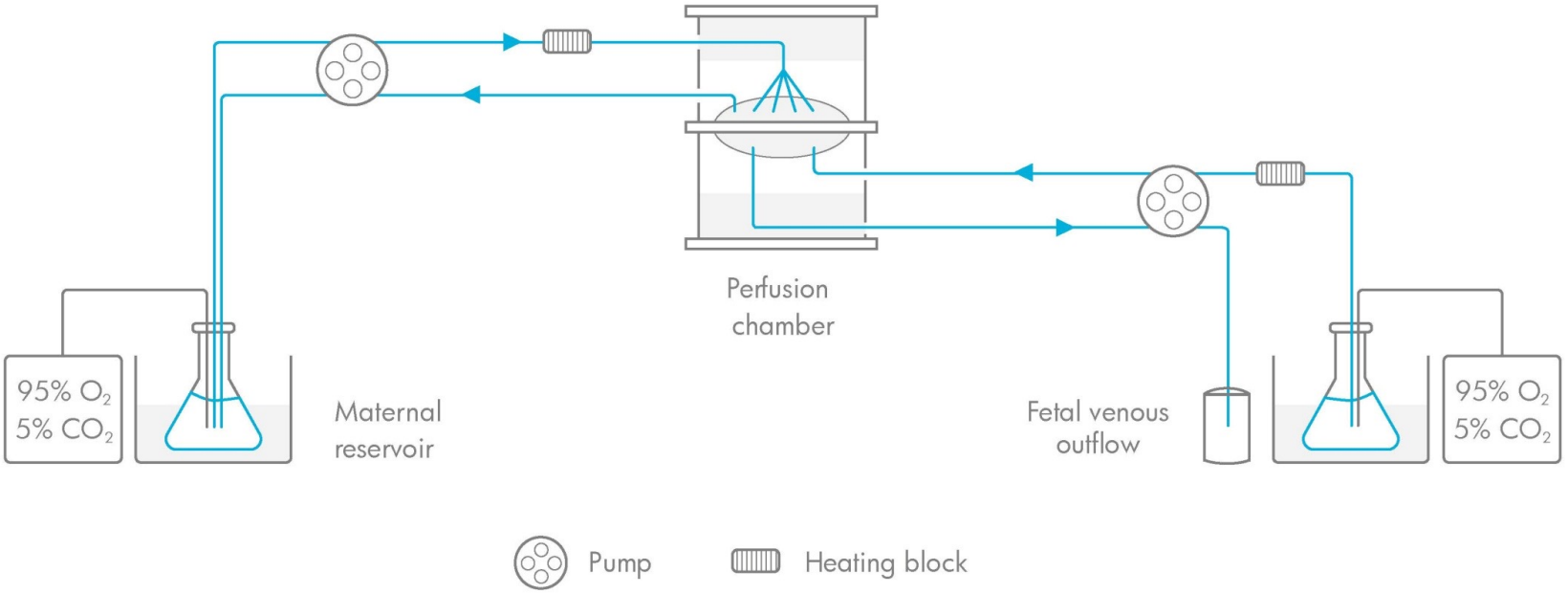
[f] MAHSC, St Mary's Hospital, NHS MFT, Manchester, M13 9WL, UK

## 12th European Placenta Perfusion Workshop (EPPW), Nijmegen

# Isolated human placenta (cotyledon) perfusion

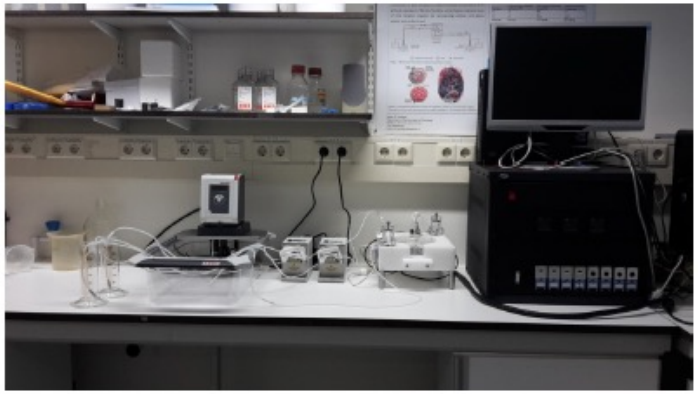


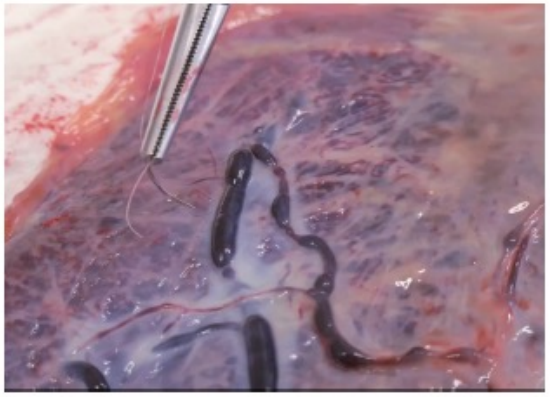

Eliesen et al. Clin Pharmacol Ther. 2020 Jul;108(1):99-106.

