Next Generation Risk Assessment of the Anti-Androgen Flutamide Including the Contribution of Its Active Metabolite Hydroxyflutamide

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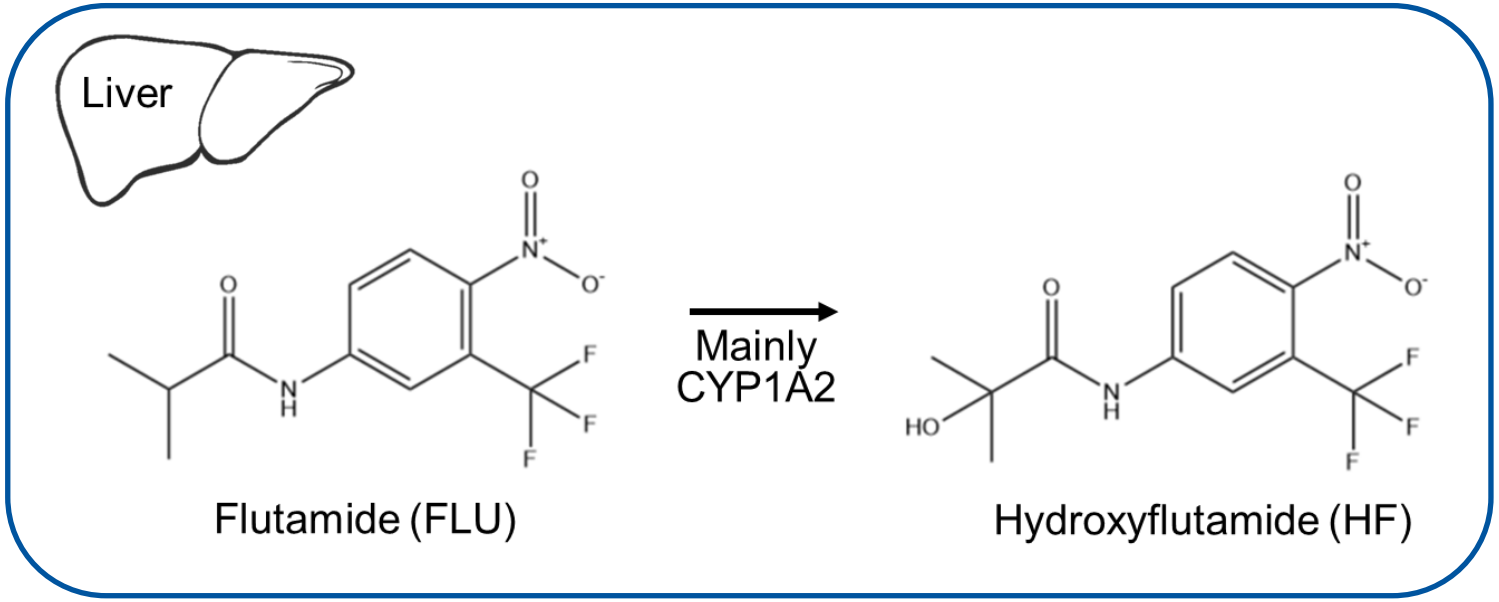
Anti-androgen Flutamide (FLU) is bioactivated to Hydroxyflutamide (HF) in the liver

