Supplementary Information on the closed-open placenta perfusion experiments workflow for

Prediction of maternal and fetal doravirine exposure by integrating physiologically-based pharmacokinetic modelling and human cotyledon perfusion experiments

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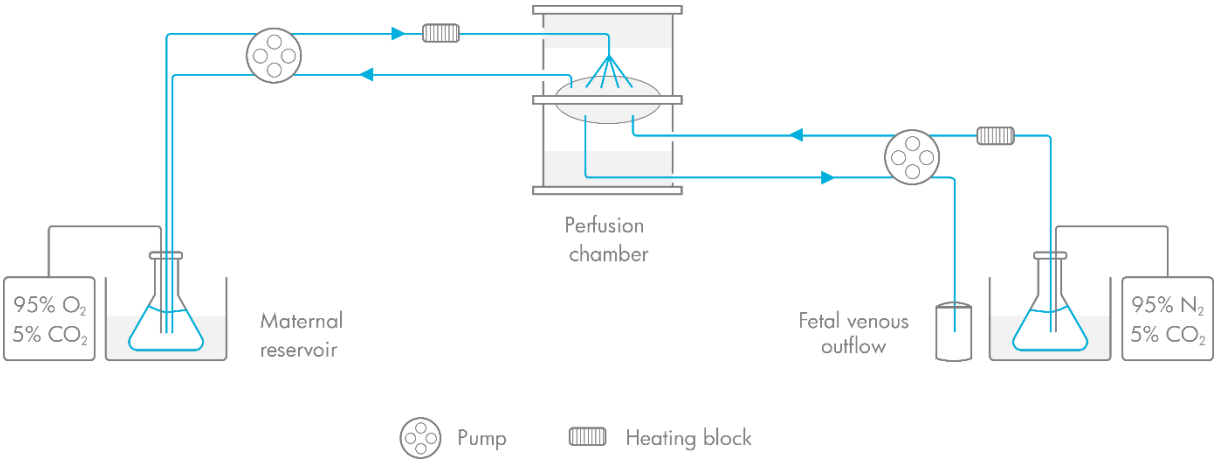
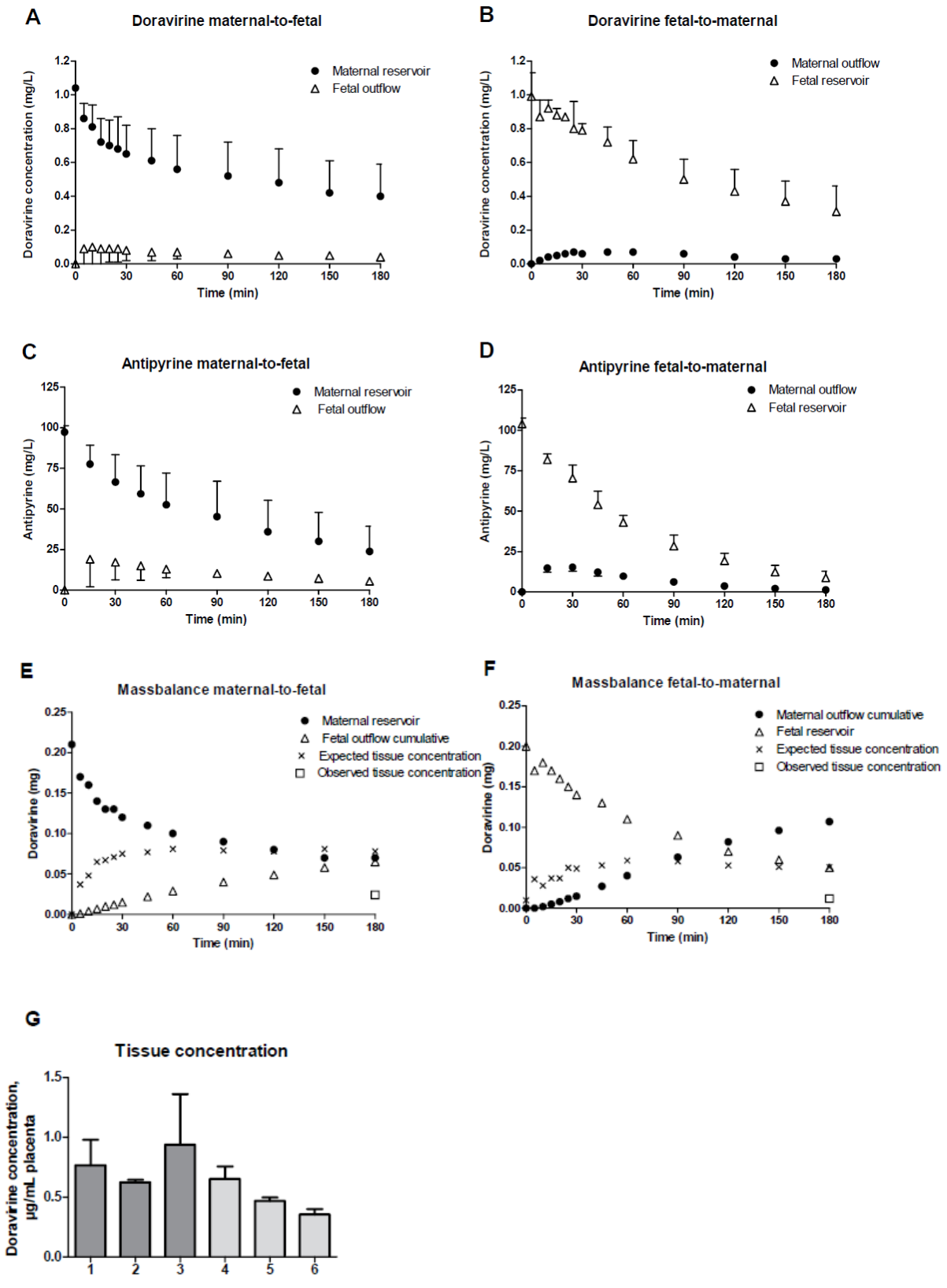