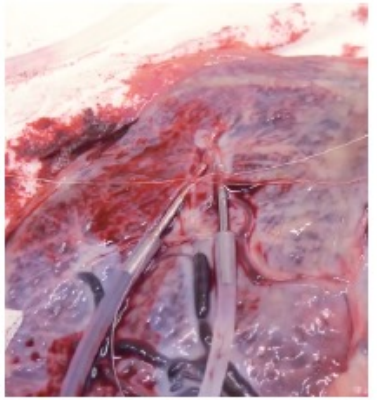
Eliesen et al. Toxicol Sci. 2017 Jun 1;157(2):500-509.

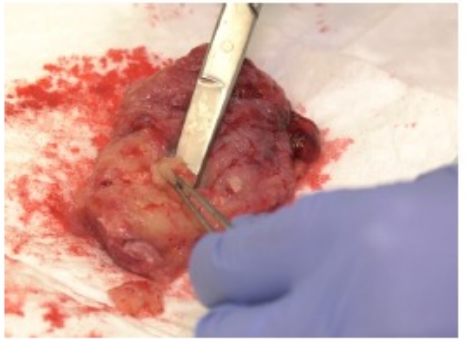
Freriksen et al. Clin Pharmacol Ther. 2020 Jun;107(6):1352-1361

# Isolated human cotyledon perfusion

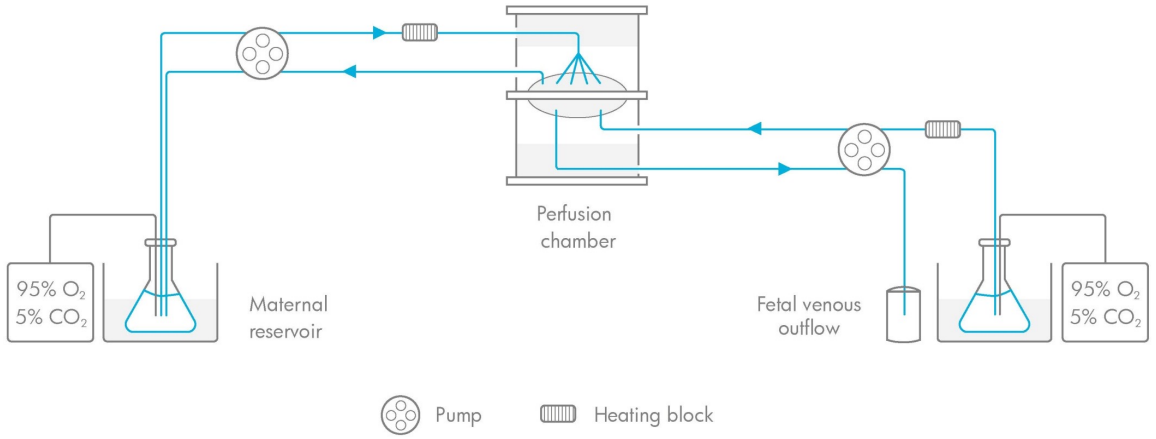






# Isolated human cotyledon perfusion




| Maternal circulation | Foetal circulation |
|---|---|
| Krebs buffer (pH 7.4) | Krebs buffer (pH 7.4) |
| Albumin (30 g/L) | Albumin (30 g/L) |
| Antipyrine (100 mg/L) | FITC-dextran (36 mg/L) |
| 95% $O_2$<br>5% $CO_2$ | 95% $O_2$<br>5% $CO_2$ |
| Flow: 12 mL/min | Flow: 6 mL/min |

3 hour placenta perfusions
(small molecules)

| Maternal circulation | Foetal circulation |
|---|---|
| RPMI (pH 7.4) | RPMI (pH 7.4) |
| Albumin (29 g/L) | Albumin (34 g/L) |
| Antipyrine (100 mg/L) | |
| 95% $O_2$<br>5% $CO_2$ | 95% $N_2$<br>5% $CO_2$ |
| Flow: 12 mL/min | Flow: 6 mL/min |

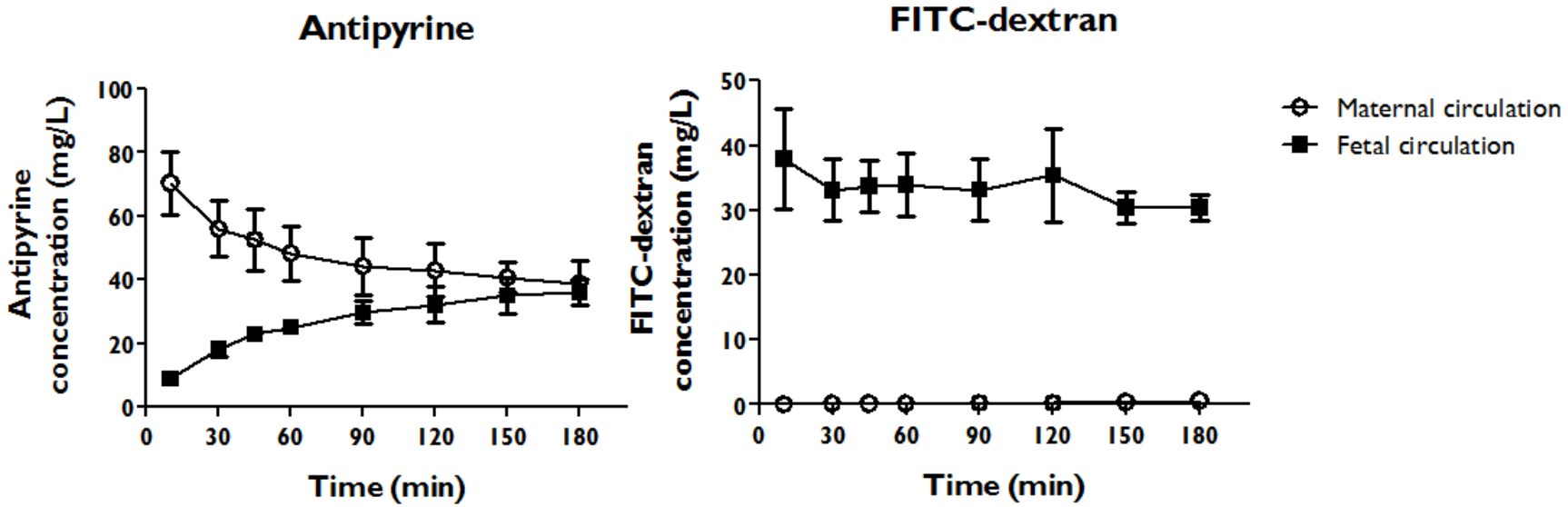
6 hour placenta perfusions
(biologics)