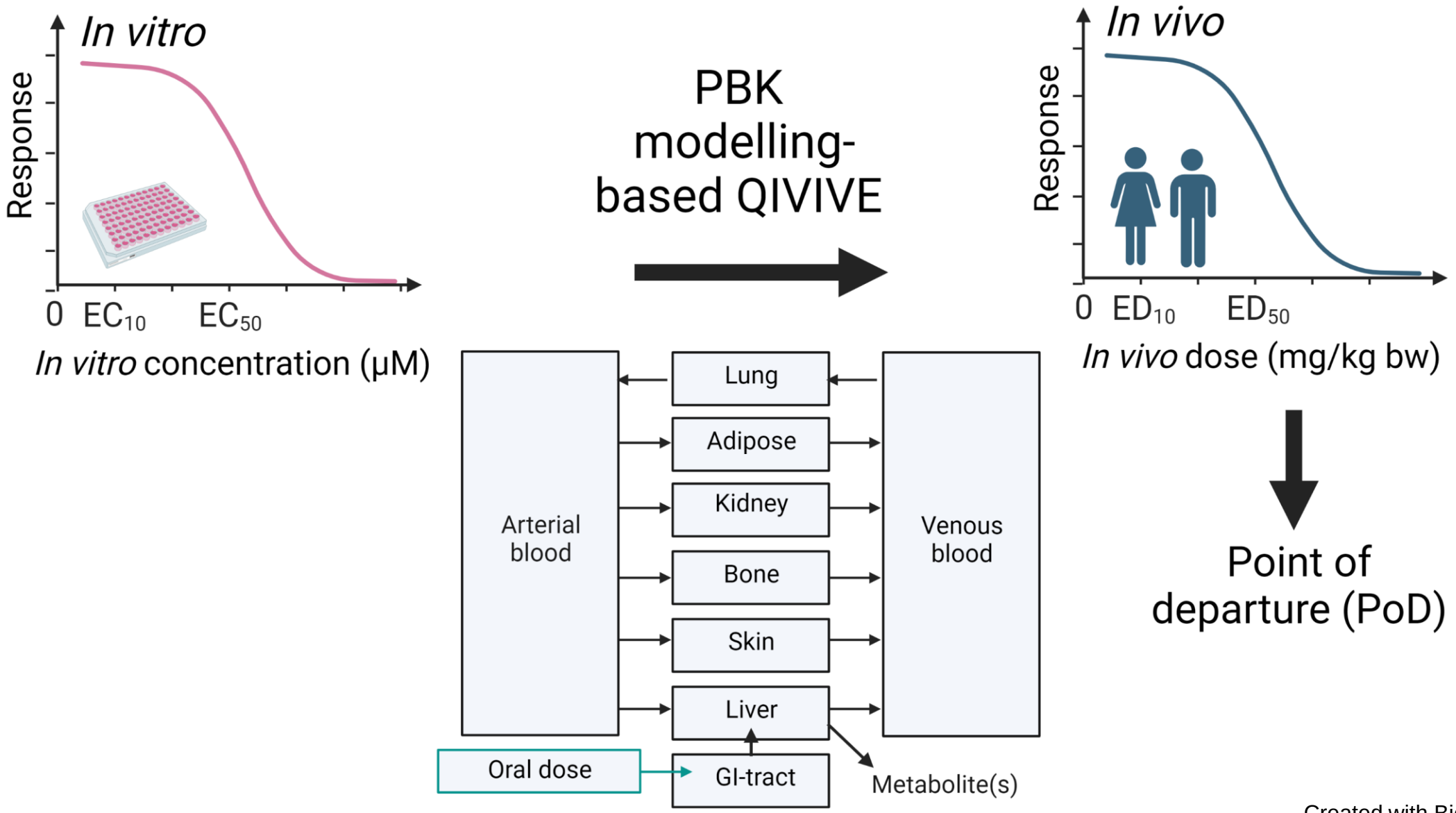


- *In vivo* anti-androgenicity FLU predominantly due to metabolite HF
- Not captured in *in vitro* androgen receptor (AR) reporter gene assay of only parent FLU

Objective:

Perform PBK modelling-based QIVIVE of the anti-androgenic activity of FLU in humans including anti-androgenic activity of HF

PBK modelling-based QIVIVE



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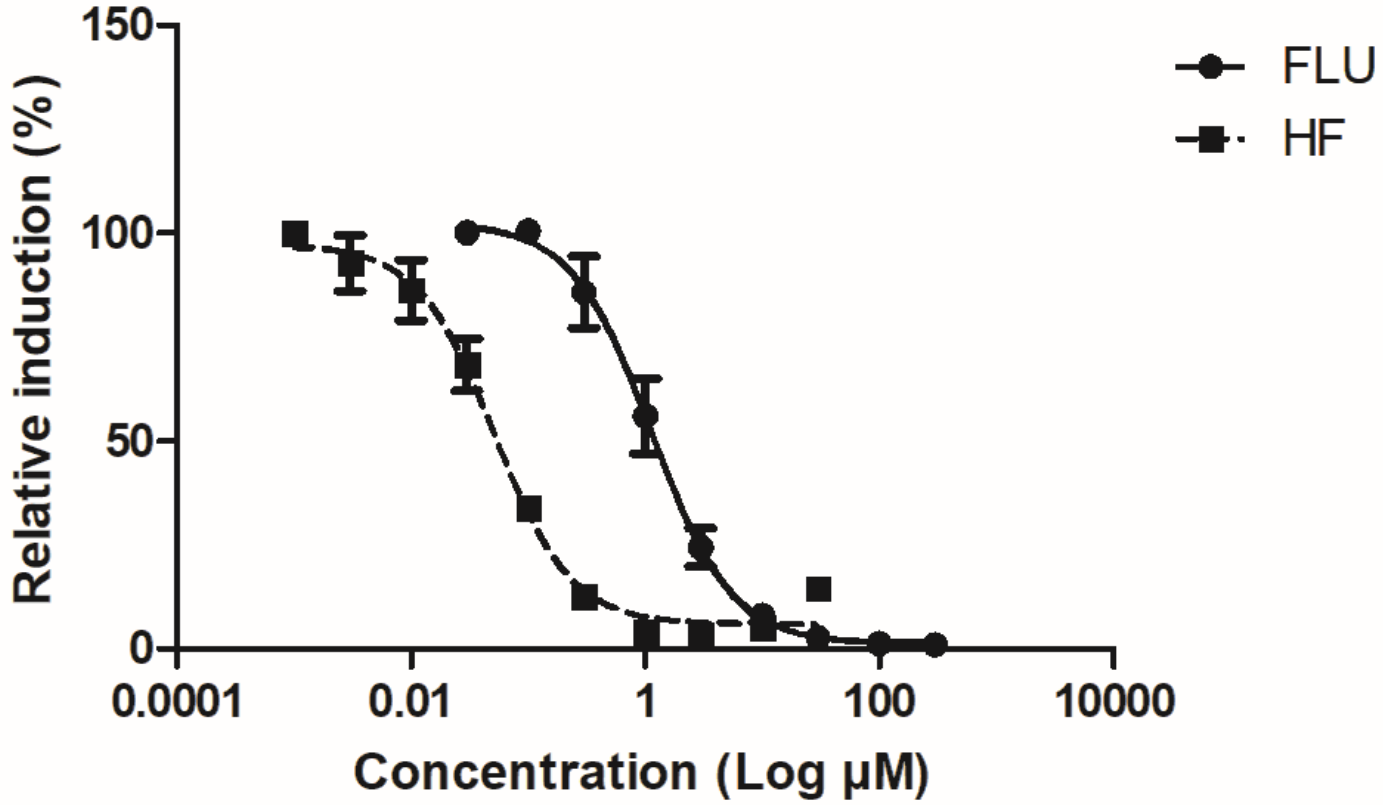
PBK modelling-based QIVIVE

Step 1	Step 2	Step 3	Step 4	Step 5
Derive *in vitro* concentration-response data of FLU and HF in the AR-CALUX assay	PBK model development describing FLU and HF kinetics in humans	Sensitivity analysis and PBK model validation with population simulation	PBK modelling-based QIVIVE of FLU - and +HF	Evaluation by benchmark dose analysis

Step 1 Determination of *in vitro* effect concentrations of FLU and HF in the AR-CALUX assay

Concentration-dependent antagonistic activity of **FLU** and **HF** on the DHT-mediated luciferase induction in the U2OS AR-CALUX reporter gene assay

