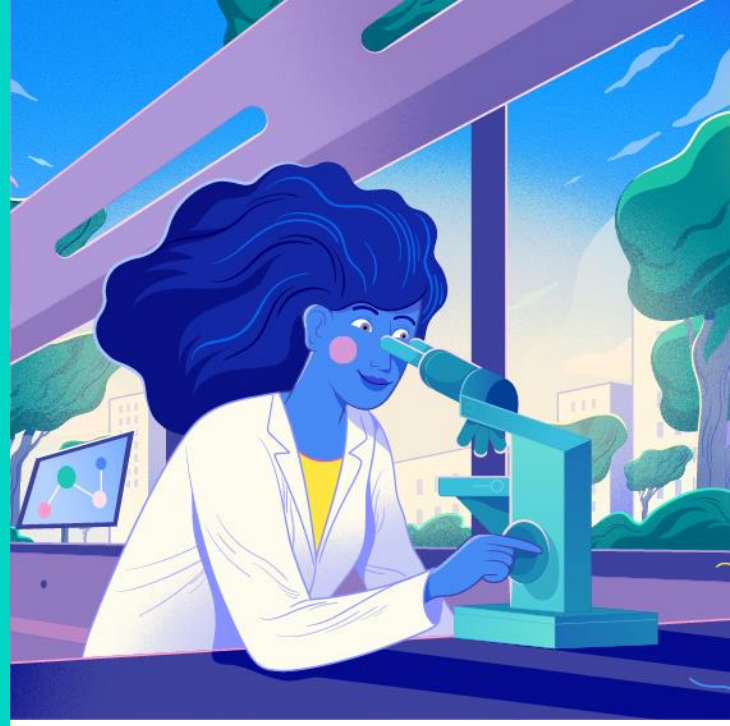
QIVIVE (quantitative in vitro-in vivo extrapolation) – bridging in vitro POD using PBK modelling.
-A case study



Hequn Li

Unilever
Safety & Environmental Assurance Centre (SEAC)

Our products must be safe

Can we make decisions on these people's safety?

The decisions we make about the safety of our products are for our consumers and workers all around the globe

Unilever

Making safety decisions without generating data in animals

- Regulations ban animal testing of cosmetic products and their ingredients in over 40 countries
- Many of our consumers do not want to buy products associated with animal testing
- Our safety assessments use a variety of non-animal approaches from QSARs/read across, ‘traditional’ in vitro approaches, and into Next Generation Risk Assessment (NGRA)

From traditional risk assessment to next generation risk assessment

Hazard ID

Potency screening

NOAEL
(mg/kg/day)

Traditional RA:

Hazard confirmation

Potency assessment

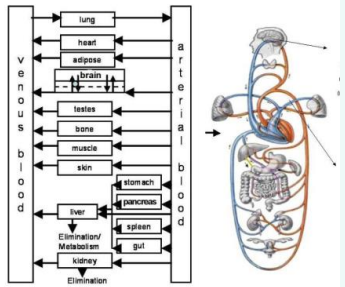
consumer exposure

internal exposure

Animal testing

- may not be relevant to human
- slower and higher cost

Animal testing for **cosmetics** is officially **banned** in 40 countries



QIVIVE with PBK modelling

Next generation

RA:

bioactivity characterization

Next generation risk assessment (NGRA):
developing and applying new approach methodologies (NAMs)
without generating new animal data

What is PBK (physiologically based kinetic) modelling?

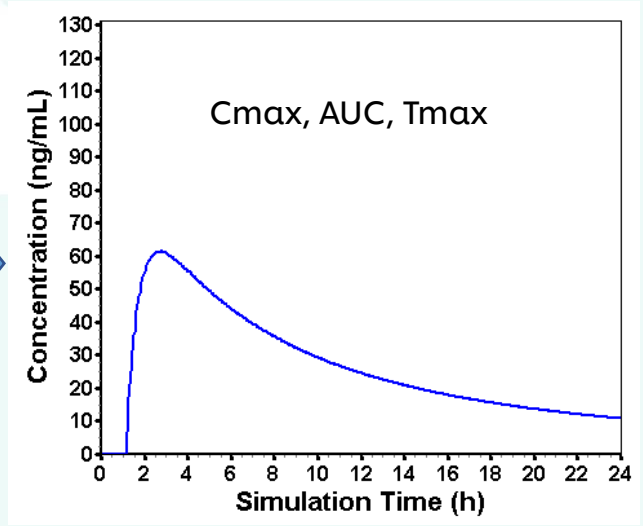
Input

ADME properties

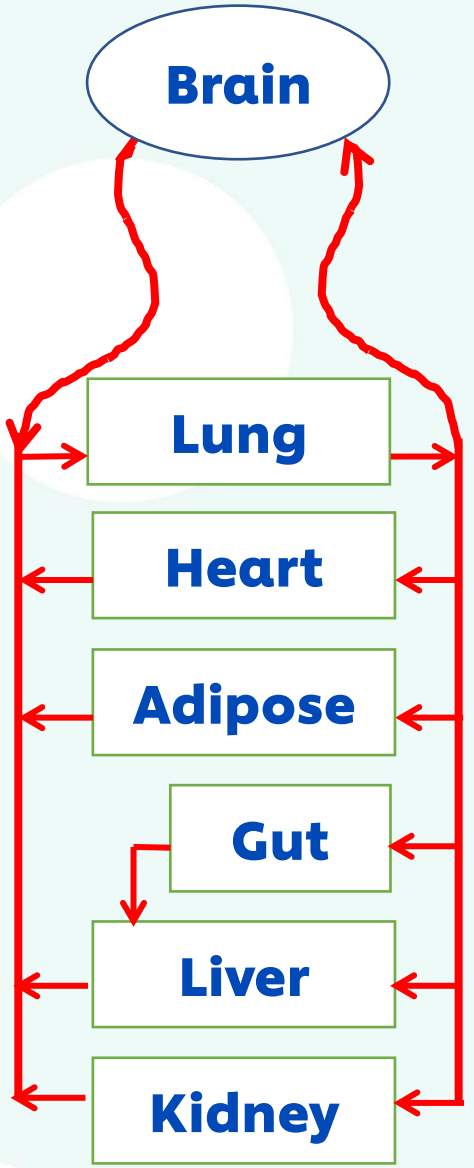
Absorption, Distribution, Metabolism, Excretion

- Physiological parameters (e.g. body weight, blood flow rates, tissue volume)
- Physico-chemical parameters (e.g. LogP, Fup, tissue/plasma partition coefficients)
- Kinetic parameters (e.g. dermal absorption, hepatic metabolism, renal excretion)
- Product use information (e.g. dose, frequency, site area, formulation)

Output



Population simulation
Uncertainty analysis



$$V_L \frac{dC_L}{dt} = Q_l\left(C_V - \frac{C_L}{P_L}\right)$$

$$V_B \frac{dC_B}{dt} = Q_B\left(C_V - \frac{C_B}{P_B}\right)$$

$$V_{SP} \frac{dC_{SP}}{dt} = Q_{SP}\left(C_V - \frac{C_{SP}}{P_{SP}}\right)$$

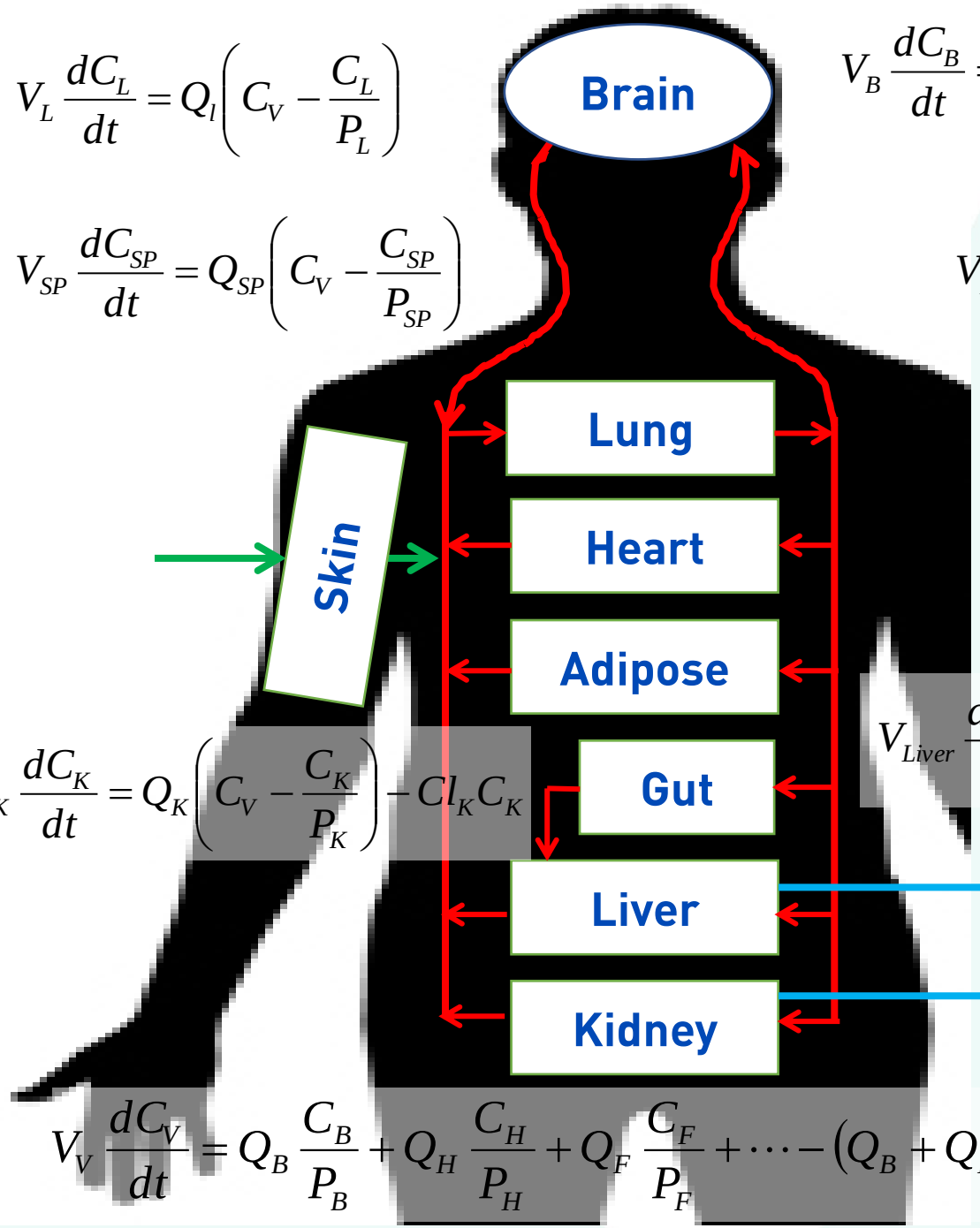
$$V_H \frac{dC_H}{dt} = Q_H\left(C_V - \frac{C_H}{P_H}\right)$$

$$V_F \frac{dC_F}{dt} = Q_F\left(C_V - \frac{C_F}{P_F}\right)$$

$$V_{RP} \frac{dC_{RP}}{dt} = Q_{RP}\left(C_V - \frac{C_{RP}}{P_{RP}}\right)$$

$$V_{Liver} \frac{dC_{Liver}}{dt} = Q_{Liver}\left(C_V - \frac{C_{Liver}}{P_{Liver}}\right) - \frac{V_{\max} C_{Liver}}{K_m + C_{Liver}}$$

$$V_K \frac{dC_K}{dt} = Q_K\left(C_V - \frac{C_K}{P_K}\right) - Cl_K C_K$$



Metabolism

Excretion

$$V_V \frac{dC_V}{dt} = Q_B \frac{C_B}{P_B} + Q_H \frac{C_H}{P_H} + Q_F \frac{C_F}{P_F} + \cdots - \left(Q_B + Q_H + Q_F + \cdots\right) C_V + dose(t)$$