Eliesen et al. Clin Pharmacol Ther. 2020 Jul;108(1):99-106.

Eliesen et al. Toxicol Sci. 2017 Jun 1;157(2):500-509.

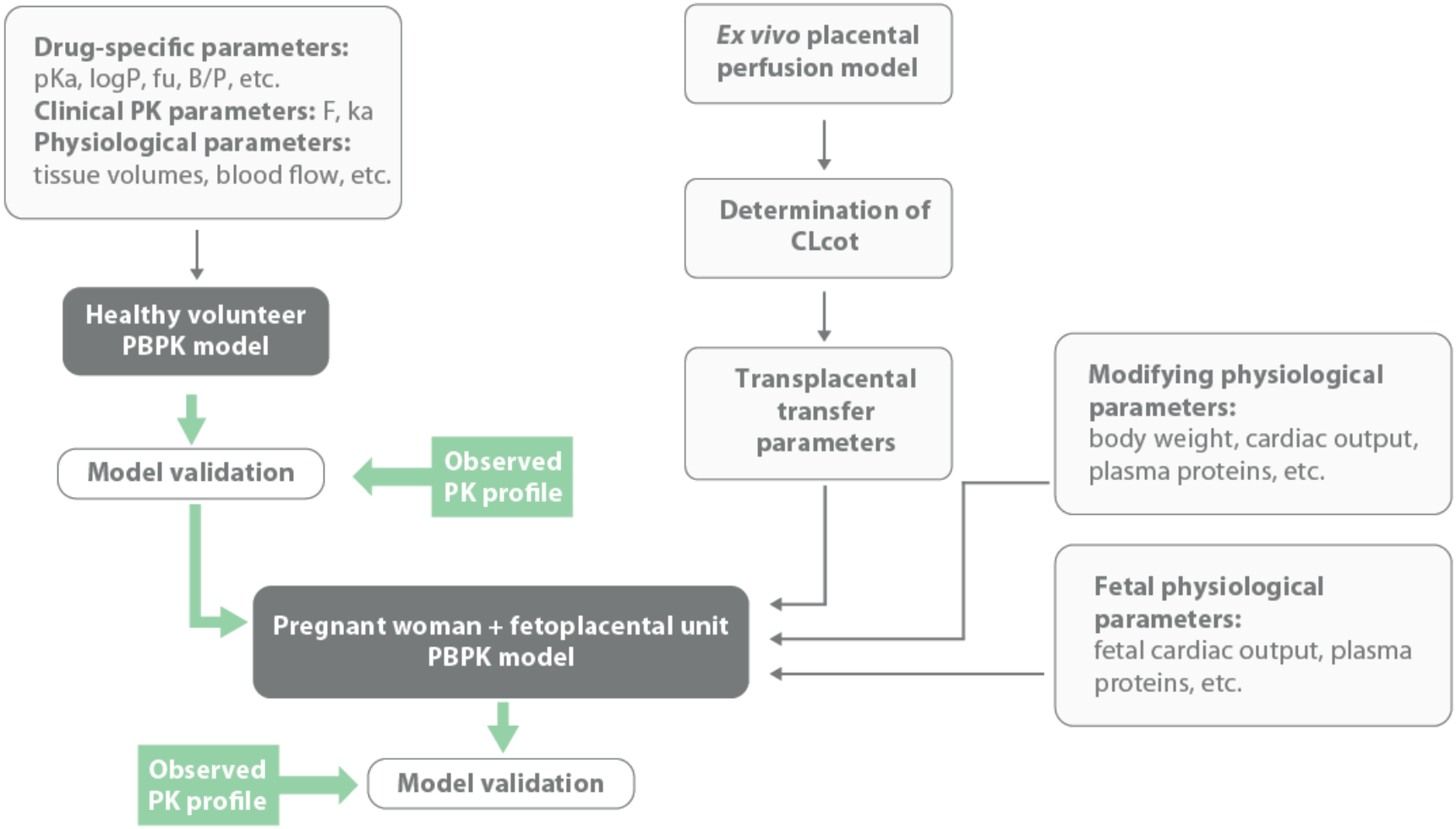
Freriksen et al. Clin Pharmacol Ther. 2020 Jun;107(6):1352-1361