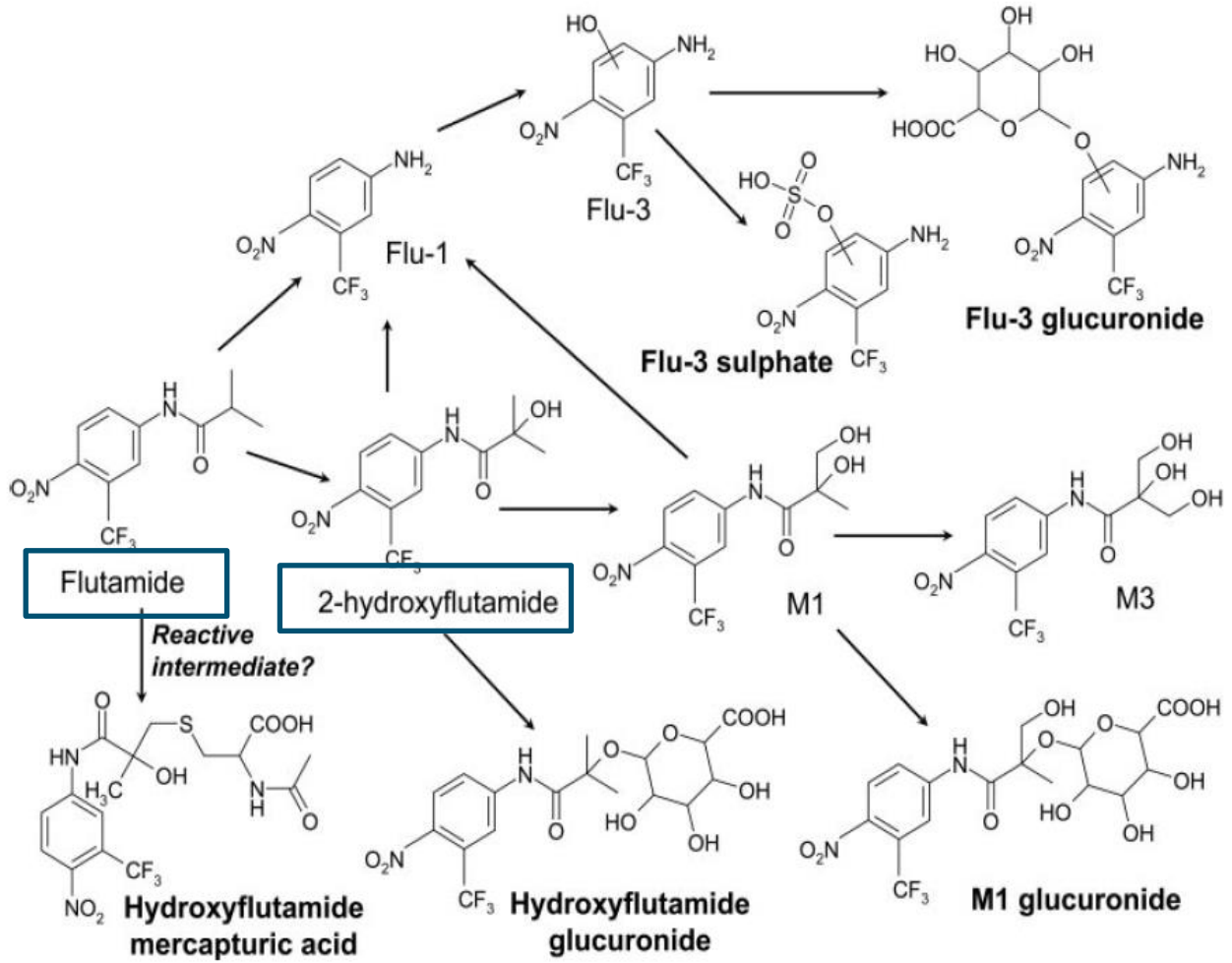


	IC_{50}
FLU	1.14 µM
HF	0.05 µM
Fold difference IC_{50}	23

Step 2 PBK model development describing FLU and HF kinetics in human

Required: Hepatic kinetic parameters FLU and HF

Metabolic scheme FLU and HF in human liver



Step 2 PBK model development describing FLU and HF kinetics in human

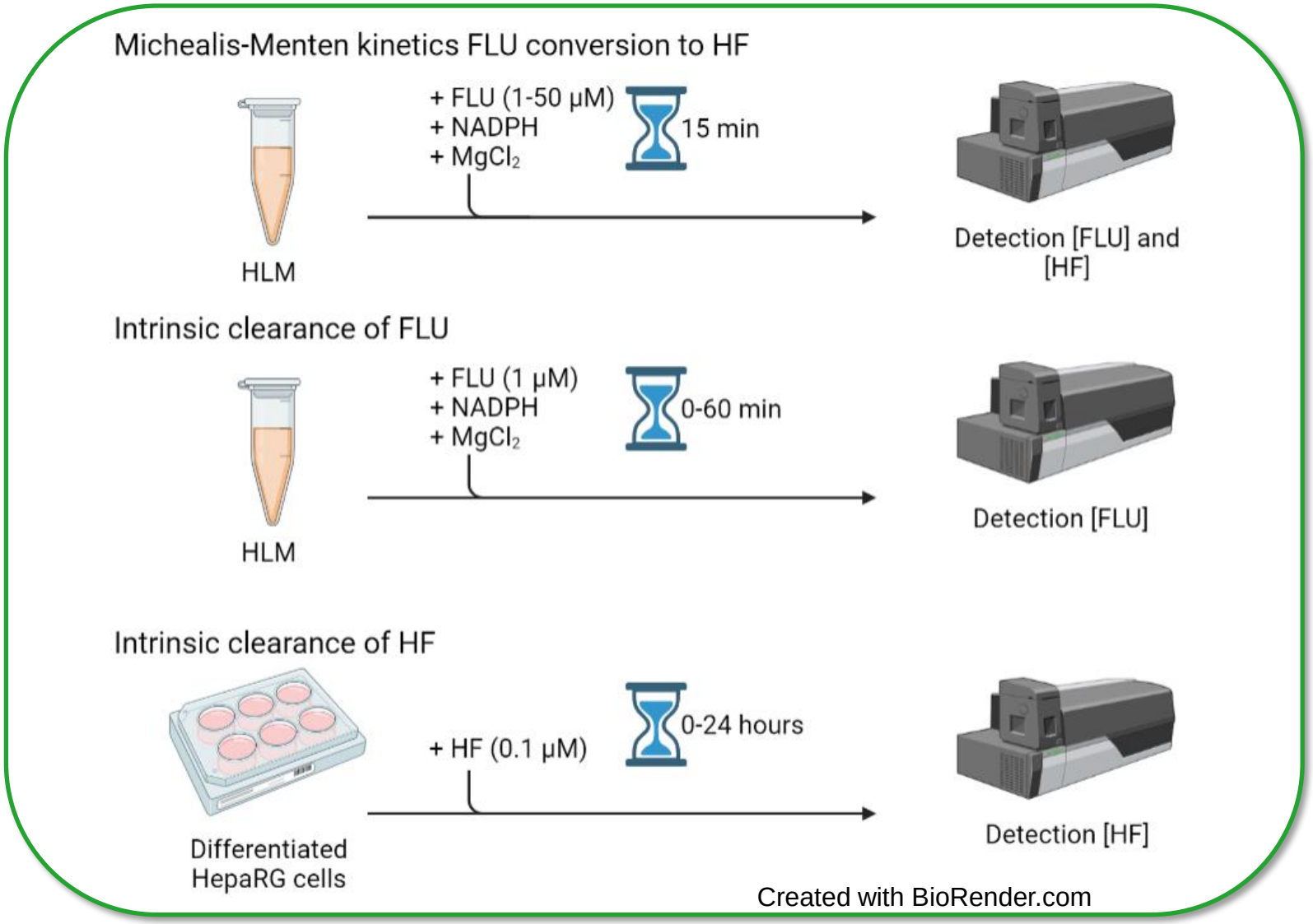
Required: *In vitro* determination hepatic kinetic parameters FLU and HF