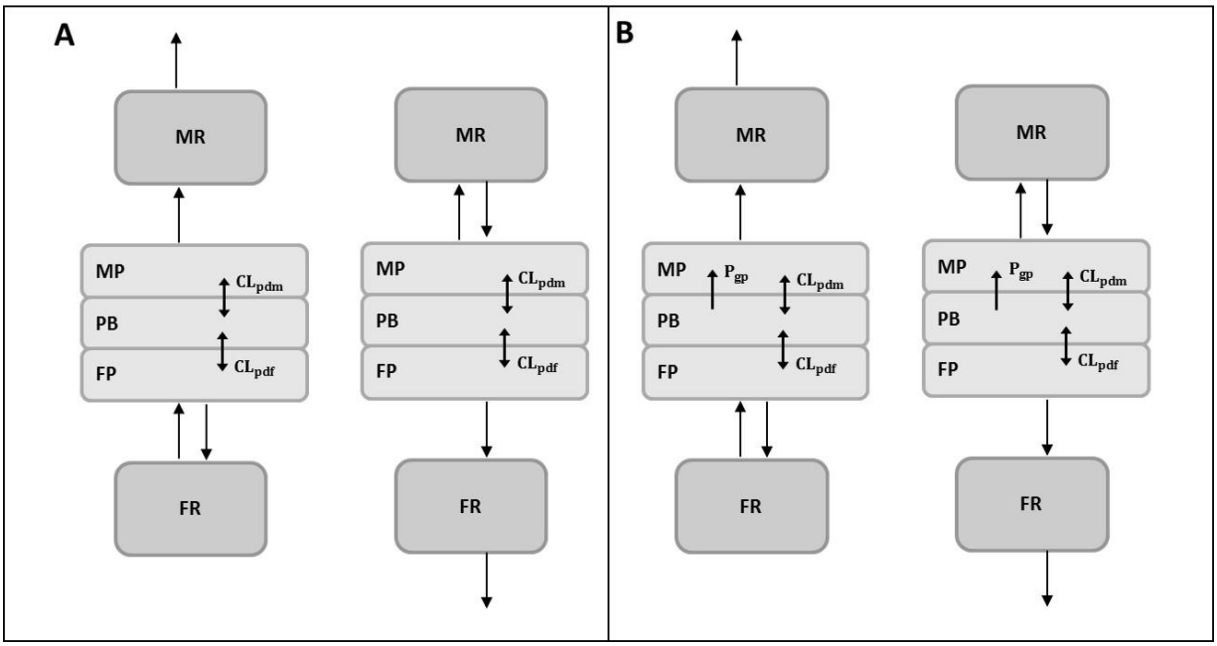
Online Resource 13: Schematic overview of the *ex vivo* dual-side placenta perfusion experiment in closed-open configuration to study placental transfer of doravirine from the maternal-to-fetal circulation and from the fetal-to-maternal circulation. Either the maternal or fetal circulation was recirculated (closed), while the other circulation was kept open. The figure shows a closed maternal circulation and a fetal open circulation.