# Typical quality control data




- ✓ Antipyrine shows extensive transfer → overlap circulations
- ✓ FITC-dextran shows poor transfer → vascular integrity

# Stepwise approach to predicting fetal drug exposure




Freriksen et al. Clin Pharmacol Ther. 2020 Jun;107(6):1352-1361

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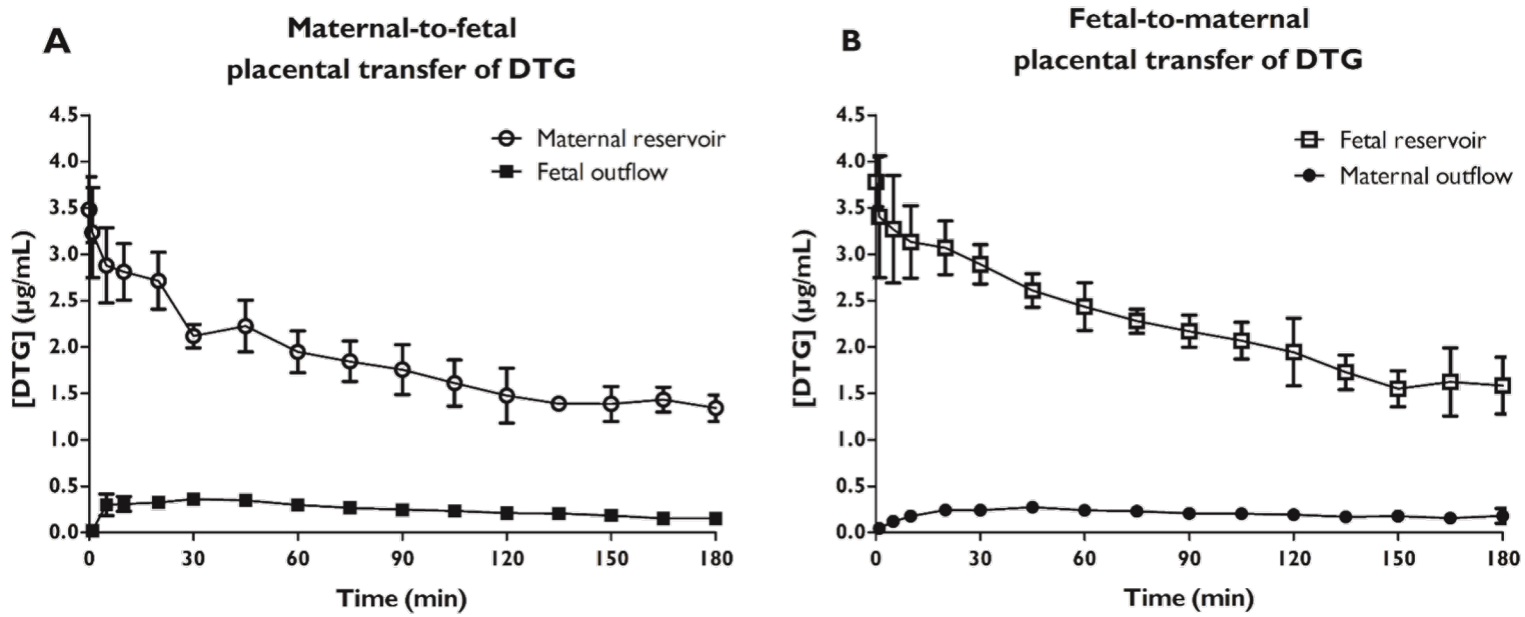
# Placental transfer *ex vivo*




Clearance values (mean ± SD):