FLU incubation with

- Human liver microsomes (HLM)

HF incubation with

- Human liver cells (HepaRG)



Step 2 PBK model development describing FLU and HF kinetics in human using GastroPlus

Kinetic parameter	Value *in vitro*
V_{max} FLU to HF → To human PK data	0.53 ± 0.08 nmol/min/mg protein
Optimized V_{max} FLU to HF[a]	
K_m FLU to HF	
CL_{int} FLU	
CL_{int} HF	

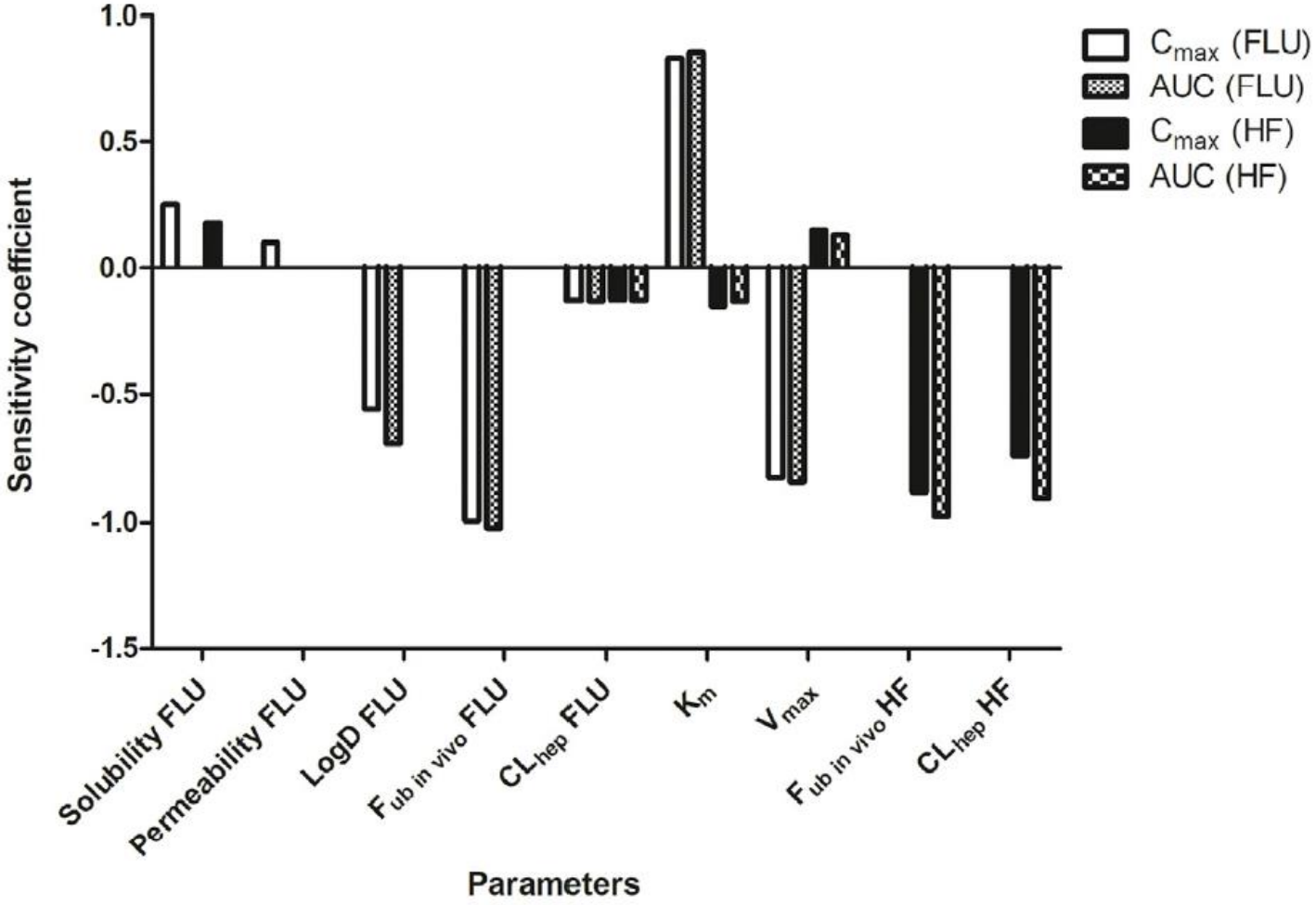
Parameters	FLU	HF
MW (g/mol)	276.22[a]	292.21[a]
LogP	3.35[a]	2.70[a]
Solubility at 25°C (mg/mL)	$5.7*10^{-3}$[b]	0.16[c]
pKa	Acid 10.54[b]	Acid 0.84[b]
	Base 0.83[b]	
P_{eff} (x 10^{-4} cm/s)	5.25[d]	
F_{ub} *in vivo*	0.20[b]	0.32[b]
R_{b2p}	0.83[b]	0.84[b]

[a] *Kim et al. (2016).*
[b] *ADMET predictor™.*
[c] *Wishart et al. (2007).*
[d] *Zuo et al. (2000).*

Step 3 Sensitivity analysis

Model parameterized for a standard human (Brown et al. 1997)	
Population	American male
Age	30
Weight	70 kg
Dose	**250 mg FLU 3x a day repeated dosing for 9 days (Radwanski et al. 1989)**