How it works

- Programming Languages
- Continuous Simulation Software
- Commercial Software
- Publicly Available Tools

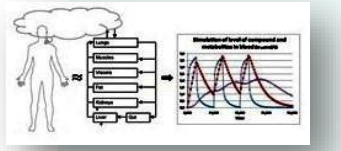
MATLAB®
The Language of Technical Computing

BERKELEY MADONNA
Modeling and Analysis of Dynamic Systems

acslX
Pharmaceutical

SIMCYP
real solutions from virtual populations

GastroPlus™



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AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY

GastroPlus


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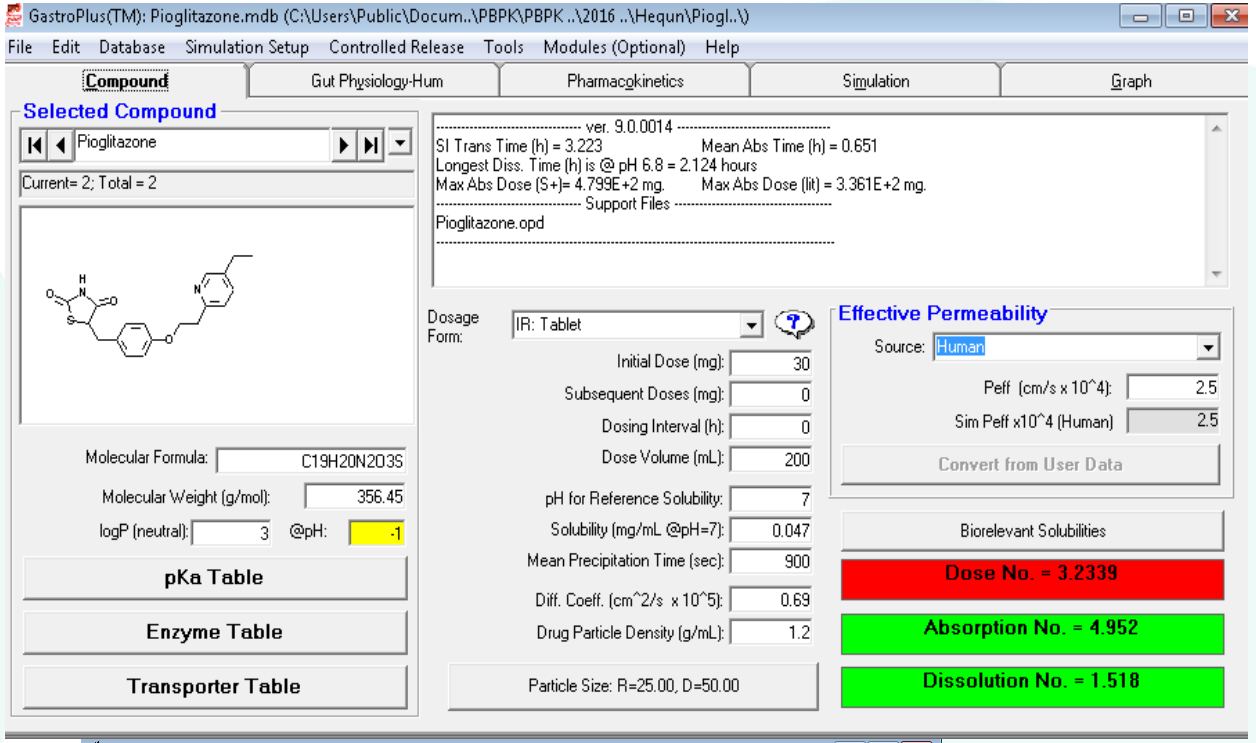
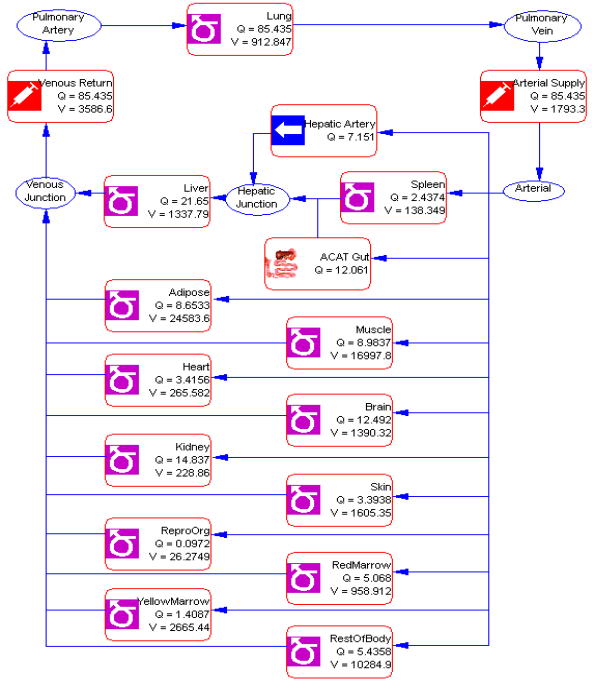
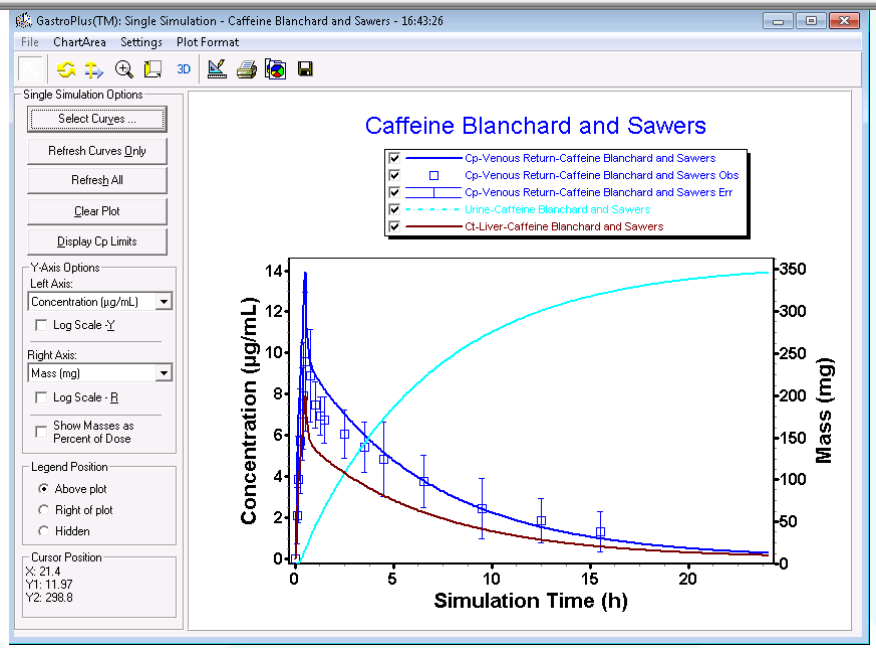

GastroPlus(TM): Pioglitazone.mdb (C:\Users\Public\Docum..\PBPK\PBPK ..\2016 ..\Hequn\Piogl..\)
File Edit Database Simulation Setup Controlled Release Tools Modules (Optional) Help
Compound
Gut Physiology-Hum
Pharmacokinetics
Simulation
Graph
Selected Compound
Pioglitazone
Current= 2; Total = 2
ver. 9.0.0014
SI Trans Time (h) = 3.223
Mean Abs Time (h) = 0.651
Longest Diss. Time (h) is @ pH 6.8 = 2.124 hours
Max Abs Dose (S+)= 4.799E+2 mg.
Max Abs Dose (lit) = 3.361E+2 mg.
Support Files
Pioglitazone.opd
Molecular Formula: C19H20N2O3S
Molecular Weight (g/mol): 356.45
logP (neutral): 3 @pH: -1
pKa Table
Enzyme Table
Transporter Table
Dosage Form: IR: Tablet
Initial Dose (mg): 30
Subsequent Doses (mg): 0
Dosing Interval (h): 0
Dose Volume (mL): 200
pH for Reference Solubility: 7
Solubility (mg/mL @pH=7): 0.047
Mean Precipitation Time (sec): 900
Diff. Coeff. (cm^2/s x10^5): 0.69
Drug Particle Density (g/mL): 1.2
Particle Size: R=25.00, D=50.00
Effective Permeability
Source: Human
Peff (cm/s x 10^4): 2.5
Sim Peff x10^4 (Human) 2.5
Convert from User Data
Biorelevant Solubilities
Dose No. = 3.2339
Absorption No. = 4.952
Dissolution No. = 1.518


Pulmonary Artery
Lung Q = 85.435 V = 912.847
Pulmonary Vein
Venous Return Q = 85.435 V = 3586.6
Arterial Supply Q = 85.435 V = 1793.3
Hepatic Artery Q = 7.151
Venous Junction
Liver Q = 21.65 V = 1337.79
Hepatic Junction
Spleen Q = 2.4374 V = 138.349
Arterial
ACAT Gut Q = 12.061
Adipose Q = 8.6533 V = 24583.6
Muscle Q = 8.9837 V = 16997.8
Heart Q = 3.4156 V = 265.582
Brain Q = 12.492 V = 1390.32
Kidney Q = 14.837 V = 228.86
Skin Q = 3.3938 V = 1605.35
ReproOrg Q = 0.0972 V = 26.2749
RedMarrow Q = 5.068 V = 958.912
YellowMarrow Q = 1.4087 V = 2665.44
RestOfBody Q = 5.4358 V = 10284.9


GastroPlus(TM): Single Simulation - Caffeine Blanchard and Sawers - 16:43:26
File ChartArea Settings Plot Format
Single Simulation Options
Select Curves ...
Refresh Curves Only
Refresh All
Clear Plot
Display Cp Limits
Y-Axis Options
Left Axis:
Concentration (µg/mL)
Log Scale -Y
Right Axis:
Mass (mg)
Log Scale -R
Show Masses as Percent of Dose
Legend Position
Above plot
Right of plot
Hidden
Cursor Position
X: 21.4
Y1: 11.97
Y2: 298.8
Caffeine Blanchard and Sawers
Cp-Venous Return-Caffeine Blanchard and Sawers
Cp-Venous Return-Caffeine Blanchard and Sawers Obs
Cp-Venous Return-Caffeine Blanchard and Sawers Err
Urine-Caffeine Blanchard and Sawers
Ct-Liver-Caffeine Blanchard and Sawers
Concentration (µg/mL)
Mass (mg)
Simulation Time (h)

Objective

ELSEVIER

Toxicology in Vitro

Volume 74, August 2021, 105171

Next generation risk assessment (NGRA): Bridging *in vitro* points-of-departure to human safety assessment using physiologically-based kinetic (PBK) modelling – A case study of doxorubicin with dose metrics considerations

Hequn Li [a], Haitao Yuan [b], Alistair Middleton [a], Jin Li [a], Beate Nicol [a], Paul Carmichael [a], Jiabin Guo [b], Shuangqing Peng [b] ✉, Qiang Zhang [c] ✉

Using the chemical doxorubicin (DOX), the objective was to evaluate the impact of dose metrics selection in the new approach method of integrating physiologically-based kinetic (PBK) modelling and relevant human cell-based assays to inform *a priori* the point of departure for human health risk.