1.03 ± 0.06 mL/min

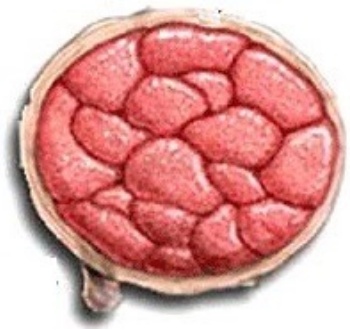
1.03 ± 0.23 mL/min

Freriksen et al. Clin Pharmacol Ther. 2020 Jun;107(6):1352-1361

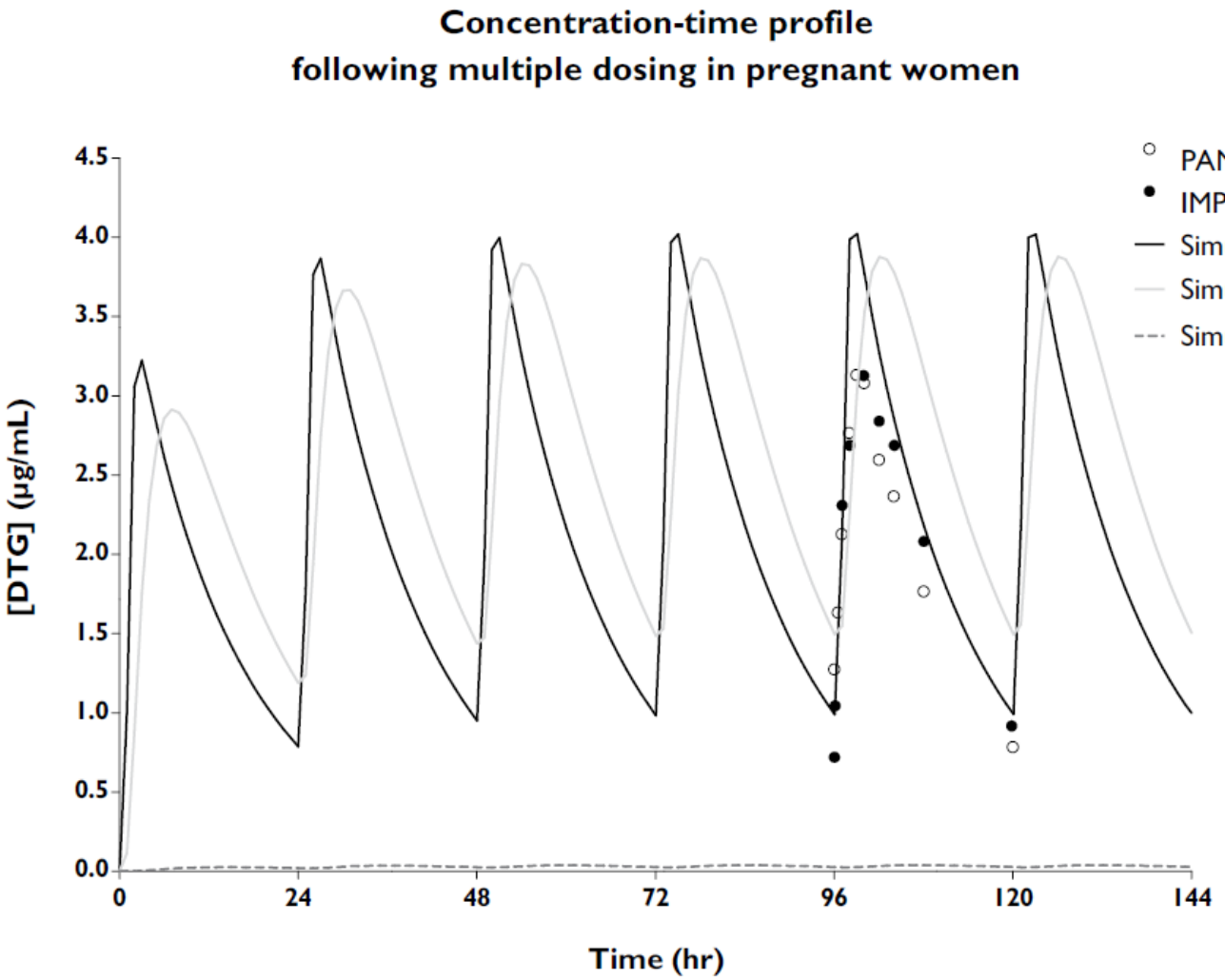
# Scaling cotyledon clearance

Correct data for ex vivo protein binding (unbound clearance)

Scale dolutegravir unbound clearance per cotyledon to whole placenta level

$Cl_{int,cotyledon}$ * N=  $Cl_{int, placenta}$

# Predicted maternal and fetal exposures




Cord blood-to-maternal blood concentration ratio

Simulated:
0.57 – 1.51

Observed:
0.64 – 1.81

Freriksen et al. Clin Pharmacol Ther. 2020 Jun;107(6):1352-1361

# But what about tissue concentrations?

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 157(2), 2017, 500–509

doi: 10.1093/toxsci/kfx063
Advance Access Publication Date: March 24, 2017
Research article

## Placental Disposition and Effects of Crizotinib: An *Ex Vivo* Study in the Isolated Dual-Side Perfused Human Cotyledon

Gaby A.M. Eliesen,* Petra van den Broek,* Jeroen J. van den Heuvel,* Albert Bilos,* Jeanne Pertijs,* Joris van Drongelen,† Frans G.M. Russel,* and Rick Greupink*,[1]

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Archives of Toxicology (2021) 95:557–571
https://doi.org/10.1007/s00204-020-02925-w

IN VITRO SYSTEMS

## Toxicity of anticancer drugs in human placental tissue explants and trophoblast cell lines

Gaby A. M. Eliesen[1] · Hedwig van Hove[1] · Maartje H. Meijer[1] · Petra H. H. van den Broek[1] · Jeanne Pertijs[1] · Nel Roeleveld[2] · Joris van Drongelen[3] · Frans G. M. Russel[1] · Rick Greupink[1]

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Toxicology in Vitro 85 (2022) 105471

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

## Predicting fetal exposure of crizotinib during pregnancy: Combining human *ex vivo* placenta perfusion data with physiologically-based pharmacokinetic modeling

Damian Roelofsen[a], Hedwig van Hove[a], Vera Bukkems[b], Frans Russel[a], Gaby Eliesen[a,1], Rick Greupink[a,*]