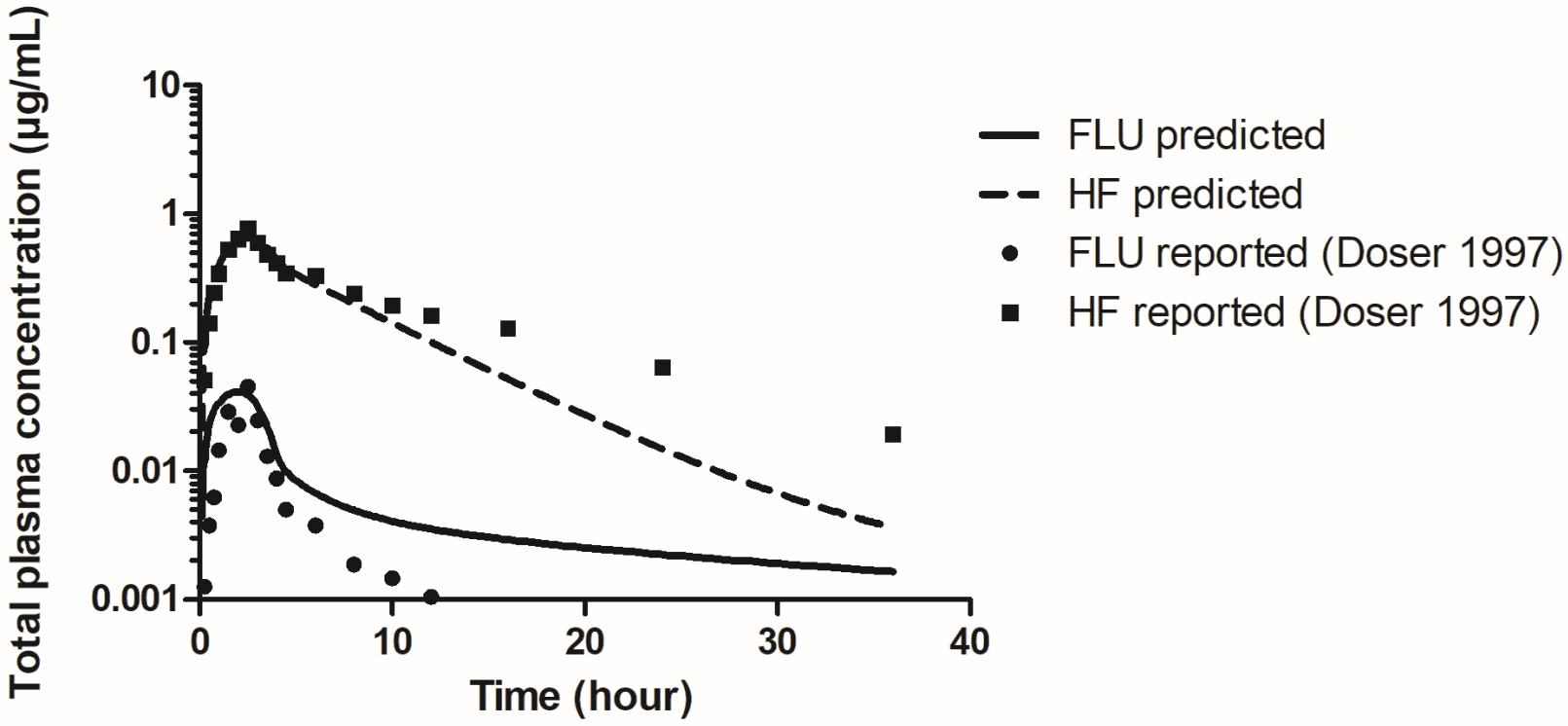
$$SC = \frac{\%\ \text{change in model outcome}}{\%\ \text{change in parameter value}}$$ (Set at 5%)



Step 3

PBK model validation

PK data from Doser et al. (1997)	
Population	Healthy females
Age	na
Weight	normal
n	19
Dose	**Single dose of 250 mg FLU**



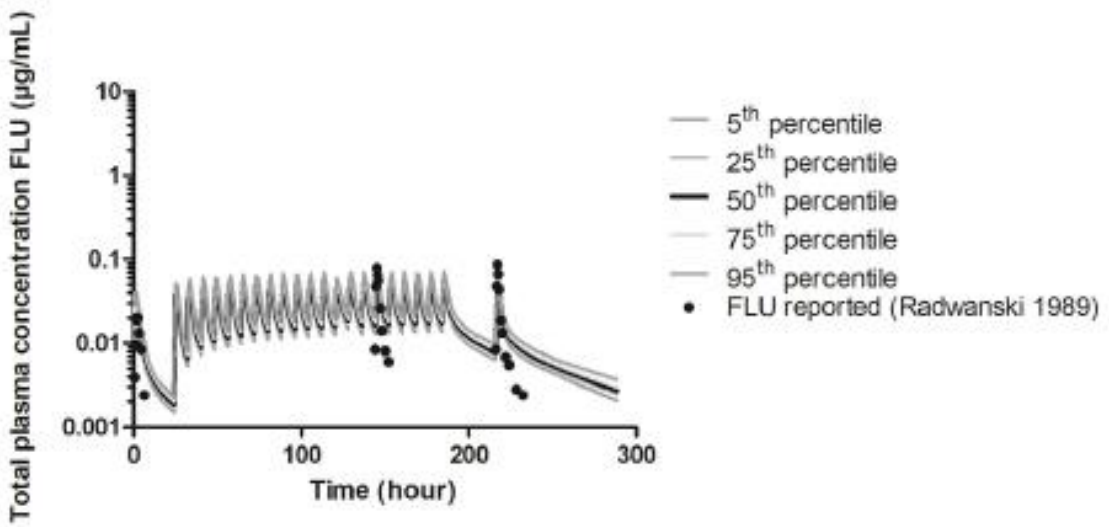
Step 3 PBK model validation with population simulation