Workflow

In vitro

- determination of in vitro key concentrations of DOX that induced cardiomyocyte toxicity

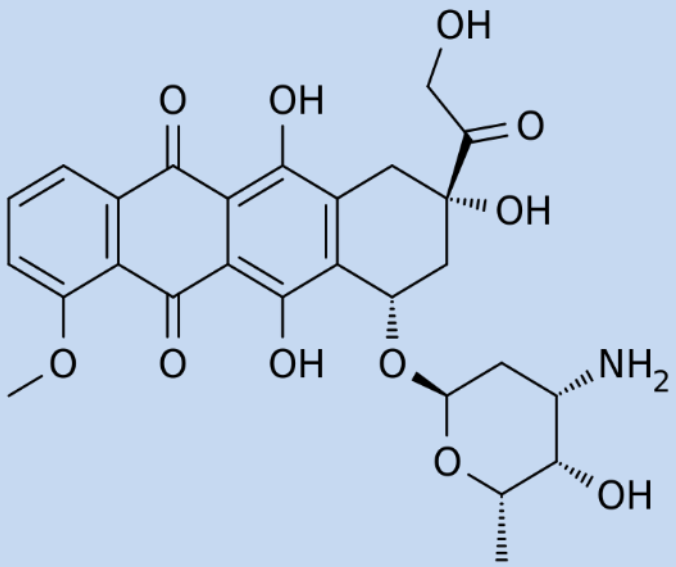
PBPK

- development of a human PBK model for DOX
- validation of the PBK model by comparing with human data

Evaluation

- Literature review on the clinical consequences of DOX treatment to identify dosing scenarios with no or mild cardiotoxicity observed
- prediction of internal key concentrations under selected clinical settings using the PBK model
- evaluation of the approach by comparison of the predicted internal doses with in vitro key concentration

Doxorubicin (Dox) case study



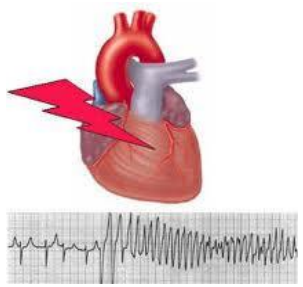
Cardiotoxic

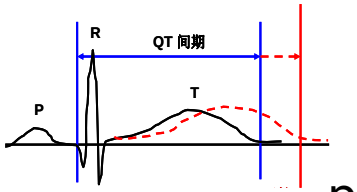
- Chemotherapy medicine used to treat cancer
- Reactive oxygen species (ROS) production increased through metabolism
- ROS causes DNA damage, lipid peroxidation and decreased glutathione levels

Cardiotoxicity of DOX in clinical

Acute cardiotoxicity

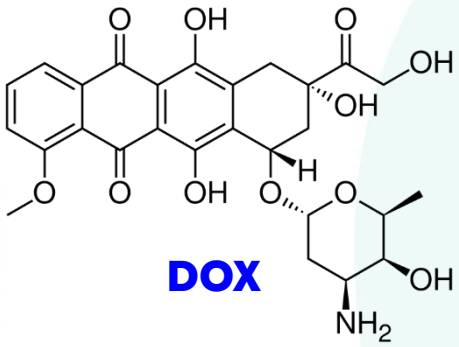
Arrhythmia





QT prolongation

Hypotension
Pericarditis



DOX

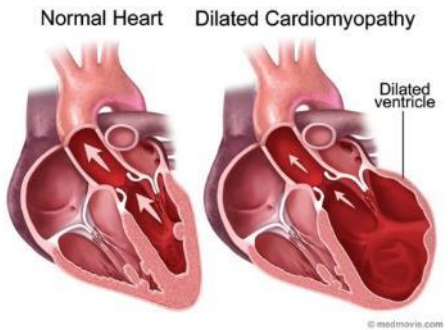
Acute toxicity:

- Non-specific ECG changes
- Reversible
- Occurs during and within 2-3 days of treatment.

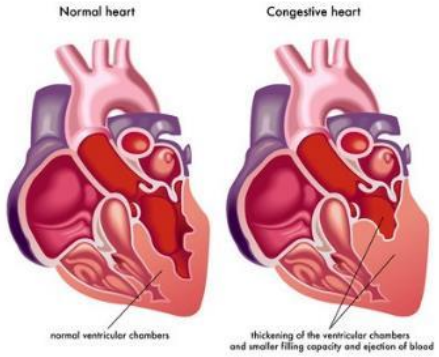
Chronic toxicity:

- Cardiomyopathy and CHF
- Dose dependent
- Irreversible

Chronic cardiotoxicity

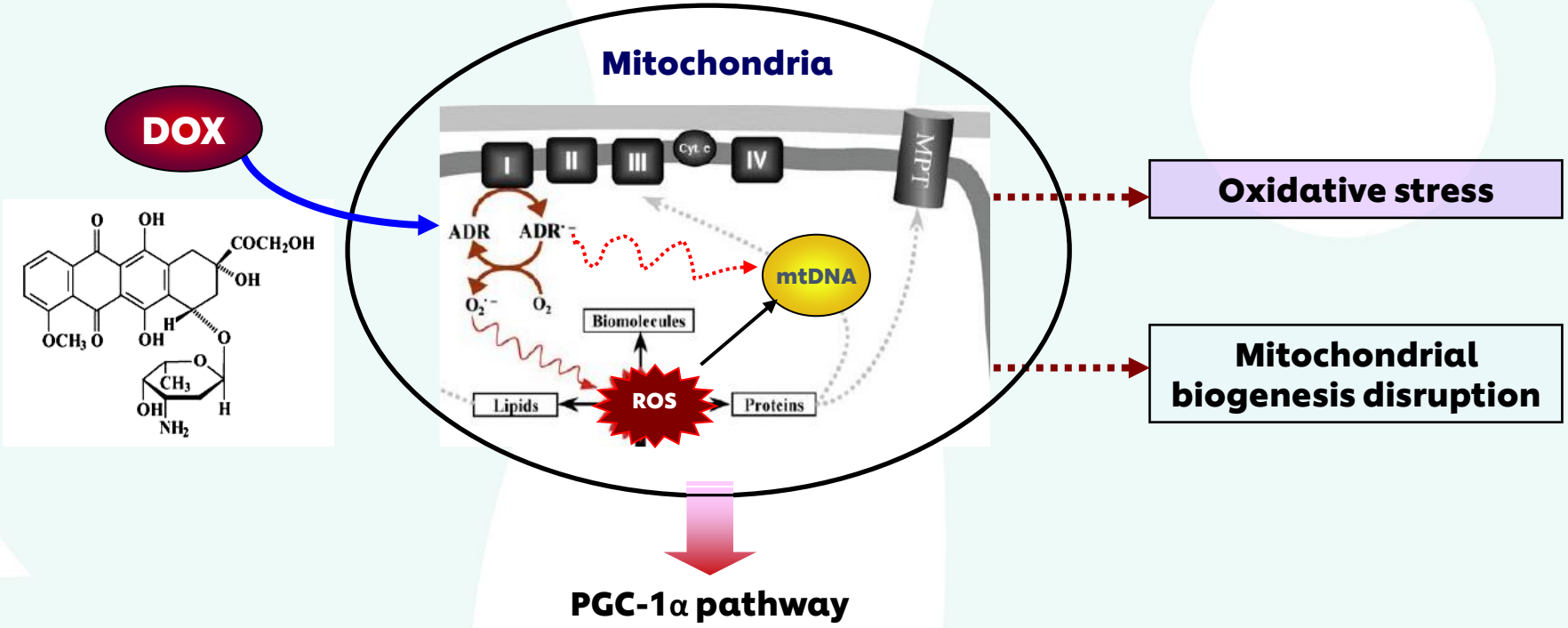


cardiomyopathy



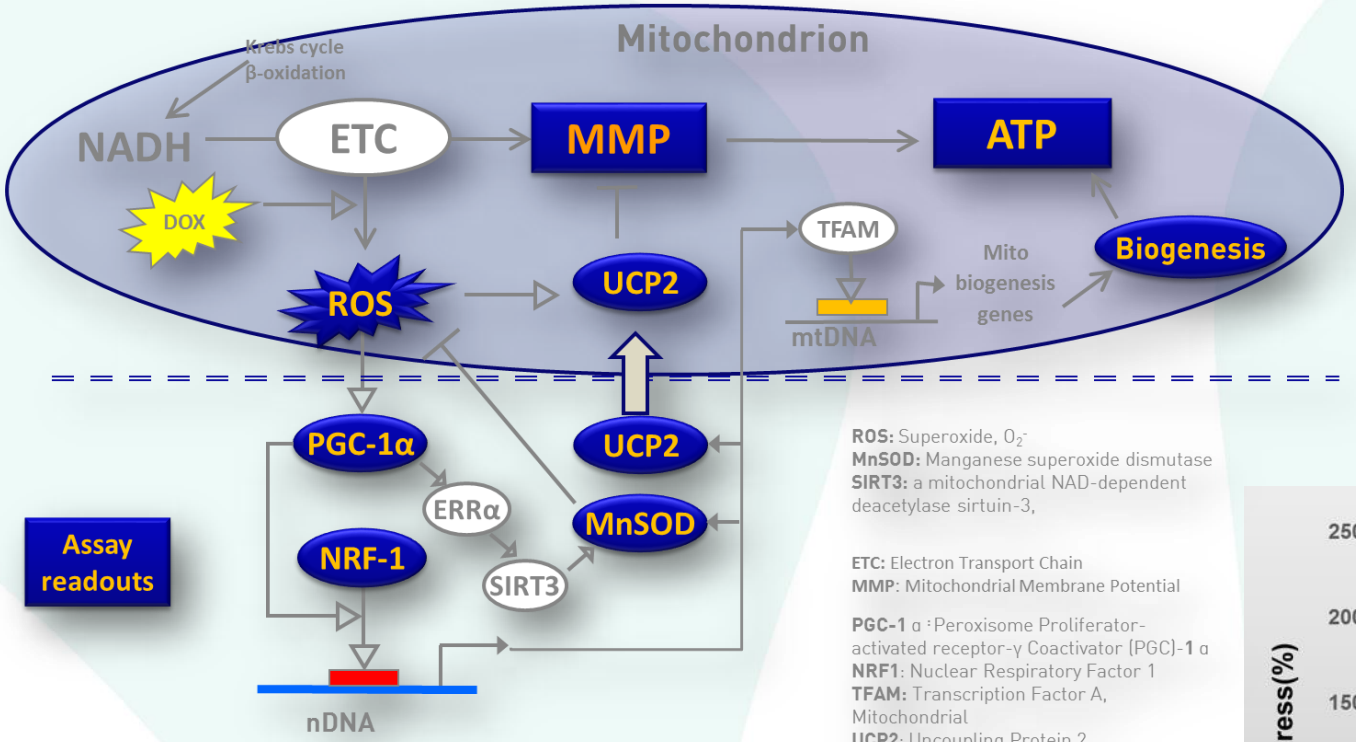
CHF (congestive heart failure)