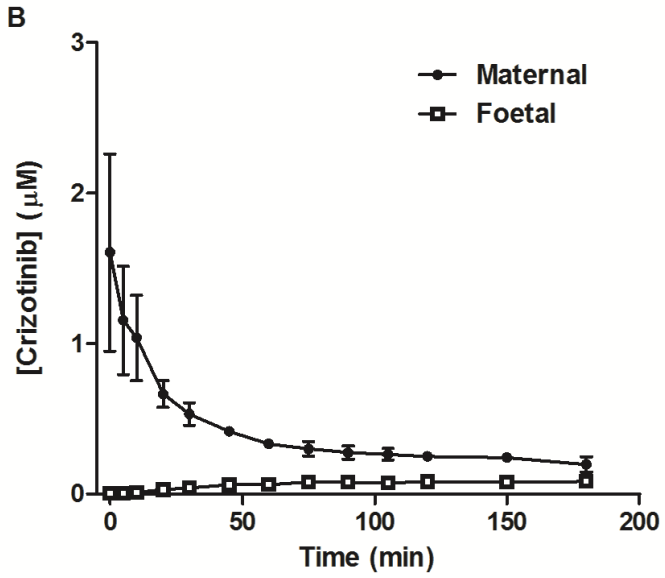
[a] Department of Pharmacology and Toxicology, Radboud University Medical Center, Nijmegen, the Netherlands
[b] Department of Pharmacy, Radboud University Medical Center, Nijmegen, the Netherlands

# Placental accumulation and effects

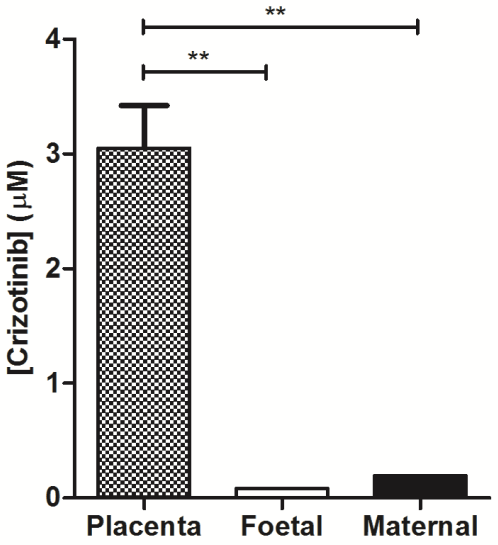
Crizotinib: Tyrosine kinase inhibitor (TKI), NSLC with ALK mutation
Limited clinical safety data for these type of drugs

Can we assess exposure and interpret potential toxicity in relation to that exposure?

Crizotinib transfer




Concentration crizotinib at the end of the placenta perfusion




*Eliesen et al, 2017, Toxicological Sciences*

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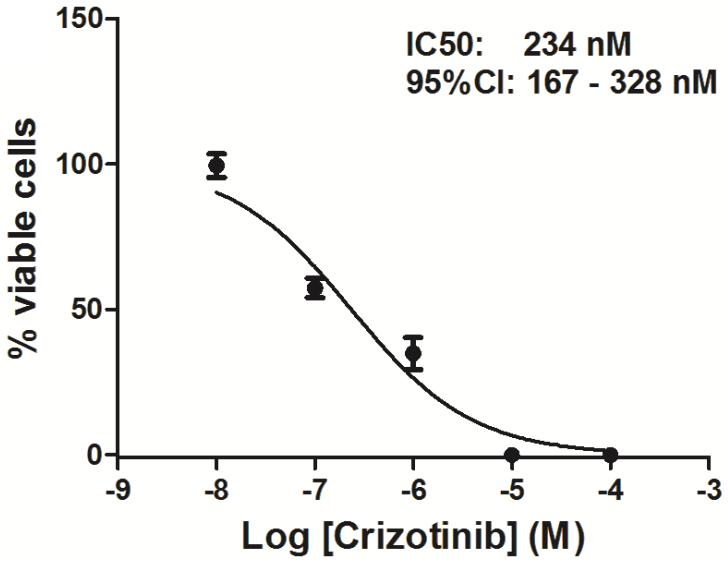
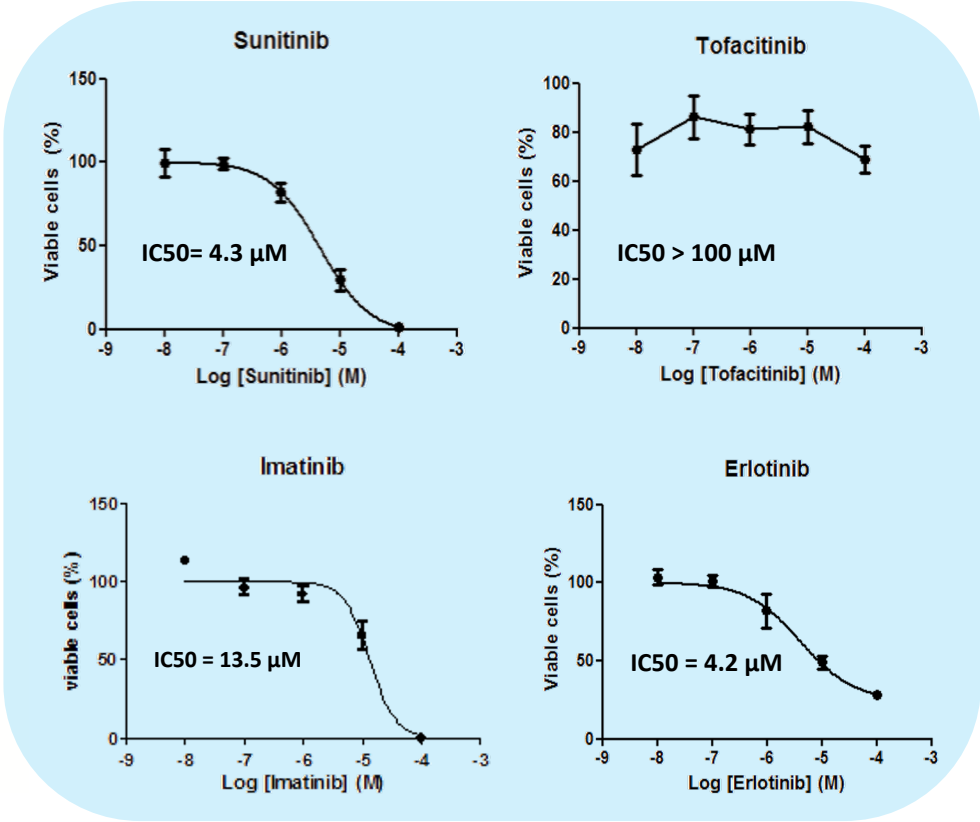
# Results cell viability assays (BeWo)