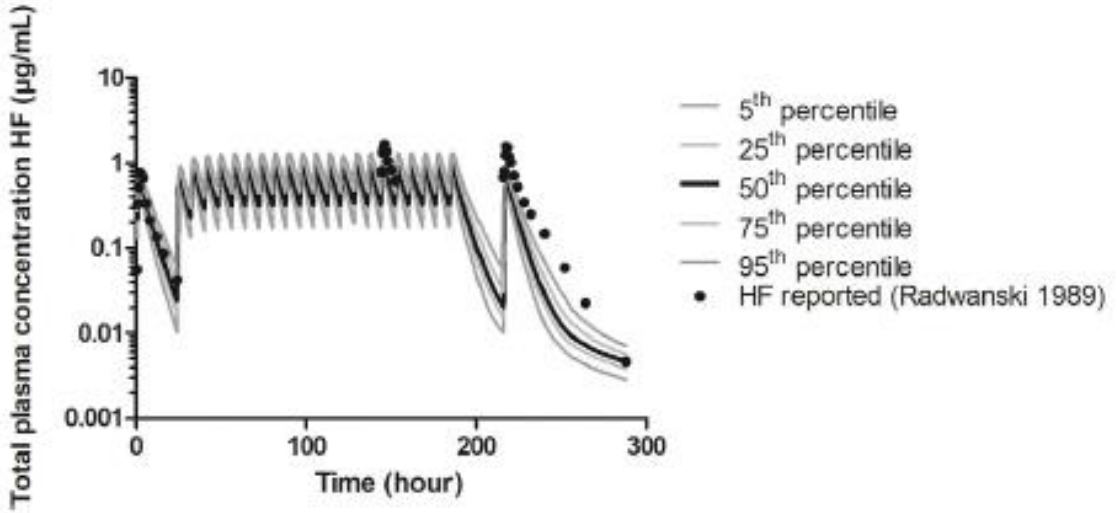
PK data from Radwanski et al. (1989)	
Population	Healthy geriatric males
Age (mean)	66
Weight (mean)	89
n	19
Dose	**250 mg FLU 3x a day repeated dosing for 9 days**

Distribution of the predictions over a healthy American Population:

n=100; male: female = 50: 50 (20-80 yo, 50-110 kg)

Confirms validity PBK model describing FLU and HF kinetics in humans





Step 4 Translation of the *in vitro* concentration-response data **FLU** to *in vivo* dose-response data, -**HF** using the PBK model developed

PBK modelling-based *in vitro* to *in vivo* extrapolation approach

1. Correct nominal *in vitro* concentrations of FLU in AR-CALUX assay for *in vitro* protein binding to obtain free *in vitro* concentrations FLU

	FLU	HF
$F_{ub\ in\ vitro}$	0.5	0.57
$F_{ub\ in\ vivo}$	0.2	0.32

free *in vitro* concentration FLU = *in vitro* concentration FLU * $f_{ub\ in\ vitro,\ FLU}$

2. Surrogate AR-CALUX based free *in vitro* concentrations FLU to free *in vivo* C_{max} values of FLU

free *in vitro* concentration FLU = free *in vivo* $C_{max,\ FLU}$

3. Model FLU doses which are required to reach these free *in vivo* C_{max} values of FLU using PBK model

Step 4 Translation of the *in vitro* concentration-response data **FLU** to *in vivo* dose-response data, **+HF** using the PBK model developed

PBK modelling-based *in vitro* to *in vivo* extrapolation approach

1. Surrogate AR-CALUX based free *in vitro* concentrations FLU to combined free *in vivo* C_{max} FLU +HF expressed in FLU equivalents

- Using the toxic equivalency factor (TEF)

	FLU	HF
$F_{ub\ in\ vitro}$	0.5	0.57
$F_{ub\ in\ vivo}$	0.2	0.32
IC_{50} (µM)	1.14	0.05
TEF	**1**	**23**

$$TEF_{HF} = IC_{50,\ FLU} / IC_{50,\ HF}$$

free *in vitro* concentrations FLU = combined free C_{max} of FLU and HF expressed in FLU equivalents

$$= \underbrace{C_{max,\ FLU} * FLU\ f_{ub\ in\ vivo} * TEF_{FLU}}_{\text{Free in vivo } C_{max,\ \mathbf{FLU}}} + \underbrace{C_{max,\ HF} * HF\ f_{ub\ in\ vivo} * TEF_{HF}}_{\text{Free in vivo } C_{max,\ \mathbf{HF\ as\ FLUeq}}}$$

