GLUCURONIDATION, SULFATION, AND PHARMACOKINETICS OF CONJUGATED METABOLITES

Swati Nagar

April 18, 2013

NJDMDG, Somerset NJ

Outline

- A renewed interest in xenobiotic conjugation
  - FDA guidelines
  - Poor IVIVE of conjugation and prediction of DDI
- Resveratrol as a model substrate
  - Tissue-specific glucuronidation and sulfation
  - Possibly active metabolites, pharmacogenetics
  - Auto-induction of glucuronidation
- Pharmacokinetics of resveratrol and its conjugates
  - Simple compartmental modeling
  - Incorporation of transporters

Why evaluate metabolite kinetics?

Guidance for Industry
Safety Testing of Drug Metabolites

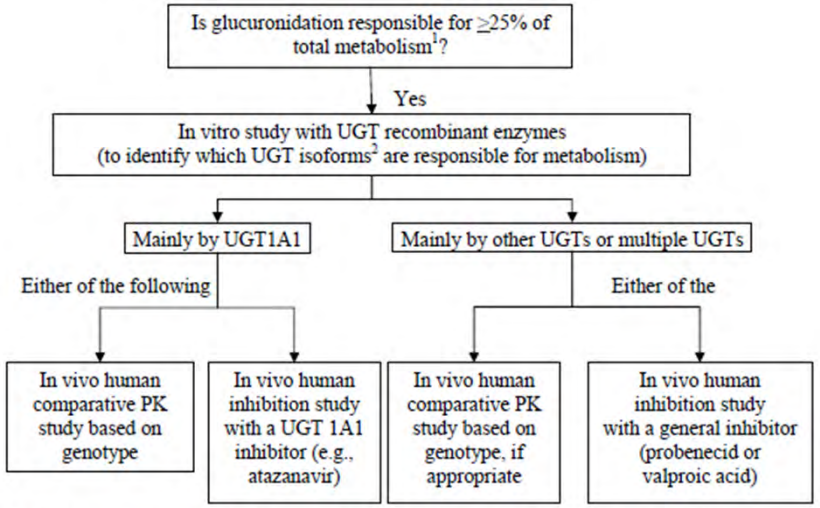
Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Figure 3. Evaluation of Investigational Drugs as UGT Substrates