## Crizotinib




## Other TKIs

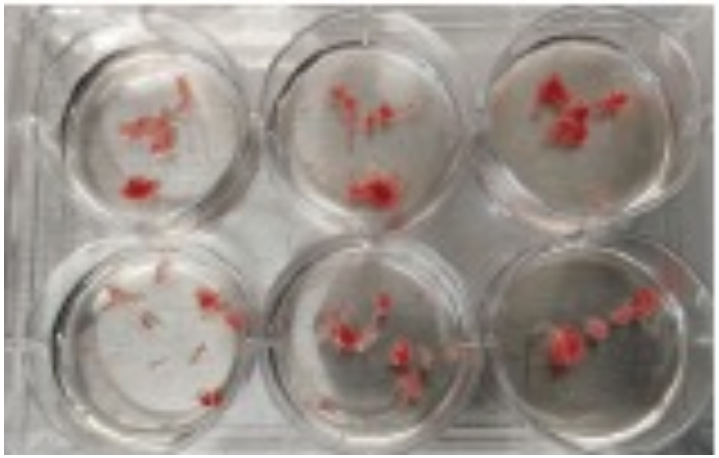



Expected tissue exposure is in the range in which crizotinib is exerts toxicity in BeWo cells

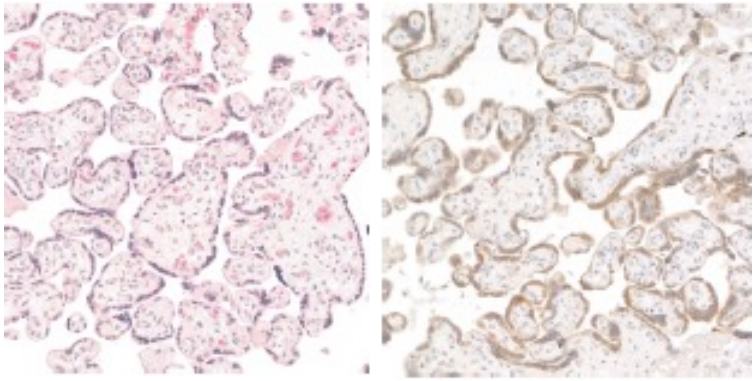
*Eliesen et al, 2017, Toxicological Sciences*