2. Model FLU doses which are required to reach these combined free C_{max} FLU and HF expressed in FLU equivalents

Assumptions TEF-based QIVIVE

3 assumptions

1. FLU and HF have same mode of action

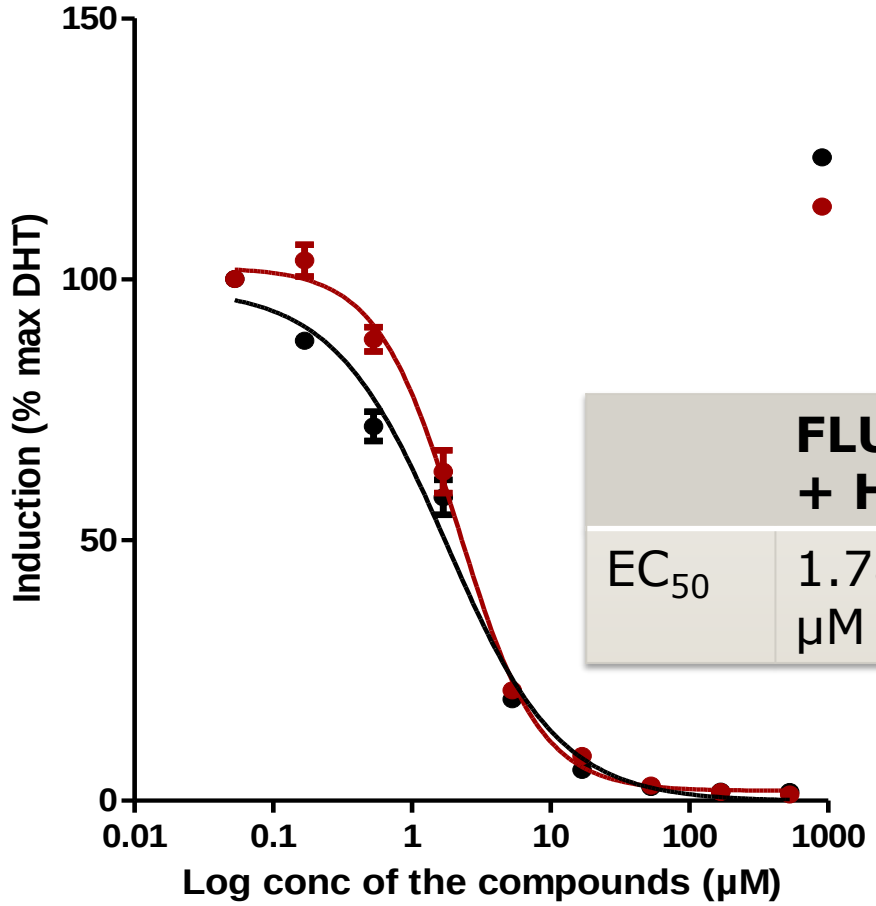
   AR inactivation

2. Concentration response curves FLU and HF are parallel

   Hillslope FLU vs HF has p value of 0.6985, so curves parallel

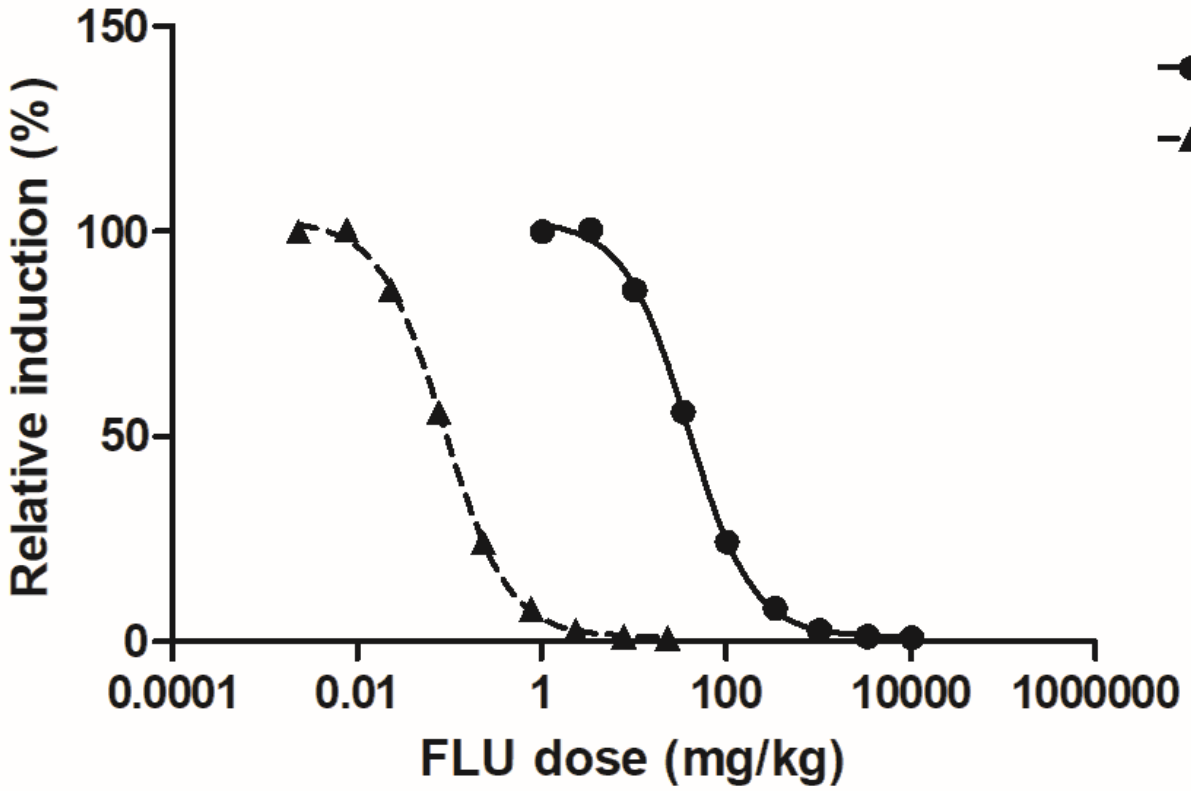
3. Toxicity is additive

   $= [FLU_{in\ vitro}] * TEF_{FLU} + [HF_{in\ vitro}] * TEF_{HF}$



	FLU + HF	FLU only
EC_{50}	1.78 µM	2.17 µM

Step 5 Evaluation of the predicted dose-dependent anti-androgenic effects of FLU, – and +HF, including BMD analysis of the predicted dose-response data