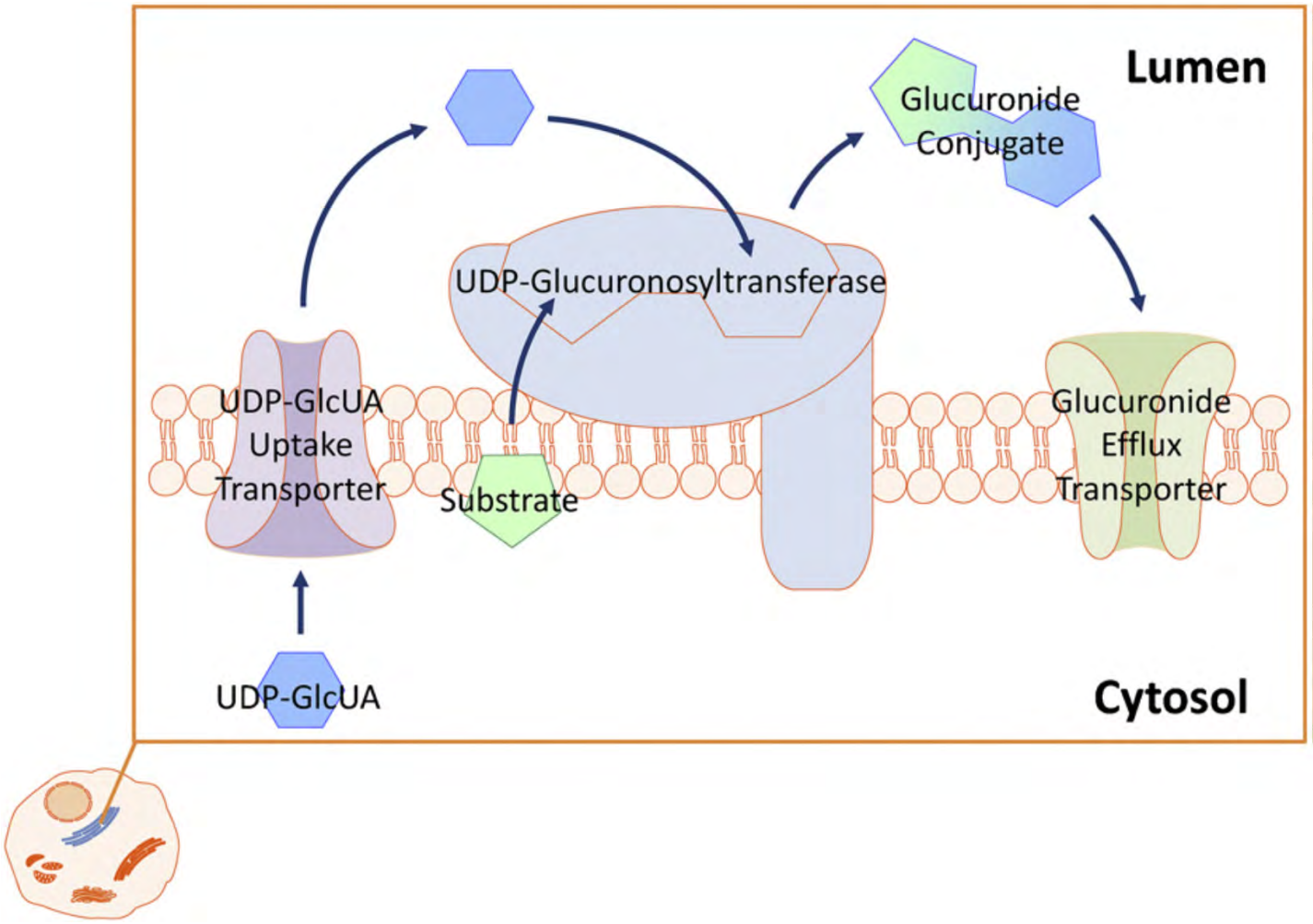


[1] In an in vitro system capable of informing contribution by UGT and non-UGT enzymes (e.g., hepatocytes or microsomes supplemented with appropriate co-factors).
[2] Main UGTs recommended to be studied: UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15.

Metabolite disposition

- Active metabolites
- Enzyme regulation
  - Enzyme inhibition
  - Enzyme induction
  - Genetic polymorphisms
- Complex disposition
  - Reversible metabolism and enterohepatic recycling
  - Interplay between DMEs and transporters

UGT cellular location and orientation



Reference: Rowland, Int J Biochem Cell Biol 2013, 45:1121–32.