Mitochondrial Mechanism of Doxorubicin-Induced Cardiotoxicity and Hypothesis



Hypothesis: DOX perturbs PGC-1α pathway to induce adaptive/ adverse response resulting in alteration of mitochondrial oxidative stress and disruption of biogenesis

In-vitro tipping point found in our collaboration work with AMMS

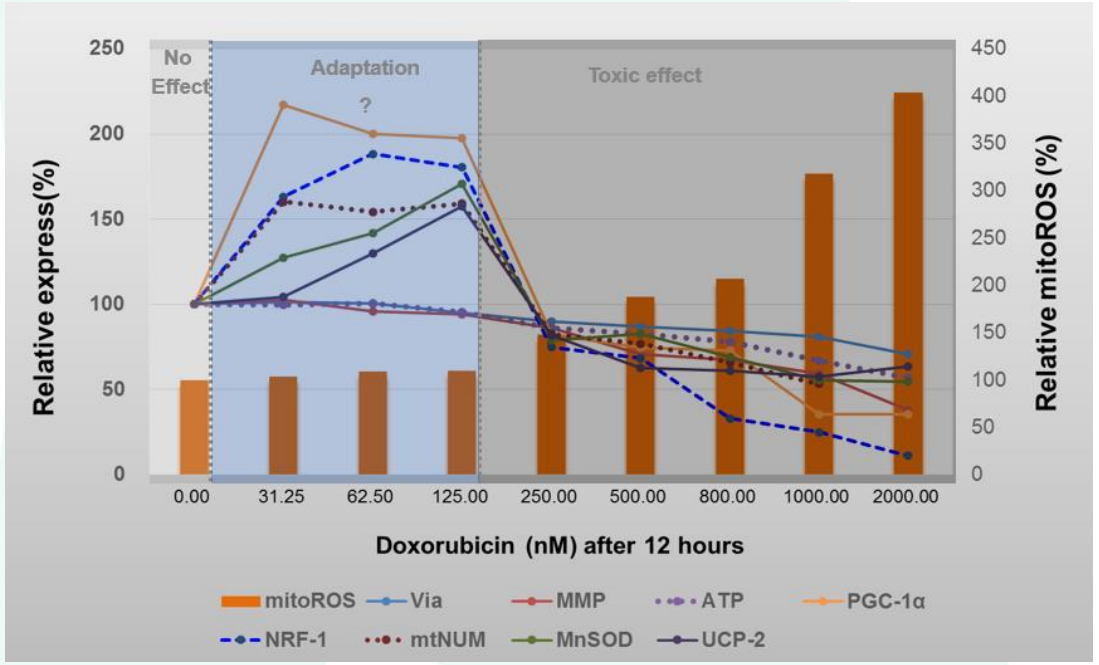


ROS: Superoxide, O_2^-
MnSOD: Manganese superoxide dismutase
SIRT3: a mitochondrial NAD-dependent deacetylase sirtuin-3,

ETC: Electron Transport Chain
MMP: Mitochondrial Membrane Potential

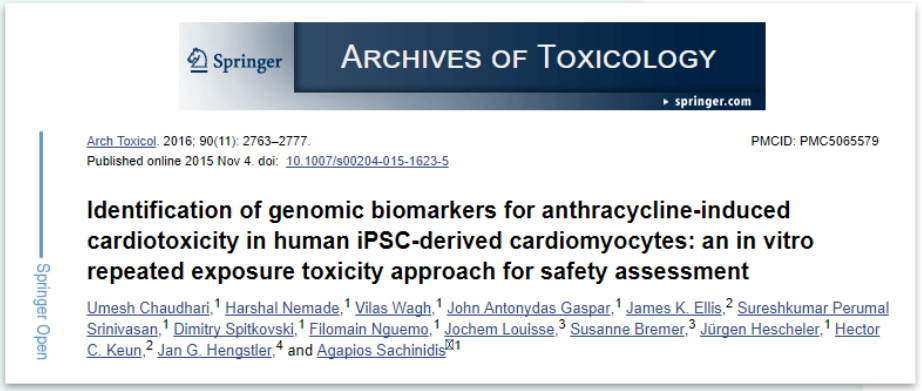
PGC-1 α : Peroxisome Proliferator-activated receptor-γ Coactivator (PGC)-**1** α
NRF1: Nuclear Respiratory Factor 1
TFAM: Transcription Factor A, Mitochondrial
UCP2: Uncoupling Protein 2

- **AC16 cardiomyocytes cell line:**
- **12h exposure**



Tipping point concentration: ***125nM @12h***

EU project – Detective



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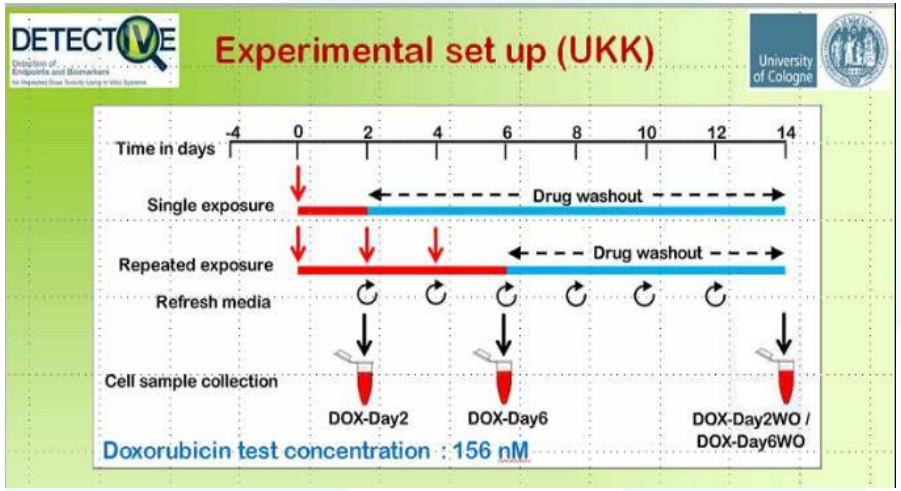
Arch Toxicol. 2016; 90(11): 2763–2777. PMCID: PMC5065579
Published online 2015 Nov 4. doi: 10.1007/s00204-015-1623-5

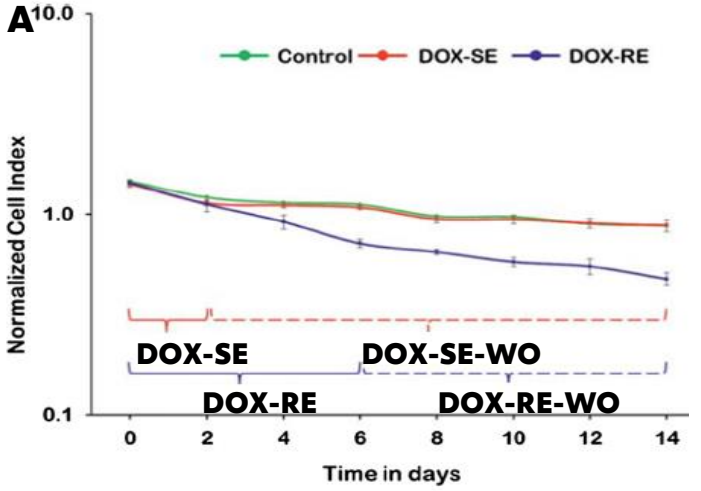
Identification of genomic biomarkers for anthracycline-induced cardiotoxicity in human iPSC-derived cardiomyocytes: an in vitro repeated exposure toxicity approach for safety assessment

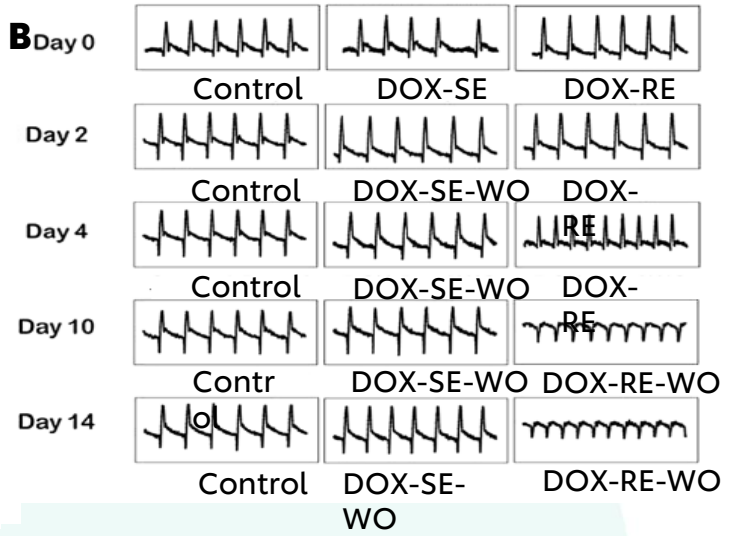
Umesh Chaudhari,[1] Harshal Nemade,[1] Vilas Wagh,[1] John Antonydas Gaspar,[1] James K. Ellis,[2] Sureshkumar Perumal Srinivasan,[1] Dimitry Spitkovski,[1] Filomain Nguemo,[1] Jochem Louisse,[3] Susanne Bremer,[3] Jürgen Hescheler,[1] Hector C. Keun,[2] Jan G. Hengstler,[4] and Agapios Sachinidis[1]

Springer Open



- **Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)**
- **Dose used was 156 nM**
- **Single and repeated exposure**
- **Functional measurements: cell viability and beating function**





No adverse effects concentration:
156nM@48h

Severe adverse effects concentration:
156nM@144h

Key concentrations derived from in vitro studies

Exposure	Cells	$C_{max,total}$ (nM)	$C_{max,free}$ (nM)	AUC_{total} (nM·h)	AUC_{free} (nM·h)	Key metrics type
Single: 125 nM 12 h	AC16	125	125	1500	1500	PoD
Single: 250 nM 12 h	AC16	250	250	3000	3000	Toxic
Single: 156 nM 48 h	hiPSC-CMs	156	131	7488	6290	PoD
Repeated: 156 nM 96 h	hiPSC-CMs	156	131	14976	12580	Toxic
Repeated: 156 nM 144 h	hiPSC-CMs	156	131	22464	18870	Toxic

Determination of free concentration:

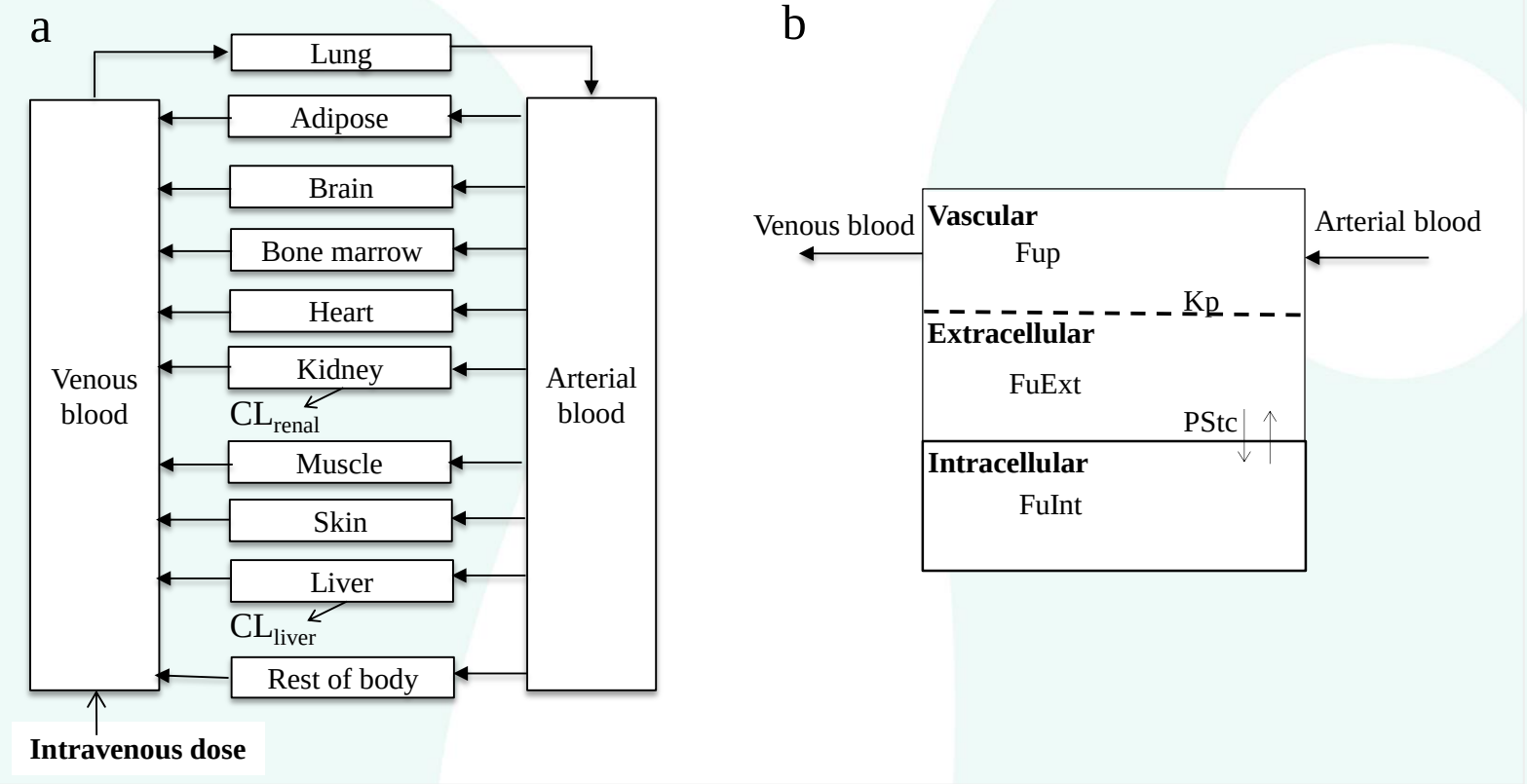
- assuming that DOX binds only to serum protein, but not to plastic,
- *in vitro* free concentration C_{free} was calculated as $C_{nominal}$*$Fu_{in\ vitro}$ (fraction of chemical unbound in the *in vitro* assay)

$$Fu_{in\ vitro} = \frac{1}{\frac{C_{albumin,in\ vitro}}{C_{albumin,plasma}}\left(\frac{1-Fup}{Fup}\right)+1}$$

Fup is the unbound fraction of chemical in human plasma , $C_{albumin,in\ vitro}$ the concentration of albumin used in the *in vitro* assay, $C_{albumin,plasma}$ the concentration of albumin in human plasma (42.5 g/L).

PBK model construction for doxorubicin

PBK structure



DOX binds to DNA

Key chemical specific parameters used in the PBK model for DOX

Parameters	Value	Source
LogP	1.27	[40]
F_{up}	0.25	[41] [42]
p*Ka*	7.34 (phenol); 8.46 (amine); 9.46 (est)	[43]
CL_{total}	0.894±0.308 L/h/kg	[19]
CL_{renal}	0.152±0.110 L/h/kg	[19]
b/p ratio	1.72±0.42	Value converted from the measured erythrocyte/plasma concentration ratio of 2.8±0.3 for DOX [44]

LogP, Logarithm of octanol-water partition coefficient;

Fup, Fraction unbound to plasma;

pKa, Logarithm of acid dissociation constant;

CL_{total}, Total clearance rate;

CL_{renal}, Renal clearance rate;

b/p ratio, blood/plasma concentration ratio.

Kp, tissue/plasma partition coefficients;
FuExt, unbound fraction in extracellular space;
FuInt, unbound fraction in intracellular space;
PStc, permeability*tissue cellular surface area product.

	Kp	FuExt	FuInt	PStc (ml/s)
Lung	0.41	0.611	0.0005	10
Adipose	0.29	0.872	0.0005	10
Liver	0.31	0.795	0.0005	25
Heart	0.37	0.680	0.0005	10
Brain	0.29	0.874	0.0005	10
Bone Marrow	0.37	0.680	0.0005	10
Kidney	0.35	0.719	0.0005	10
Muscle	0.30	0.839	0.0005	10
Skin	0.46	0.546	0.0005	10
Rest of body	0.34	0.732	0.0005	10
Method	*Poulin and Theil, 2000; Poulin and Theil, 2002*		*optimized*	*optimized*

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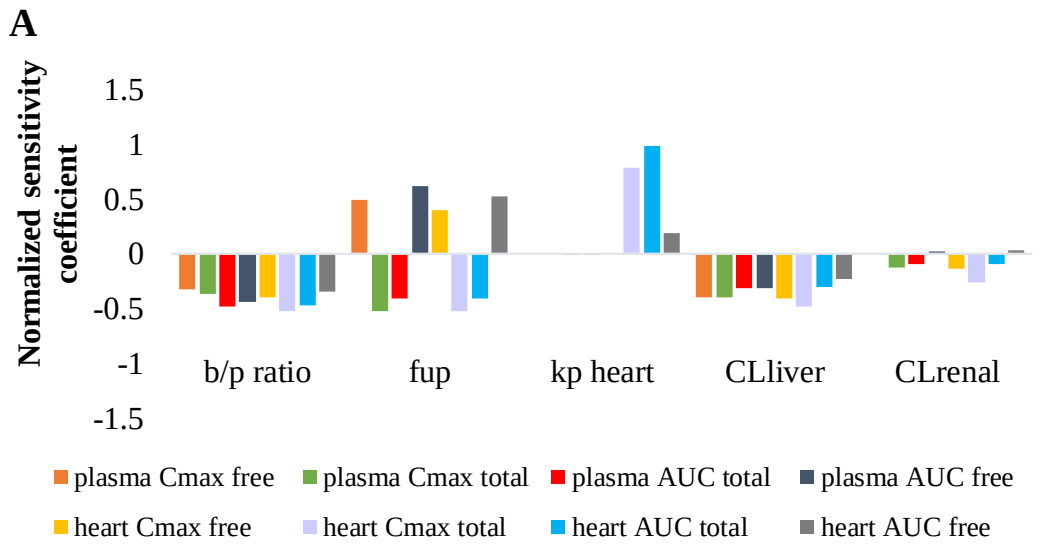
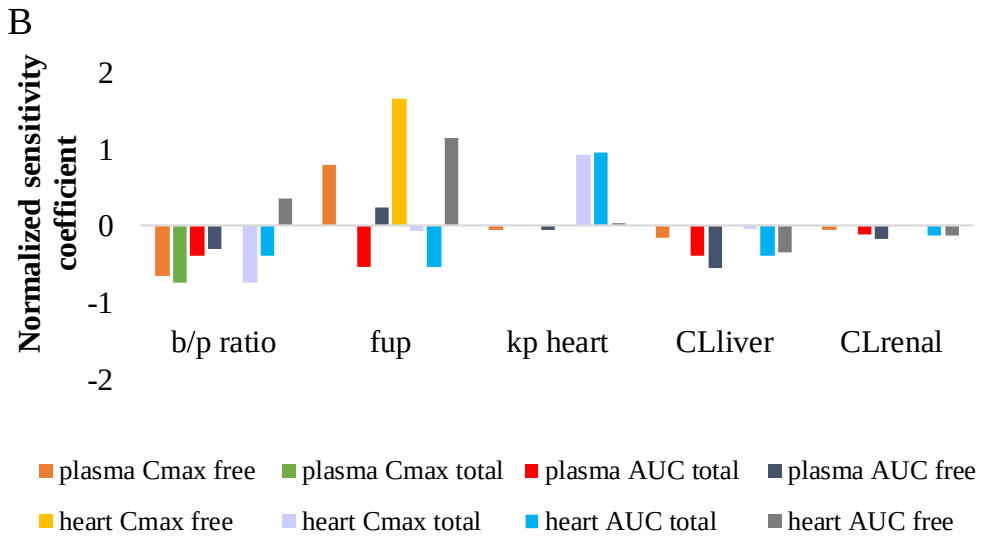
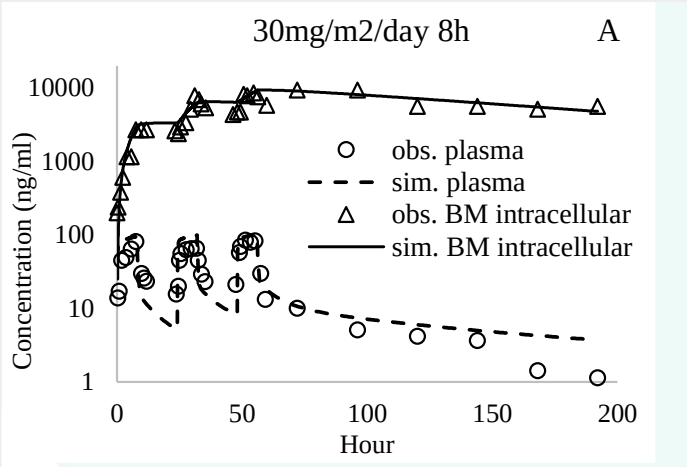
Local sensitivity analysis