Online Resource 14: Overview of the main characteristics of the placentas used for the *ex vivo* dual-side placenta perfusion experiments in closed-open configuration

Closed-open perfusion						
Placenta nr.	**Maternal age, years**	**Medication**	**Gestational age at delivery, weeks**	**Birth weight neonate, g**	**Mode of delivery**	**Cotyledon weight, g**
1	32	None	38	3950	Caesarean section	30.8
2	35	None	40	3274	Vaginal	41.4
3	30	None	39	3655	Caesarean section	39.4
4	28	None	39	3530	Caesarean section	35.5
5	34	None	39	4035	Caesarean section	22.5
6	25	None	38	2895	Caesarean section	22.5



Online Resource 15: Results of the *ex vivo* human cotyledon perfusion experiments in closed-open configuration. Placental transfer of doravirine in A) maternal-to-fetal-direction B) and fetal-to-maternal-direction. Placental transfer of antipyrine in C) maternal-to-fetal-direction D) and fetal-to-maternal-direction. Doravirine mass balance E) in maternal-to-fetal-direction and F) fetal-to-maternal-direction. G) Doravirine concentration in the placental tissue at the end of the experiment n = 6 placentas and 3 tissue samples per placenta. Data are shown as mean ± SD

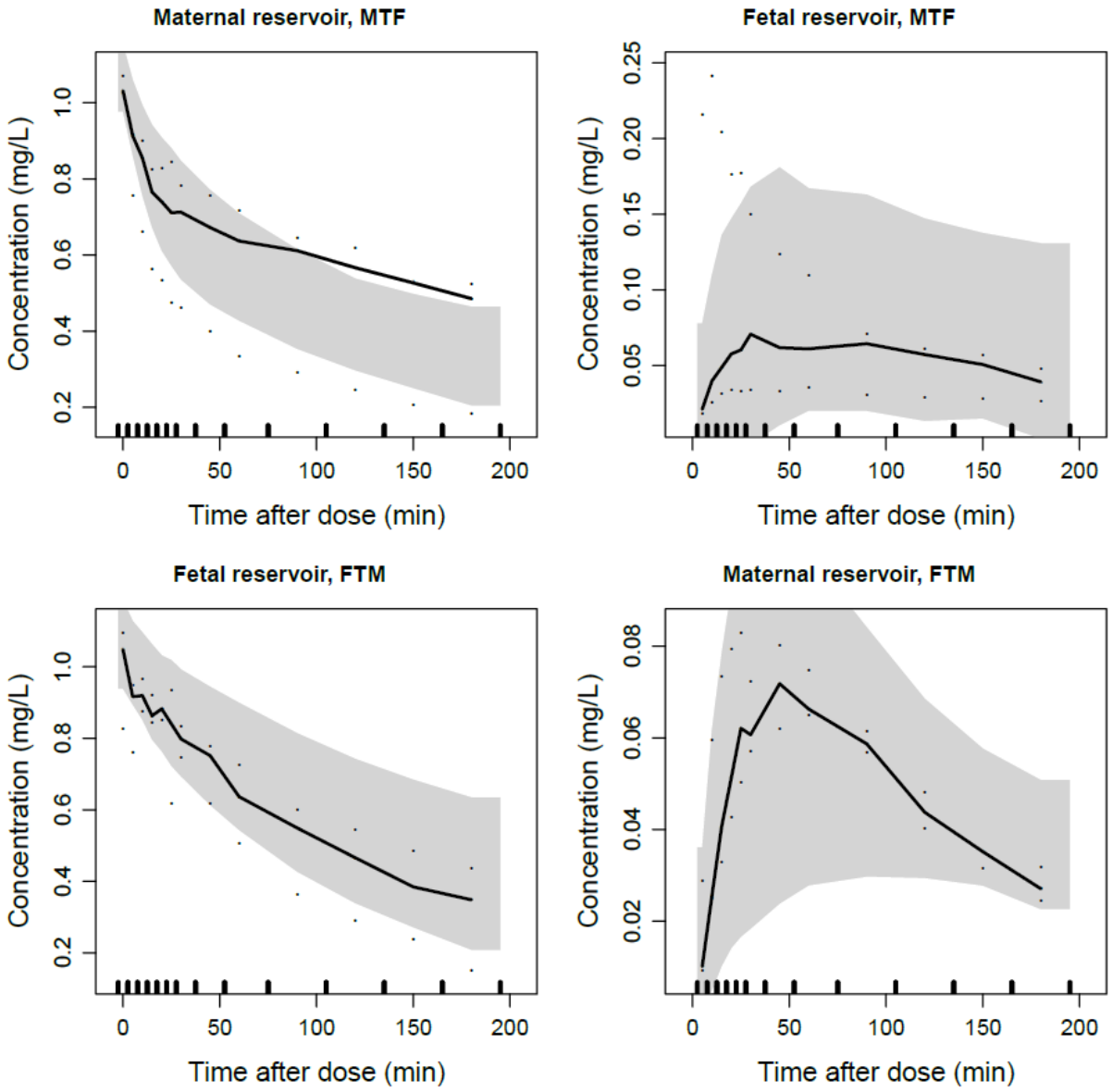


Online Resource 16: Schematic overview of the tested mechanistic placenta model structures to estimate intrinsic placental transfer parameters of doravirine based on *ex vivo* perfusion data in closed-open configuration. A) representing simple diffusion transfer model. B) representing diffusion model combined with P-gp mediated active transport over the maternal-facing placental barrier. Doravirine was added to the closed fetal reservoir (FR, left in A and B) of closed maternal reservoir (MR, right in A and B); these models were run simultaneously while the relevant compartments were turned on based on the dosing compartment covariate. MR; maternal reservoir, MP; maternal part of the placenta, BP; barrier of the placenta, FP; fetal part of the placenta, FR; fetal reservoir, Qm; maternal blood flow, Qf; fetal blood flow, CLm; clearance between maternal part of the placenta and the placental barrier, CLf; clearance between fetal part of the placenta and the placental barrier, P-gp; p-glycoprotein-mediated active transport.