Glucuronidation and DDIs

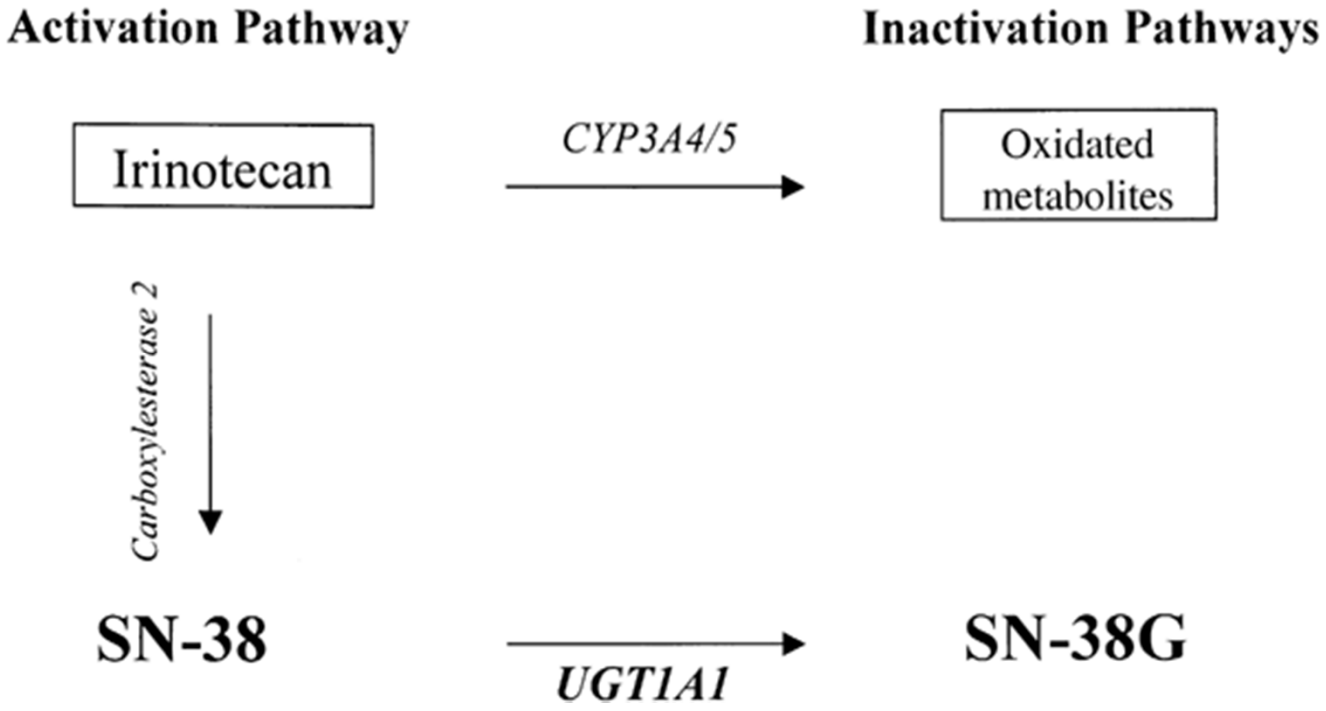
- Much evidence in vitro for both UGT inhibition and induction
- Not many clinical studies
- Complicating factors
  - In vitro protocols not standardized across labs
  - Overlapping substrate specificity, compensatory mechanisms
  - In vivo inhibitor concentrations below their Ki
  - Therefore, AUCi/AUC not as dramatic as with CYPs

References: Kiang et al, Pharmacol Therap 2005, 106:97-132.
Miners et al, Biochem Pharmacol 2006, 71: 1531-39.