# Results cell viability assays (villous explants)

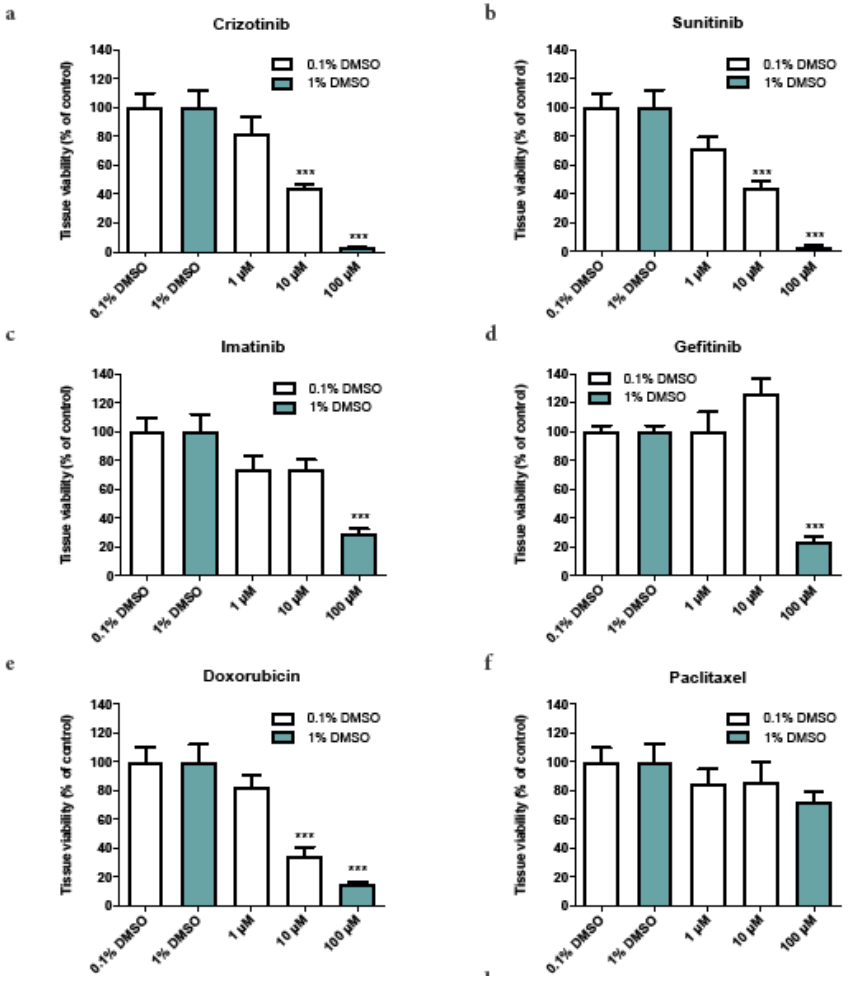
Villous explants in culture medium



HE and HCG staining of explants



Cytotoxicity




Crizotinib is less potent in primary placental tissues than in BeWo cells

*Eliesen et al, 2022, Archives of Toxicology*

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# Combine with PBPK modeling

## Predicted steady state concentrations of crizotinib, 2 dd 250 mg, gestational week 40 Including prediction of population variability.

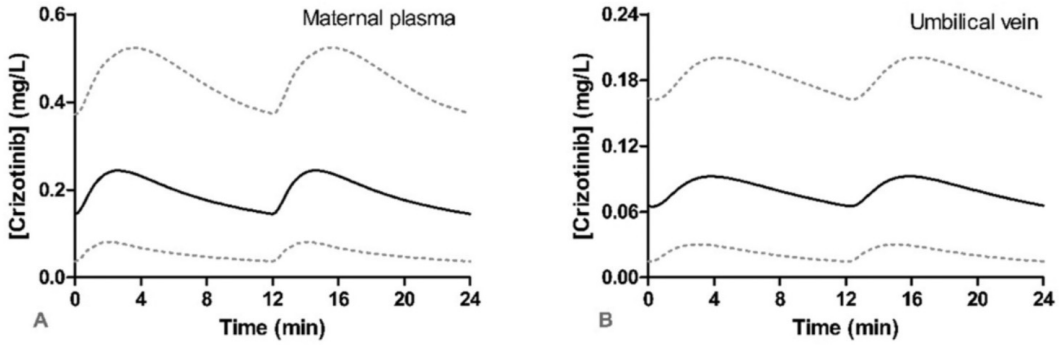


**Fig. 3.** Mean predicted concentration-time profile of the A) maternal plasma concentration of crizotinib during gestational week 40 at steady-state and B) umbilical vein concentration of crizotinib during gestational week 40 at steady-state. Crizotinib is given 250 mg BID. Grey dotted lines indicate the 5th and 95th percentiles for the mean concentrations predicted in the virtual population.

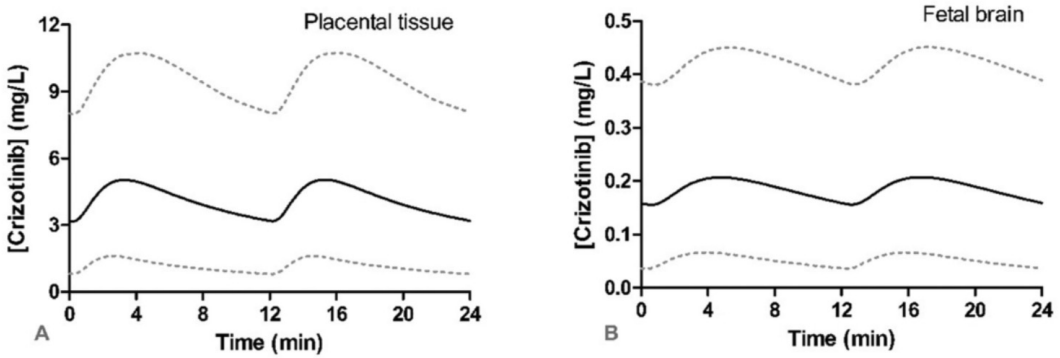


**Fig. 4.** Mean predicted concentration-time profile of the **A)** placental tissue concentration of crizotinib during gestational week 40 at steady-state and **B)** fetal brain concentration of crizotinib during gestational week 40 at steady-state. Crizotinib is given 250 mg BID. Grey dotted lines indicate the 5th and 95th percentiles for the mean concentrations predicted in the virtual population.

*Roelofsen et al, 2022, Toxicol in Vitro*

# Summary

Human placental tissues and cells may be used to:

Aid in non-clinical and early clinical toxicological risk assessment by providing information on exposure, particularly in combination with PBPK modeling.

Study adverse effects of drugs on the level of the placenta.

In case of marketed drugs, provide additional pieces of evidence, next to clinical pharmacology data, to help guide clinical drug dosing during pregnancy (e.g. for fetal therapy).

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## Thank you for your attention!

## Questions?

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