Online Resource 17: Parameters of the mechanistic placenta model

Parameter	Input	Reference
V_{MR}, mL	200 or 3	Experimental condition, based on dosing compartment
V_{MP}, mL	5.08	11.55% of total placental volume (1). Standardized for placenta of 42g = 44.02 mL (2).
V_{PB}, mL	4.86	11.05% of total placental volume (1). Standardized for placenta of 42g = 44.02 mL (2).
V_{FP}, mL	3.63	8.25% of total placental volume (1). Standardized for placenta of 42g = 44.02 mL (2).
V_{FR}, mL	200 or 3	Experimental condition, based on dosing compartment
$Q_{M,}$ mL/min	12	Experimental condition
$Q_{F,}$ mL/min	6	Experimental condition
FU	0.529	Measured
FU_p	0.01	Estimated with Simcyp® PBPK simulator version 20, based on the physiochemical properties.
$CL_{PDM,}$ mL/min	To be estimated	
$CL_{PDF,}$ mL/min	To be estimated	
$CL_{P\text{-}gp,}$ mL/min	To be estimated	

Abbreviations: V_{MR}; volume maternal reservoir, V_{MP}; volume maternal part of the placenta, V_{PB}; volume placental barrier, V_{FP}; volume fetal part of the placenta, V_{FR}; volume fetal reservoir, Q_M; maternal blood flow, Q_F; fetal blood flow, FU; *ex vivo* fraction unbound, FU_P; fraction unbound in placental barrier, CL_{PDM}; clearance between maternal part of the placenta and the placental barrier, CL_{PDF} clearance between fetal part of the placenta and the placental barrier, $CL_{P\text{-}gp}$; P-glycoprotein-mediated active transport (or other efflux transporter at the syncytiotrophoblast-blood interface).



Online Resource 18: Visual predictive check of the final mechanistic placenta model (simulations *n*=1000) with the observed data of the *ex vivo* human cotyledon perfusion experiments in closed-open configuration. The observations of the perfusion experiments are indicate by the black dots. The median of the observations is indicated by the continuous black line. The gray shaded area indicate the 95% confidence interval around the median of the simulated data. Vertical markers indicate the bins around the sampling points. MTF; maternal-to-fetal perfusion experiment, FTM; fetal-to-maternal perfusion experiment.

Online Resource 19: Final estimates of the intrinsic placental transfer parameters based on the perfusion data from the closed-open perfusion experiment using the mechanistic placenta model.

Parameter	Parameter estimate	95%CI from SIR
CL_{pdm}, mL/min [a]	11.0	5.6 - 22.7
CL_{pdf}, mL/min [a]	4.3	2.3 – 8.3
IIV CL_{pdm}, %	Fixed op 100 [b]	
IIV CL_{pdf}, %	Fixed op 100 [b]	
Additive residual error after dosing in maternal compartment, µg/mL	0.00188	0.00128 - 0.00250
Additive residual error after dosing in fetal compartment, µg/mL	0.00007	0.00003 - 0.00014
Proportional residual error after dosing in maternal compartment,%	4.5 [b]	0.7 - 9.0 [C]
Proportional residual error after dosing in fetal compartment, %	8.7 [b]	6.7 – 11.8 [C]

Abbreviations: CL_{pdm}; clearance between maternal part of the placenta and the placental barrier, CL_{pdf}; clearance between fetal part of the placenta and the placental barrier, IIV; interindividual variability, 95%CI; 95% confidence interval, SIR; sampling importance resampling

[a] A typical cotyledon weighs 42g, assuming to be equal to 44.02 mL

[b] Transformed from log normal variance to % coefficient of variation with √(exp(variance)-1)

[c] Transformed individual SIR results from log normal variance to %coefficient of variation with √(exp(variance)-1) for calculation of the 95% confidence interval

References

(1) Barker, G., Boyd, R.D., D'Souza, S.W., Donnai, P., Fox, H. & Sibley, C.P. Placental water content and distribution. *Placenta* **15**, 47-56 (1994).

(2) Wolf, H., Oosting, H. & Treffers, P.E. Placental volume measurement by ultrasonography: evaluation of the method. *Am J Obstet Gynecol* **156**, 1191-4 (1987).