UGT2B7 inhibition – fluconazole and AZT

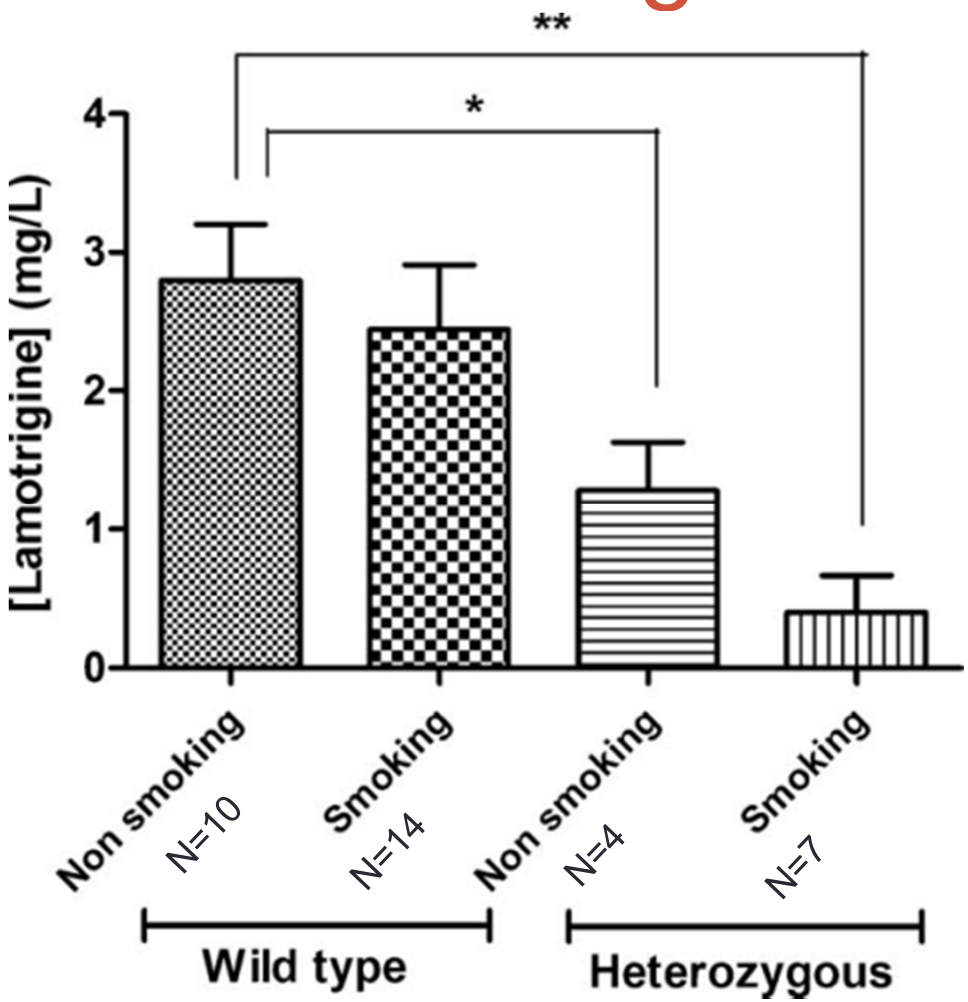
- In HIV-infected males (n=12), fluconazole significantly decreased CL/F of AZT, and increased formation of AZT-G
- AUCi/AUC=1.92
  - Sahai et al, J Infect Disease 1994, 169, 1103-7.
- IVIVE was possible when using a Ki estimate with either HLM or UGT2B7 (with BSA)
  - Uchaipichat et al, Br J Clin Pharmacol 2006, 61, 427-439.

UGT1A1 and irinotecan



References:Ando et al, Ann Oncol 1998, 9: 845-7.
Desai et al, Oncogene 2003, 22:6621–28.