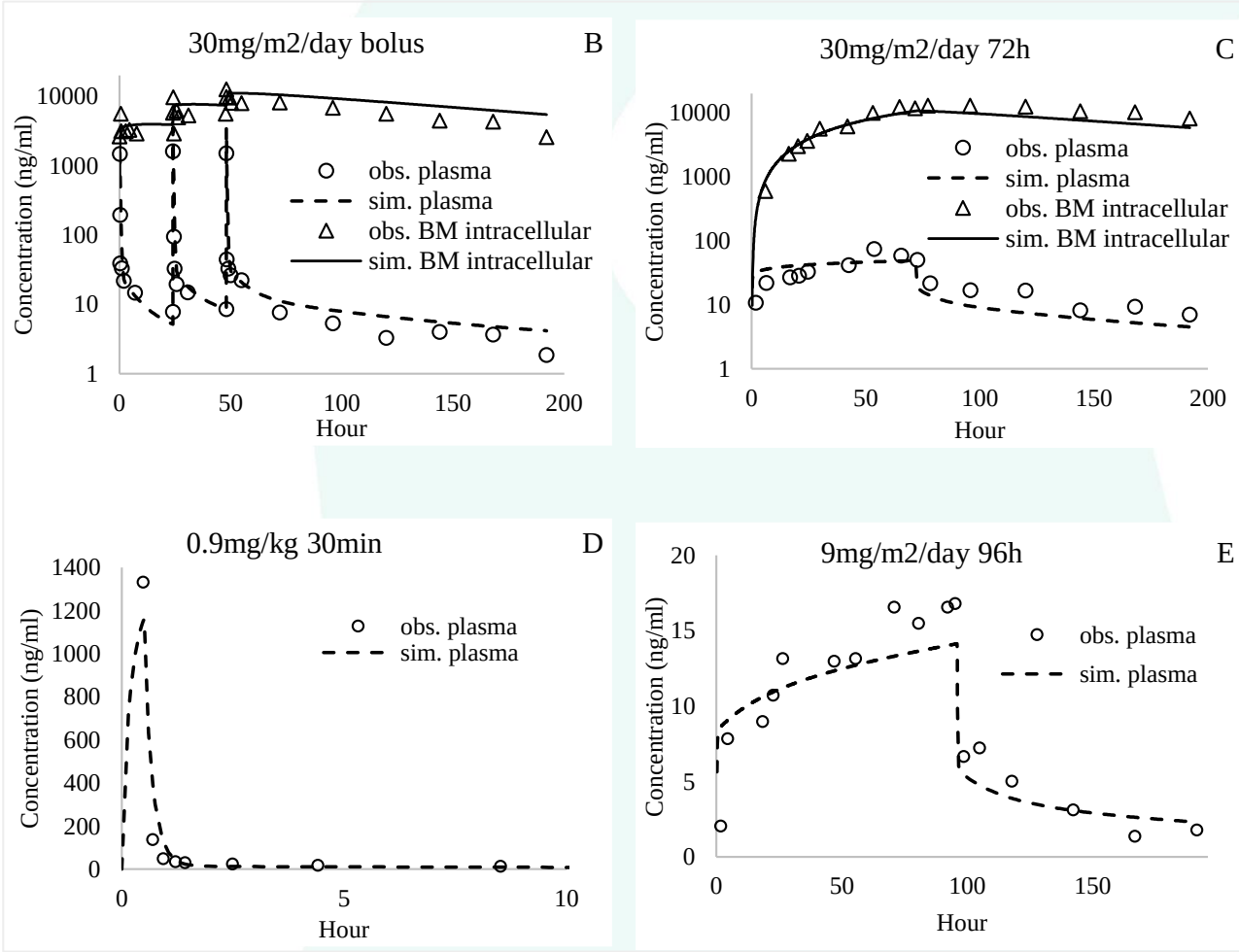


All model parameters with normalized sensitivity coefficients smaller than -0.03 and larger than 0.03 are shown.

PBK model development and verification against human PK data



Model development



Model verification

Comparison of observed and PBK simulated plasma and bone marrow C_{max} and $AUC_{0-\infty}$ of DOX following i.v. administration of various dosing regimens.

			(A) 8-hour infusion of 30 mg/m2/day DOX	(B) Bolus injection of 30 mg/m²/day DOX	(C) 72-h infusion of 30 mg/m²/day DOX	(D) 30-min infusion of 0.9 mg/kg/day DOX	(E) 96-h infusion of 9 mg/m2/day DOX
C_{max} (ng/ml)	Plasma	observed	85.1	1627.1	74.4	1331.3	16.8
		simulated	78.8	3443.3	43.4	1160.7	14.1
		fold difference	***1.1***	***2.1***	***1.7***	***1.1***	***1.2***
	Bone marrow intracellular	observed	9380.4	12625.3	12857.8		
		simulated	10565.2	14123.6	13028.7		
		fold difference	***1.1***	***1.1***	***1.0***		
$AUC_{0-\infty}$ (ng/ml·h)	Plasma	obs	2996.3	2346.4	4674.8	1035.9	1626.4
		simulated	3032.2	3903.9	3680.6	1158.8	1945.7
		fold difference	***1.0***	***1.7***	***1.3***	***1.1***	***1.2***
	Bone marrow intracellular	obs	1147827.2	1047141.6	1787445.7		
		simulated	1251425.5	1604248.9	1517515.8		
		fold difference	***1.1***	***1.5***	***1.2***		

Literature search on clinical cardiotoxicity data of DOX

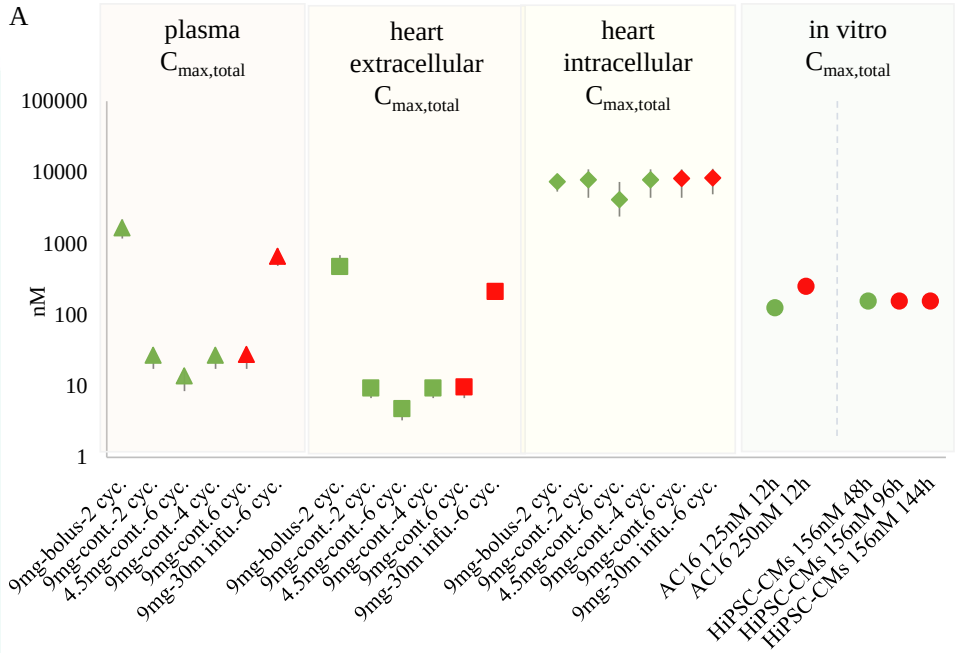
Participants	Age (years)	No. of patients	Intervention[a]	DOX regimens			Toxicity evaluation criterion	Follow-up duration	Cardiotoxicity observed			
				Dose (mg/m²/day)	i.v. injection type	duration			After 1st treatment	After 1st cycle	During whole study	CHF[b]
Adults with multiple myeloma (MM)	41-75	37	PAD/TD	4.5	Continuous infusion	6 cycles	CTC	27-month (13-39)	no	no	no	no
Adults with relapsed MM	37-66	4	PAD	4.5	Continuous infusion	4 cycles	CTC	not mentioned	no	no	no	no
Adults with MM	15-66	32	PAD	9	Bolus injection	2 cycles	CTC	49.5-momth (30.5-68.1)	no	no	no	no
Adults with untreated MM	15-65	139	VAD	9	30 min infusion	6 cycles	WHO	at least 12-month	One patient had cardiac dysrhythmias and one had myocardial infraction			no
Adults with relapsed or refractory MM		13	iPAD	9	30 min infusion	6 cycles	CTC	21-month	no	no	no	no
Adults with newly diagnosed MM	34-65	20	PAD	9	Continuous infusion	2 cycles	CTC	24-month	no	no	no	no
Adults with relapsed MM	37-66	14	PAD	9	Continuous infusion	4 cycles	CTC	not mentioned	no	no	no	no
Adults with MM	29-80	50	VAD	9	Continuous infusion	6 cycles	cardiac examination was carried out before each cycle	70-month	no	no	One patient developed cardiotoxicity	no
Women with epithelial ovarian carcinoma	39-73	17	paclitaxel and DOX	7.5,10,12.5,15	Continuous infusion	3 cycles	CTC	not mentioned	no	Two subjects had an asymptomatic drop in left ventricular function		no

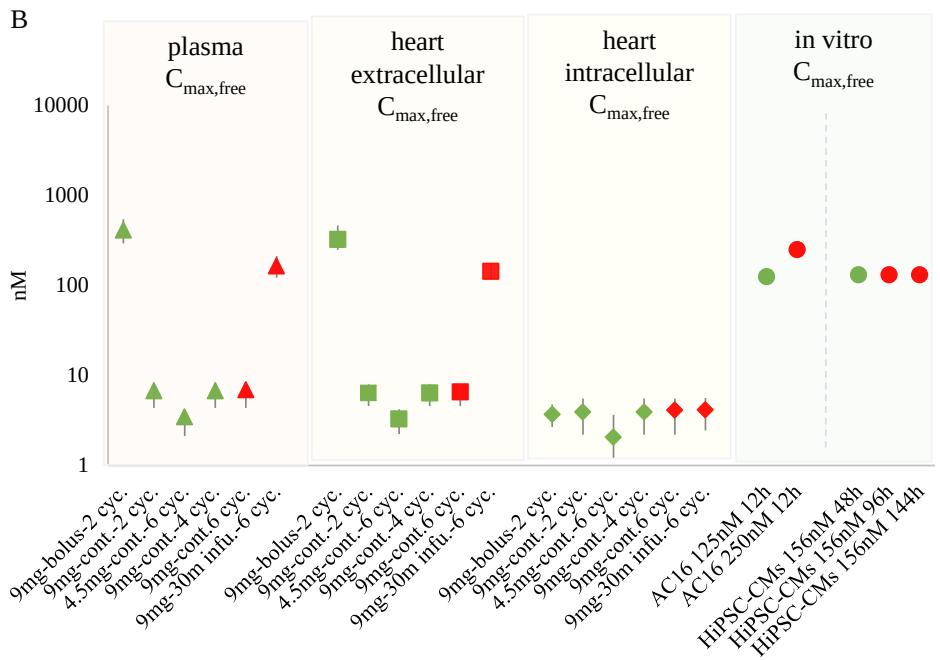
For 4 days as a cycle, repeated every 3 weeks,

Summary of the dosing regimens with no or mild cardiotoxicity

DOX dosing regimens				Cardiotoxicity observed
Dose (mg/m²/day)	**i.v. injection type**	**Cycle**	**Number of cycles in total**	
4.5	Continuous infusion	Treated for 4 days as a cycle, repeated every three weeks	6 cycles	No cardiotoxicity observed
9	Bolus injection		2 cycles	No cardiotoxicity observed
9	30 min infusion		6 cycles	Mild cardiotoxicity observed
9	Continuous infusion		2 cycles	No cardiotoxicity observed
9	Continuous infusion		4 cycles	No cardiotoxicity observed
9	Continuous infusion		6 cycles	Mild cardiotoxicity observed

These dosing regimens were selected and simulated with the PBK model to make predictions on AUC and C_{max} in plasma and tissue.