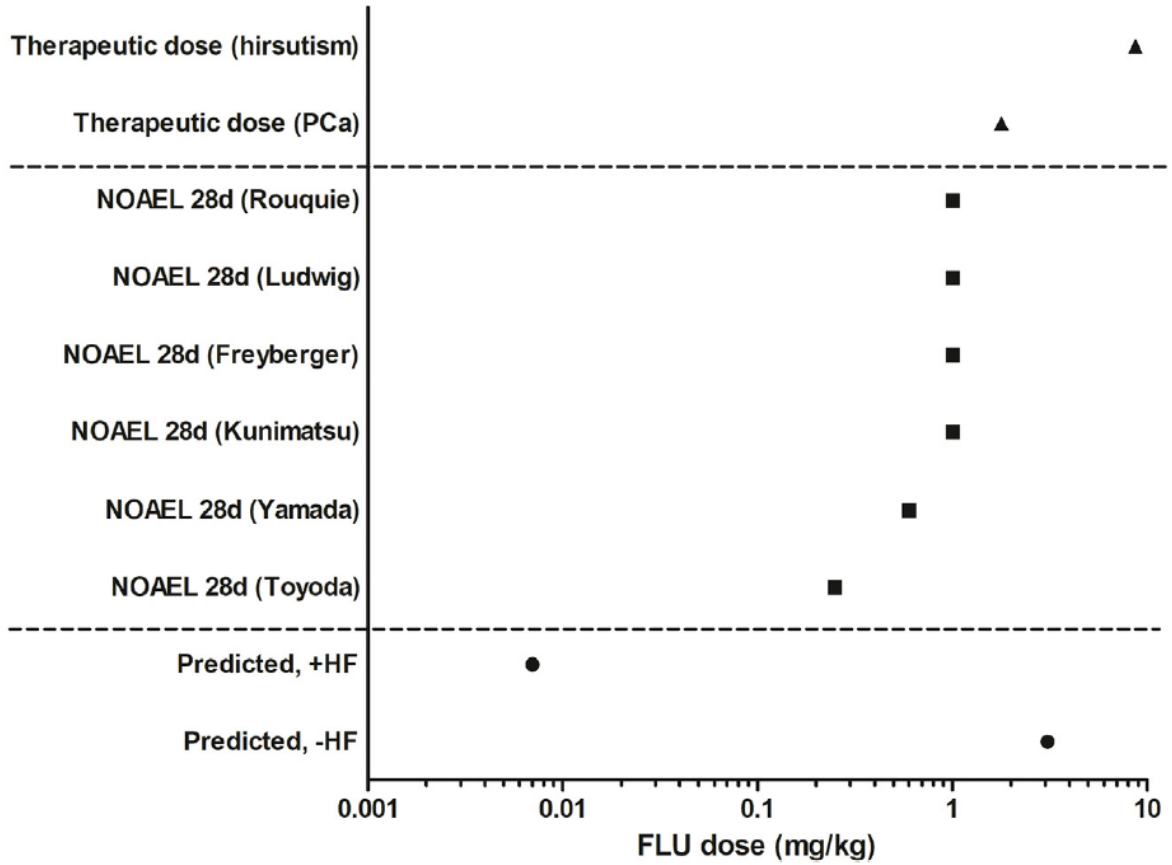


	FLU -HF	FLU +HF
$BMDL_{05}$ (mg/kg)	3.08	0.007

BMDS3.2.1 software (US EPA)

Including the contribution of HF in QIVIVE predicting the *in vivo* anti-androgenic activity of FLU results in **440 fold** lower $BMDL_{05}$

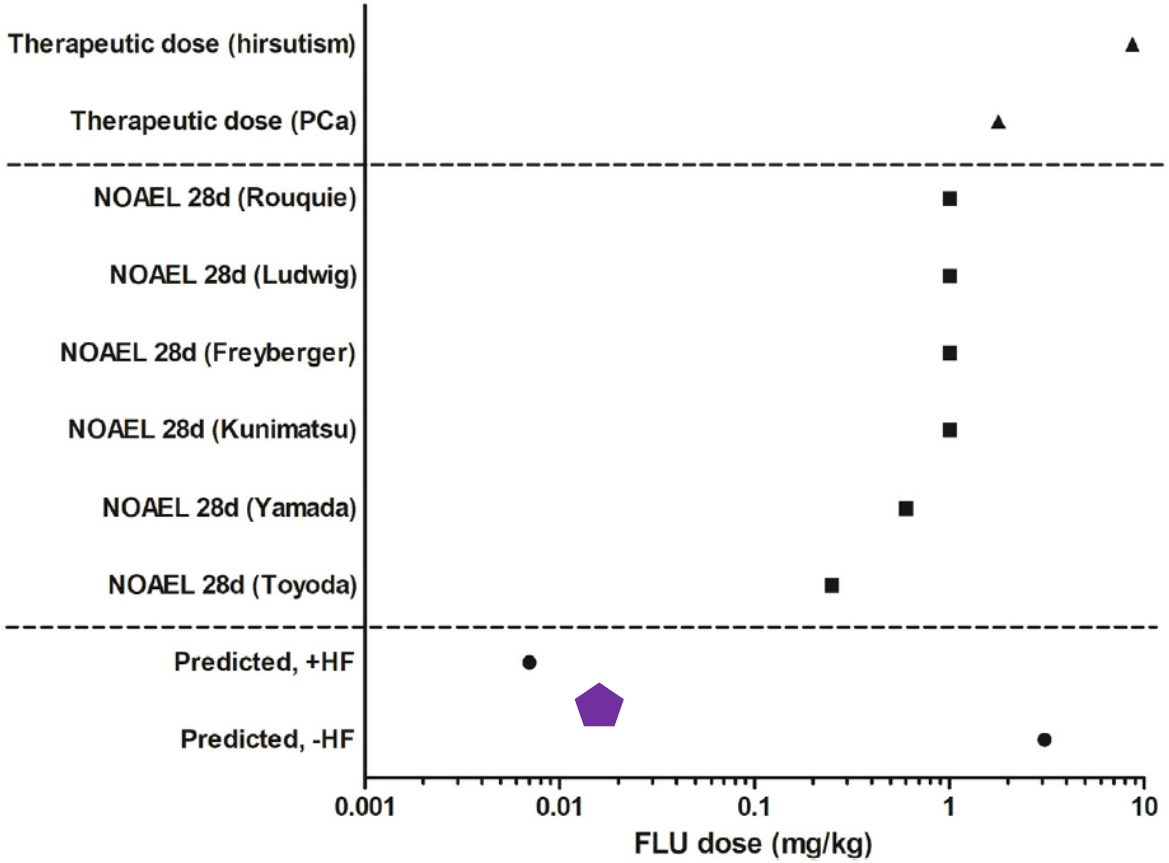
Step 5 Evaluation of the predicted dose-dependent anti-androgenic effects of FLU, – and +HF, including BMD analysis of the predicted dose-response data



- **PoD FLU –HF** comparable to therapeutic active doses FLU
- **PoD FLU +HF** 35 fold lower than lowest reported NOAEL

PBK modelling-based QIVIVE of the *in vitro* anti-androgenic response of FLU including the contribution of HF is protective to predict *in vivo* anti-androgenic activity

Step 5 Evaluation of the predicted dose-dependent anti-androgenic effects of FLU, – and +HF, including BMD analysis of the predicted dose-response data



35 fold difference in *in vitro* derived PoD and animal derived PoD

- Rat lower conversion rate FLU to HF and lower FLU clearance
- At similar exposure level FLU and bioavailability, humans expected to have higher HF levels than rats

Exchanging human V_{max} with rat V_{max} in PBK model:

$BMDL_{05}$ of FLU +HF = 0.014 mg/kg (⬟)

= 17-fold lower than lowest animal-PoD

At similar exposure level FLU and bioavailability, in humans higher anti-androgenicity, justifying lower PoD

Discussion & conclusion

- *In vitro* derived PoD more conservative than animal derived PoD
  - Species differences in toxicokinetics
  - Disruption at molecular versus organ/tissue level
- Use of uncertainty factors (UFs) in IVIVE
  - Interindividual differences
  - UF interspecies differences exchanged by UF for uncertainty using *in vitro* and *in silico* assays

- Including contribution of toxicokinetics and toxicodynamics metabolite important in setting PoD based on PBK-modelling based IVIVE
- *In vitro* derived PoD FLU +HF protective for human health
- NGRA not to predict animal PoDs but to protect human health

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