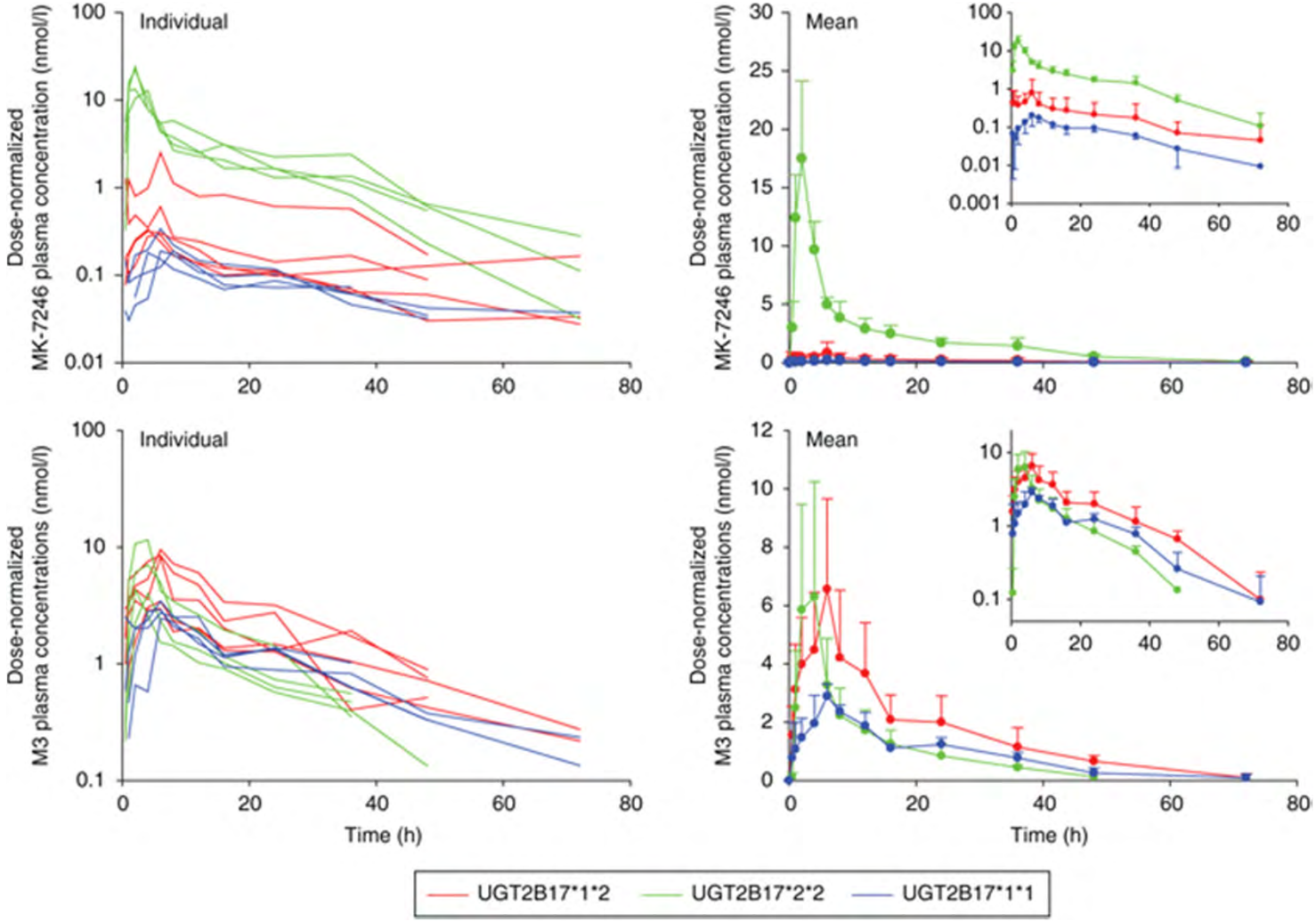
UGT1A4 and lamotrigine



L48V, exon 1

Reference:Gulcebi et al, Epilepsy Res 2011, 95: 1-8.