A
plasma
$C_{max,total}$
heart
extracellular
$C_{max,total}$
heart
intracellular
$C_{max,total}$
in vitro
$C_{max,total}$
nM
100000
10000
1000
100
10
1
9mg-bolus-2 cyc.
9mg-cont.-2 cyc.
4.5mg-cont.-6 cyc.
9mg-cont.-4 cyc.
9mg-cont.6 cyc.
9mg-30m infu.-6 cyc.
AC16 125nM 12h
AC16 250nM 12h
HiPSC-CMs 156nM 48h
HiPSC-CMs 156nM 96h
HiPSC-CMs 156nM 144h


B
plasma
$C_{max,free}$
heart
extracellular
$C_{max,free}$
heart
intracellular
$C_{max,free}$
in vitro
$C_{max,free}$
nM
10000
1000
100
10
1
9mg-bolus-2 cyc.
9mg-cont.-2 cyc.
4.5mg-cont.-6 cyc.
9mg-cont.-4 cyc.
9mg-cont.6 cyc.
9mg-30m infu.-6 cyc.
AC16 125nM 12h
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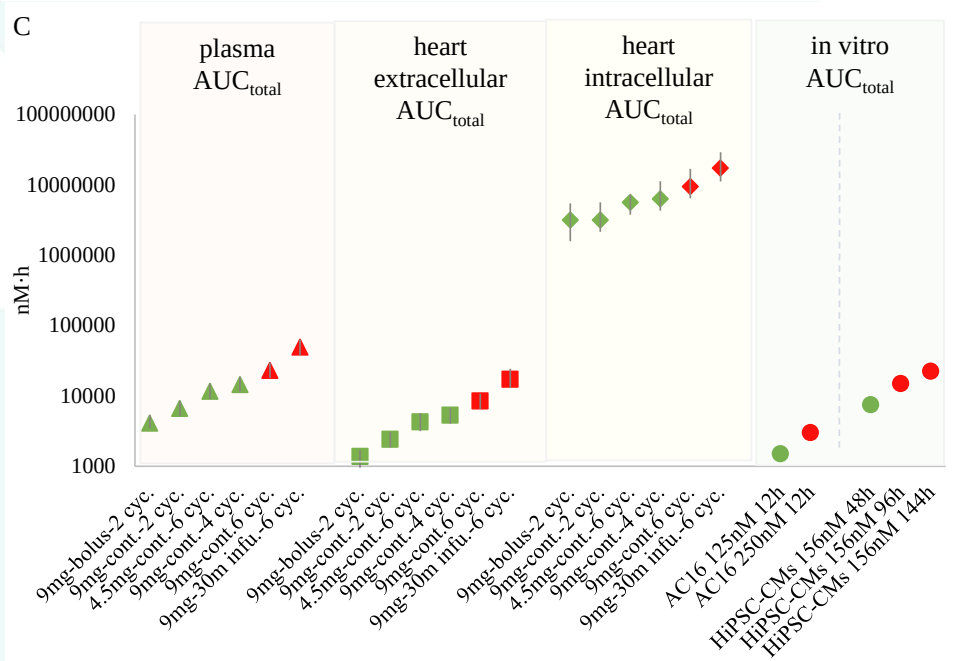
C
plasma
AUC_{total}
heart
extracellular
AUC_{total}
heart
intracellular
AUC_{total}
in vitro
AUC_{total}
nM·h
100000000
10000000
1000000
100000
10000
1000
9mg-bolus-2 cyc.
9mg-cont.-2 cyc.
4.5mg-cont.-6 cyc.
9mg-cont.-4 cyc.
9mg-cont.6 cyc.
9mg-30m infu.-6 cyc.
AC16 125nM 12h
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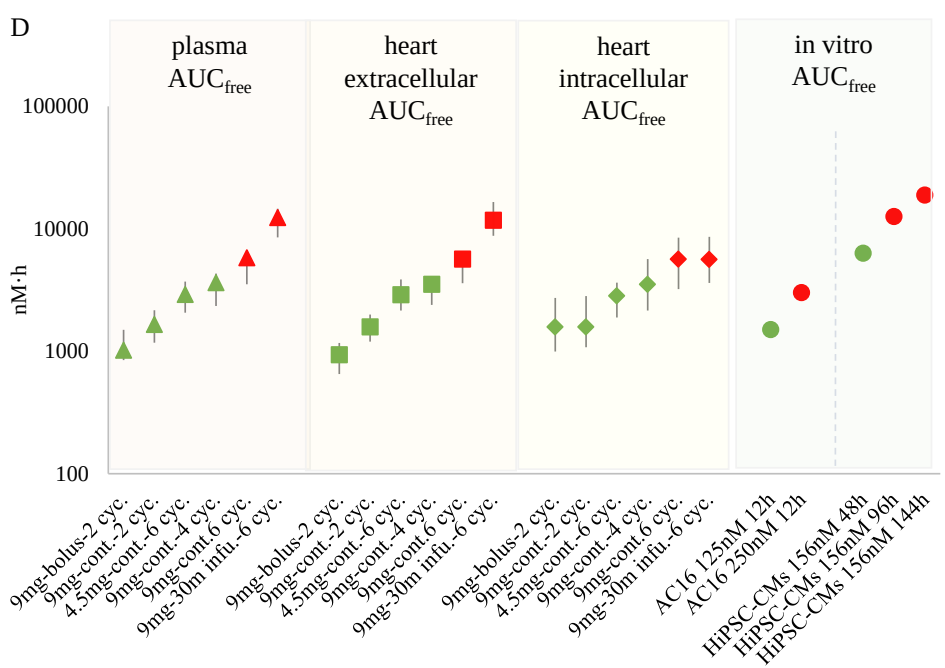
D
plasma
AUC_{free}
heart
extracellular
AUC_{free}
heart
intracellular
AUC_{free}
in vitro
AUC_{free}
nM·h
100000
10000
1000
100
9mg-bolus-2 cyc.
9mg-cont.-2 cyc.
4.5mg-cont.-6 cyc.
9mg-cont.-4 cyc.
9mg-cont.6 cyc.
9mg-30m infu.-6 cyc.
AC16 125nM 12h
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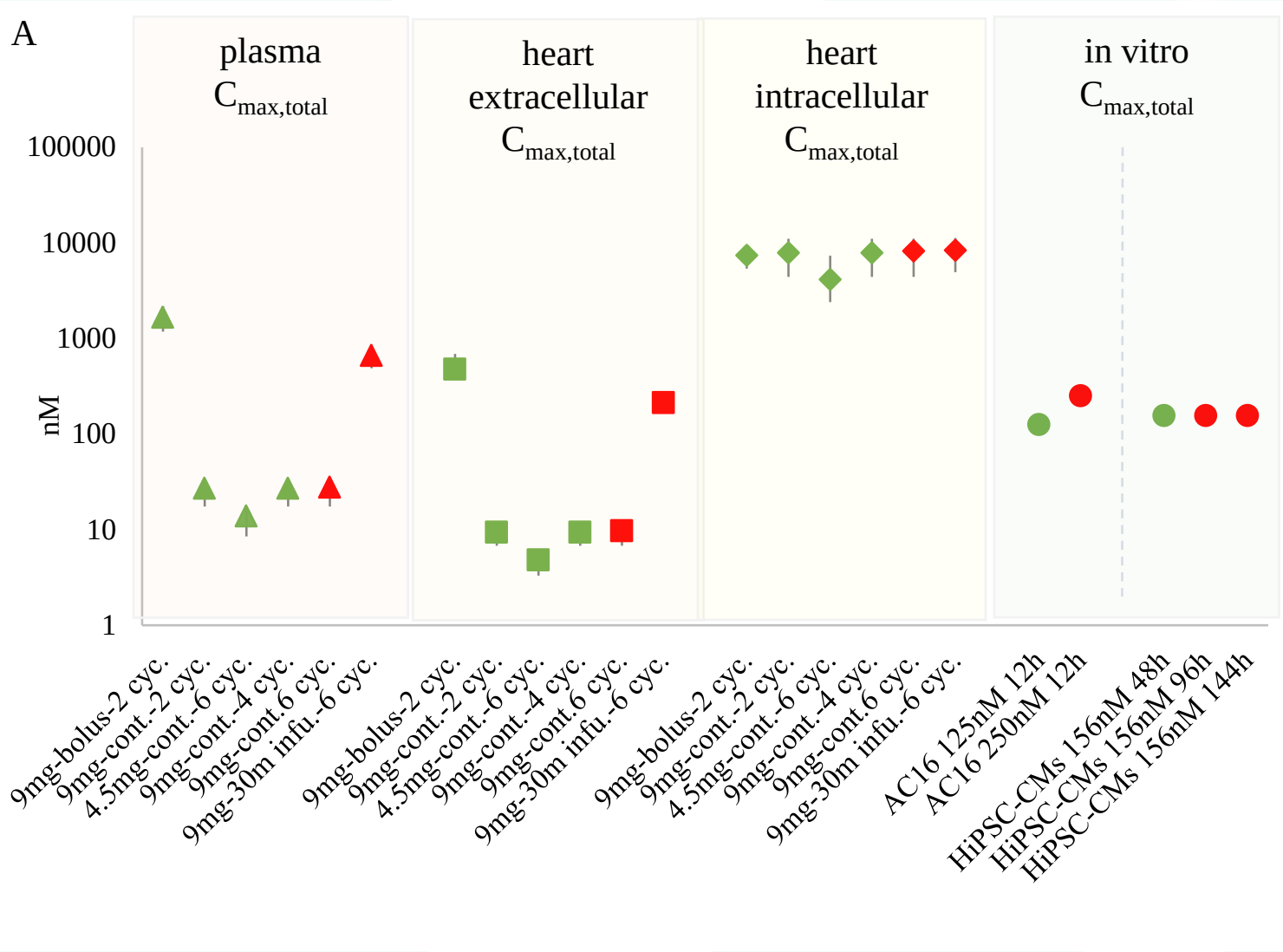
Quantitative interpretation of in vitro key findings by comparing with PBK model-predicted internal doses of human clinical exposure