UGT2B17 and MK-7246



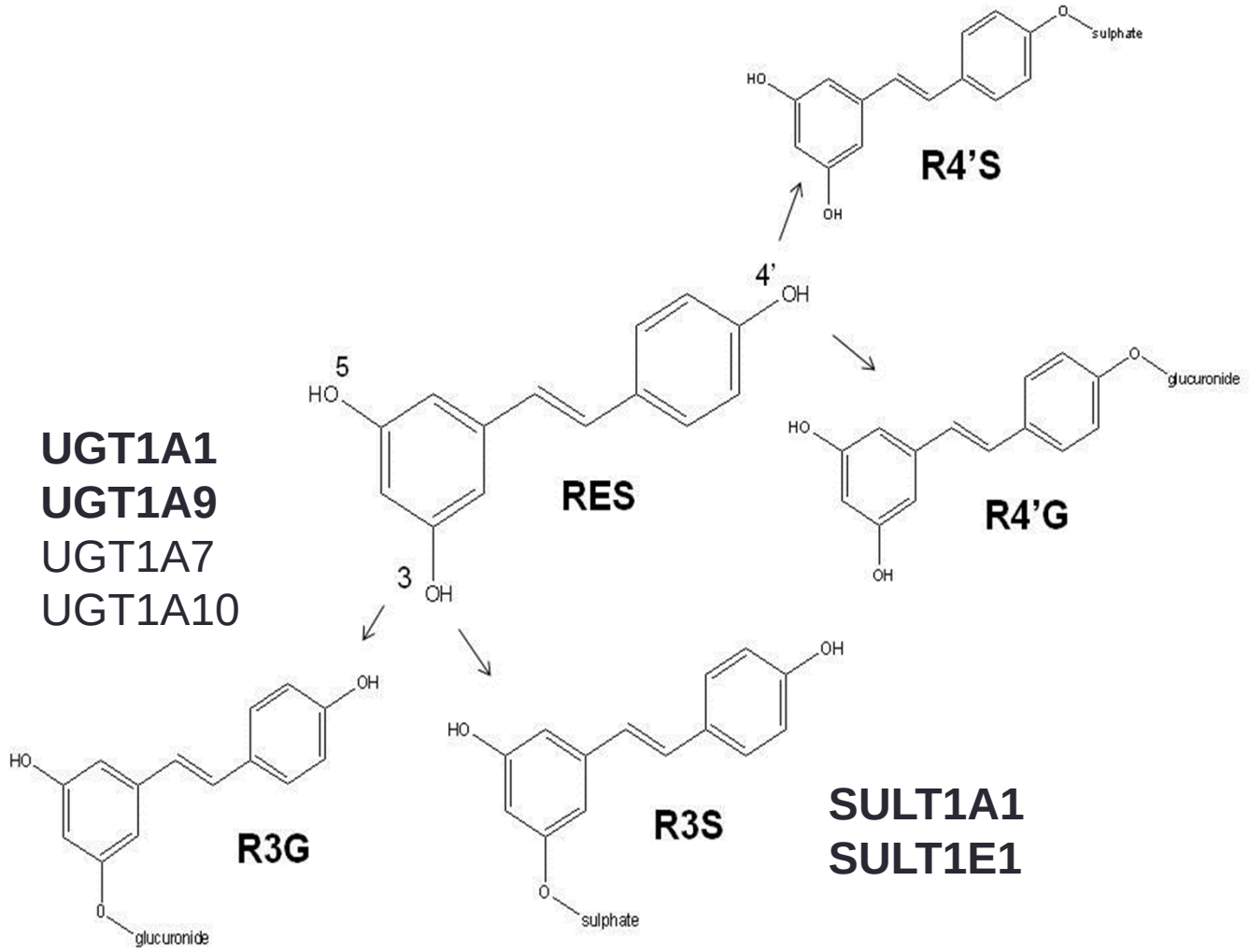
Reference:Wang et al, CPT 2012, 92: 96-102.

Outline

- A renewed interest in xenobiotic conjugation
  - FDA guidelines
  - Poor IVIVE of conjugation and prediction of DDI
- Resveratrol as a model substrate
  - Tissue-specific glucuronidation and sulfation
  - Possibly active metabolites, pharmacogenetics
  - Auto-induction of glucuronidation
- Pharmacokinetics of resveratrol and its conjugates
  - Simple compartmental modeling
  - Incorporation of transporters

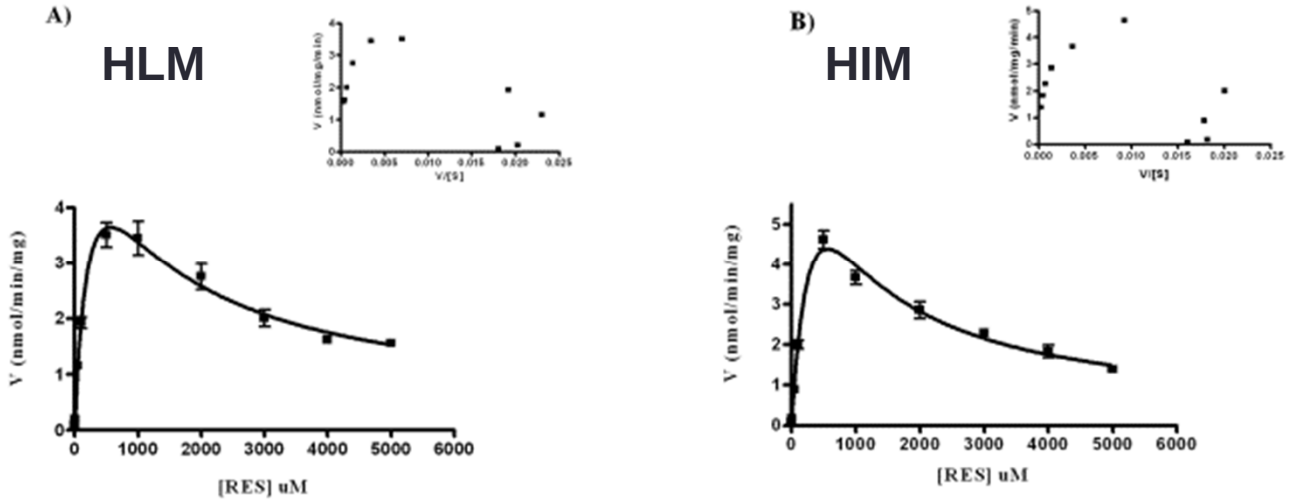
Resveratrol as a model substrate

- Substrate for glucuronidation
- Substrate for sulfation
- Possible transporter involvement
- Possibly active metabolites
- Possible role in DME regulation
- Human studies indicate poor F
- Potential chemopreventive
- High profile research on its anti-cancer and anti-obesity potential
- NIH programmatic interest
  - Hope of $$



References: Aumont et al, Arch Biochem Biophys 2001; 393: 281 – 289.
Miksits et al, Xenobiotica 2005; 35: 1101-19.
Brill et al, Pharm Pharmacol 2006; 58: 469 – 479.
Sabolovic et al, Biopharm Drug Dispos 2006; 27: 181 – 189.
Nagar et al, Mol Pharmacol 2006; 69: 2084 – 2092.

Tissue-specific RES conjugation



Conjugation Product	Protein Source	V_{max}	K_m	K_i	Type of Fit	Goodness of Fit (r^2)
R3G	HLM	7.4 ± 0.25	280.4 ± 21.6	1022 ± 71.5	PSI	0.91
	HIM	12.2 ± 0.34	505.4 ± 29.4[a]	600.8 ± 20.5[a]	PSI	0.95
R4'G	HLM	V_{max1} = 0.45 ± 0.01 V_{max2} = 1.3 ± 0.03	K_{m1} = 65.2 ± 29 K_{m2} = 1685 ± 106.8	na	BPM	0.96
	HIM	8.9 ± 0.14	454.5 ± 21.8	564.3 ± 38.1	PSI	0.95

Reference: Iwuchukwu and Nagar, DMD 2008; 36: 322 – 330.

RES is conjugated in the lung – in vitro

Conjugation product	Protein source	Vmax (pmol/min/mg)	Km (uM)	Ki (uM)	Goodness of Fit (r2)	Type of Fit
R3G	Mouse lung S9	324.40 ± 13.05	7.34 ± 1.60	6632 ± 1198	0.93	Partial substrate inhibition
R3S	Mouse lung S9	7.05 ± 0.28	2.69 ± 0.45	2021 ± 717.6	0.96	Partial substrate inhibition
R3S	Human lung S9	16.15 ± 0.48	4.45 ± 0.79	23238 ± 7305	0.95	Partial substrate inhibition

Reference: Sharan and Nagar, DMD 2013, 41: 1163 – 69.