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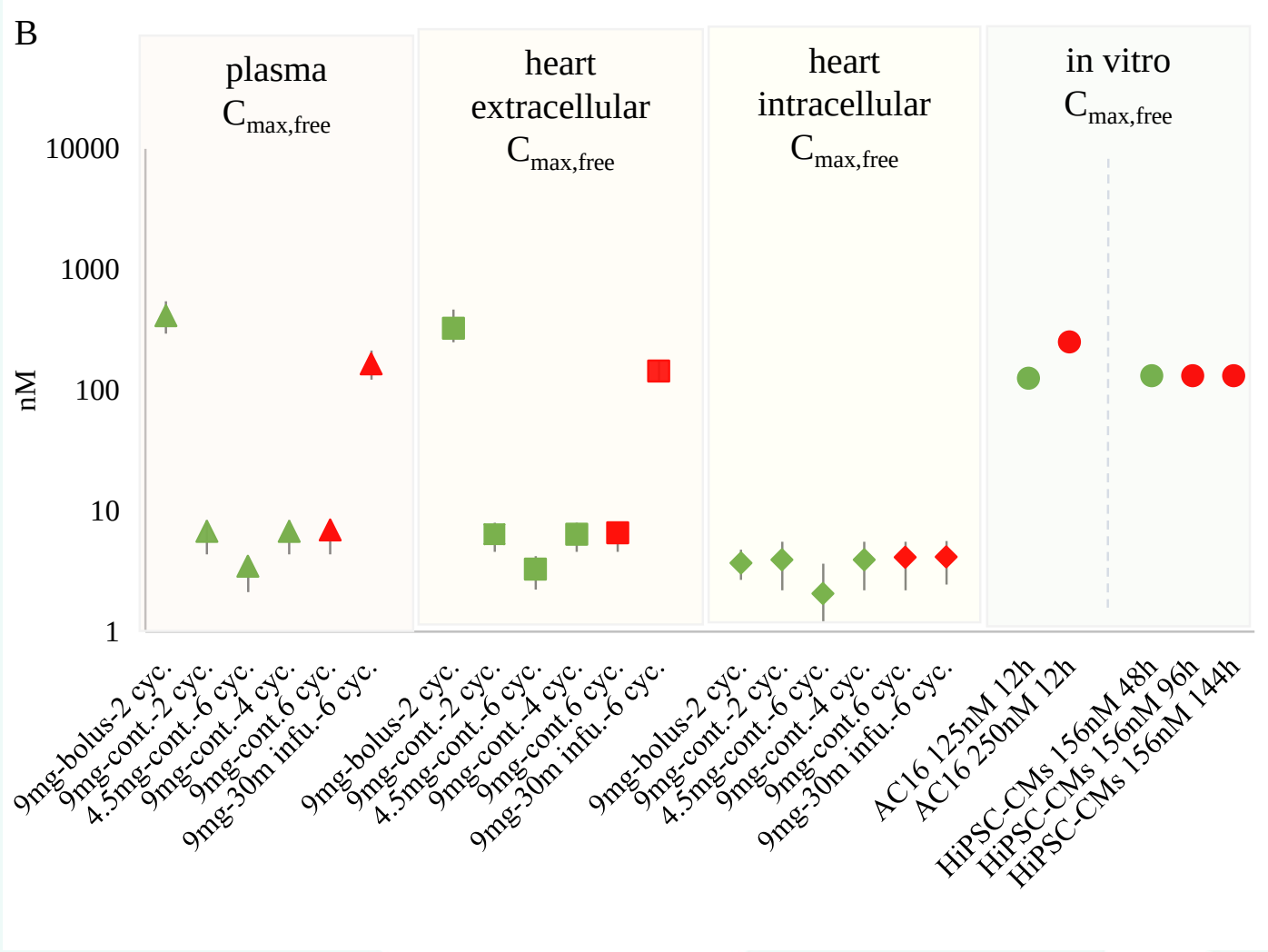
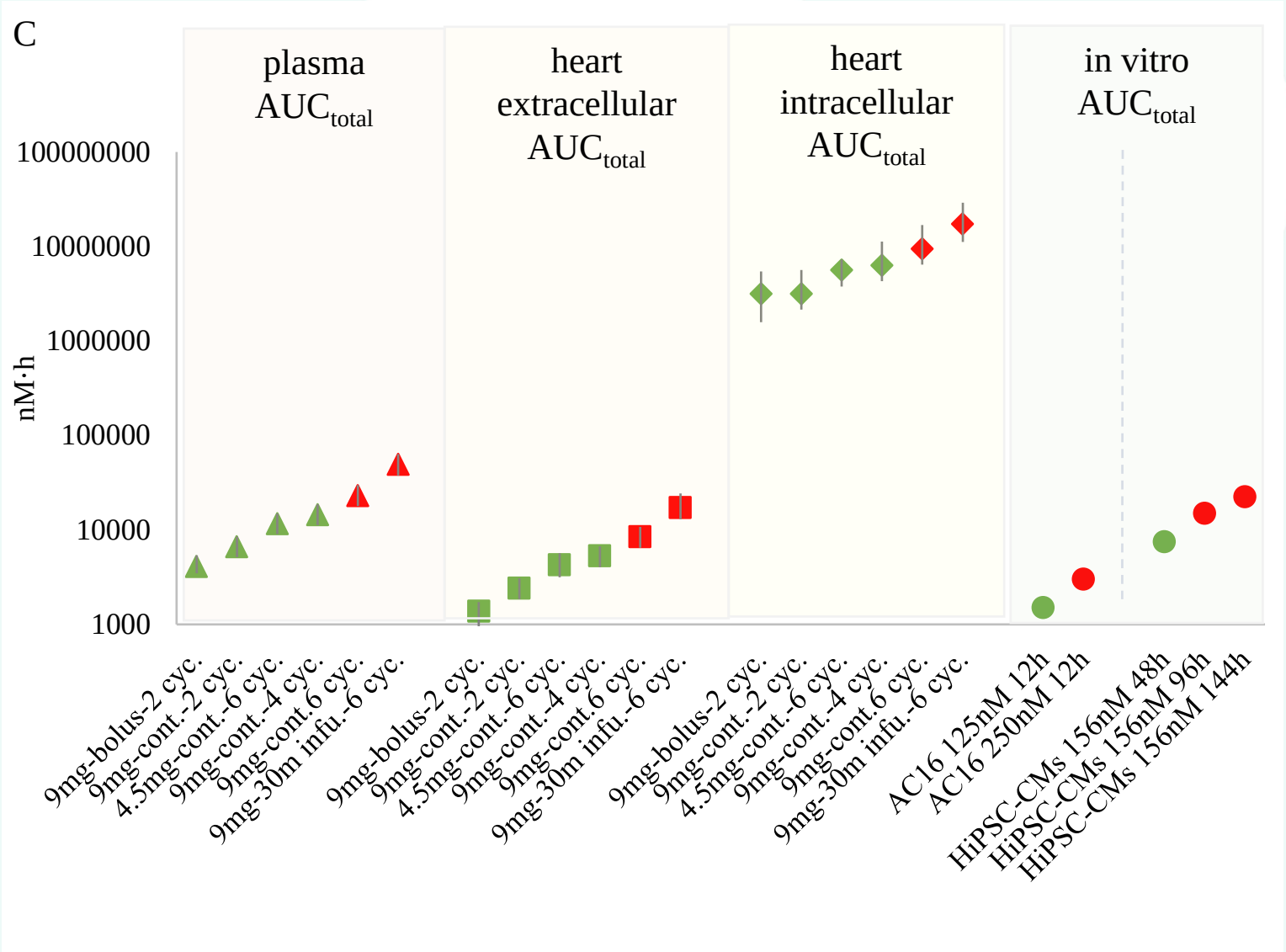
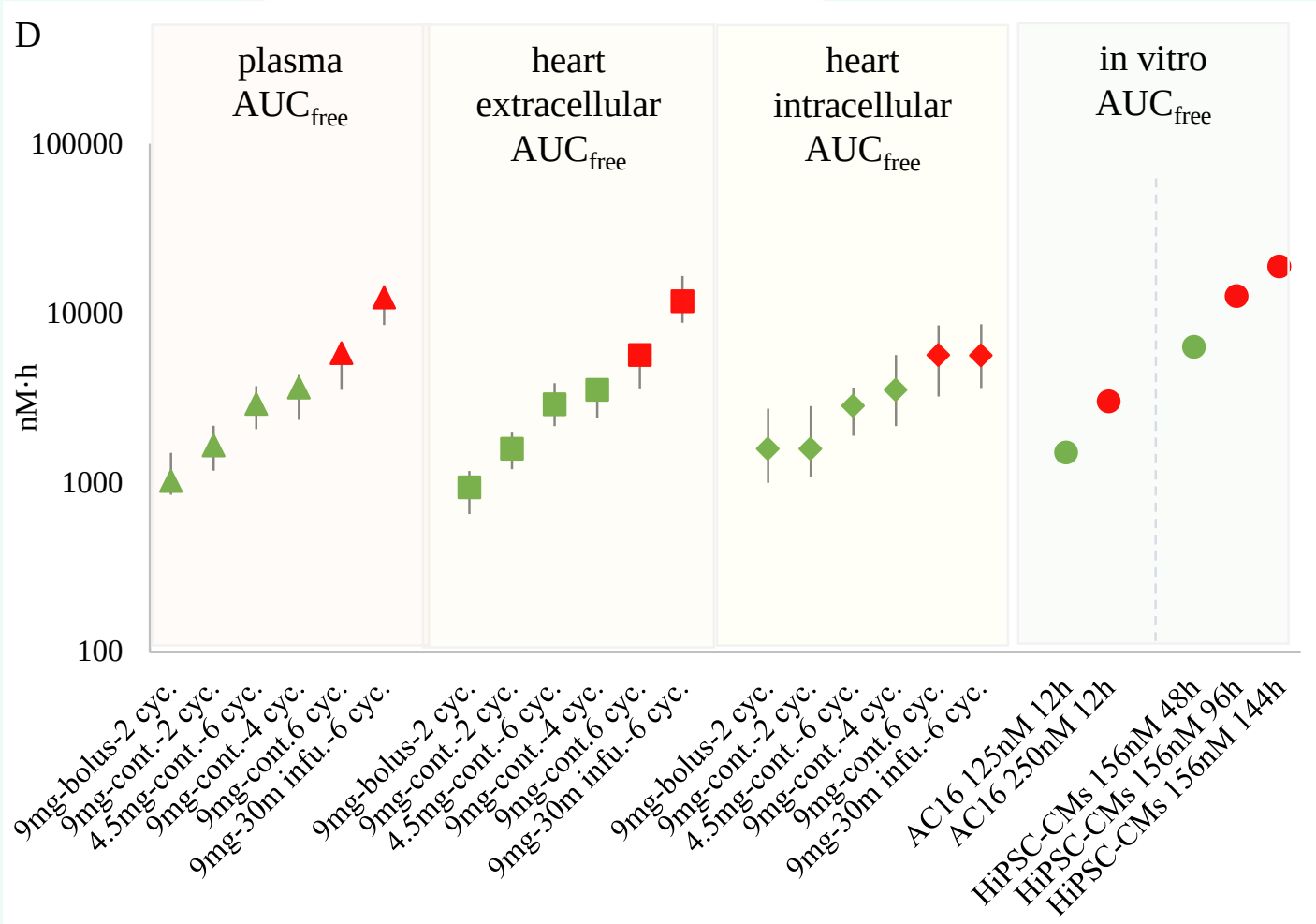


Quantitative interpretation of in vitro key findings by comparing with PBK model-predicted internal doses of human clinical exposure



Quantitative interpretation of in vitro key findings by comparing with PBK model-predicted internal doses of human clinical exposure



it is possible to combine PBK modelling of human exposure with in vitro-derived toxicity information to predict the potential risk of different exposure levels in humans

Conclusion

- Combined with PBK modelling, the *in vitro* information obtained from toxicity pathway-based cell assays is useful in informing human cardiovascular risk of DOX.
- The unbound heart AUC and plasma AUC are good metrics to link *in vitro* findings to human risk of DOX on cardiotoxicity as in vitro PoD has shown good predictivity on human safe exposure level when these two metrics were used.
- The DOX AUC metric appeared to be more conservative than the C_{max} metric from the human safety perspective.

Challenges ahead

- How good are in vitro assays?
  - reliability of in vitro model
  - duration of exposure (e.g. Repeat dose)
  - in vitro kinetics (e.g. active transporters, metabolism)
- Clinical data
  - chronic effects are more prevalent – pose challenge to be compared to in vitro data
- IVIVE
  - Some uncertainty related to tipping points (e.g., pathway models)
  - What about using cellular concentrations as the dose metric to make the link?
  - Predicting heart concentration: DOX is reported to be actively transported the predictions could be wrong

More case studies are needed!

Acknowledgement

Alistair Middleton, Jin Li, Beate Nicole, Paul Carmichael, Carl Westmoreland

Haitao Yuan, Shuangqing Peng, Jiabin Guo

Qiang Zhang

Ian Sorrell