RES conjugation in the lung – in vivo

RES	RES 15 mg/kg i.a. (n = 5)	RES 15 mg/kg i.v. (n = 5)	Units
AUC0-inf	**591.08 ± 167.29**	**294.98 ± 137.87***	**min*uM**
Cl	118.77 ± 33.36	280.04 ± 158.25	mL/min/kg
Vss	37.59 ± 23.70	34.90 ± 20.10	L/kg
t1/2	190.58 ± 69.65	101.30 ± 43.41*	min
R3G			
AUC0-inf	**921.23 ± 457.07**	**2268.35 ± 517.00***	**min*uM**
R3S			
AUC0-inf	174.94 ± 45.75	157.21 ± 77.77	min*uM

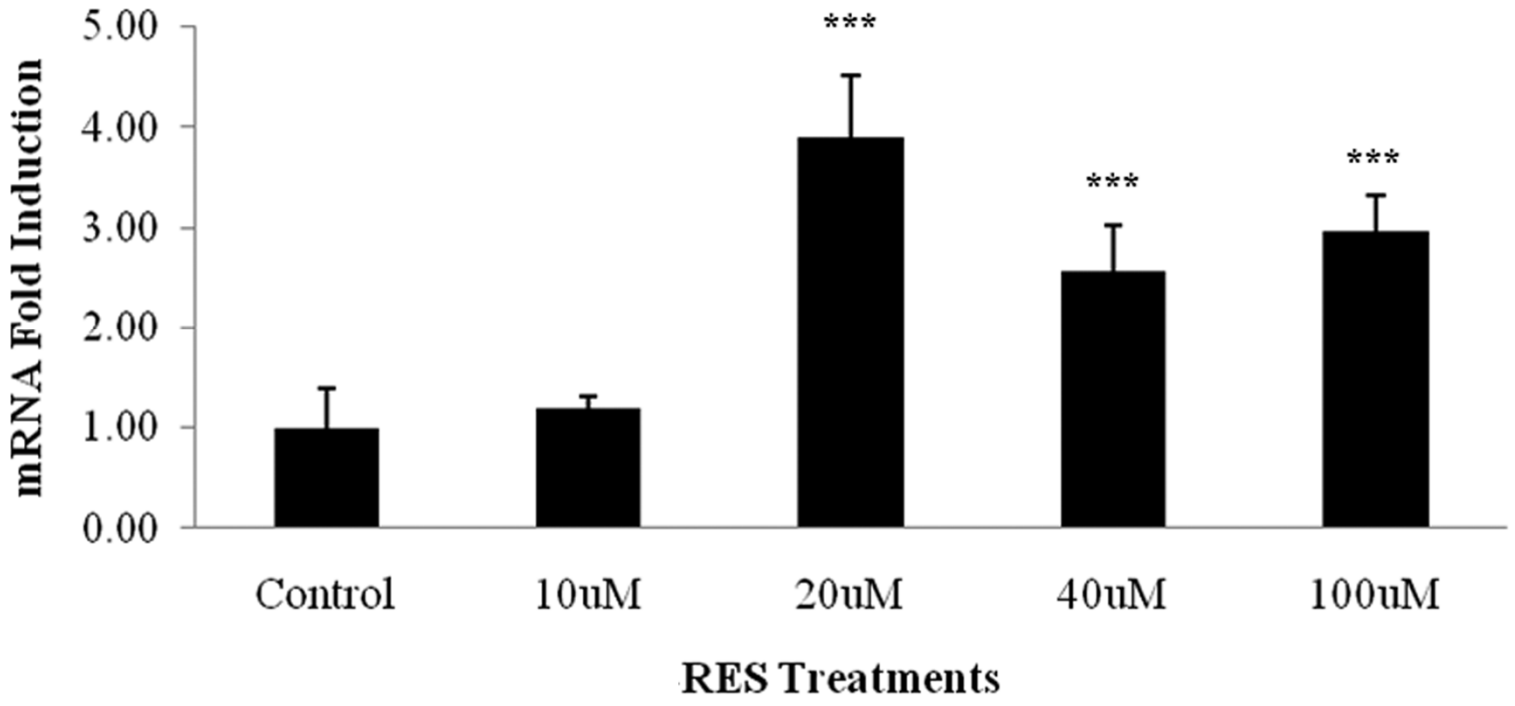
Reference: Sharan and Nagar, DMD 2013, 41: 1163 – 69.

Implications of tissue-specific conjugation

- IVIVE of clearance needs to incorporate extrahepatic tissue metabolism

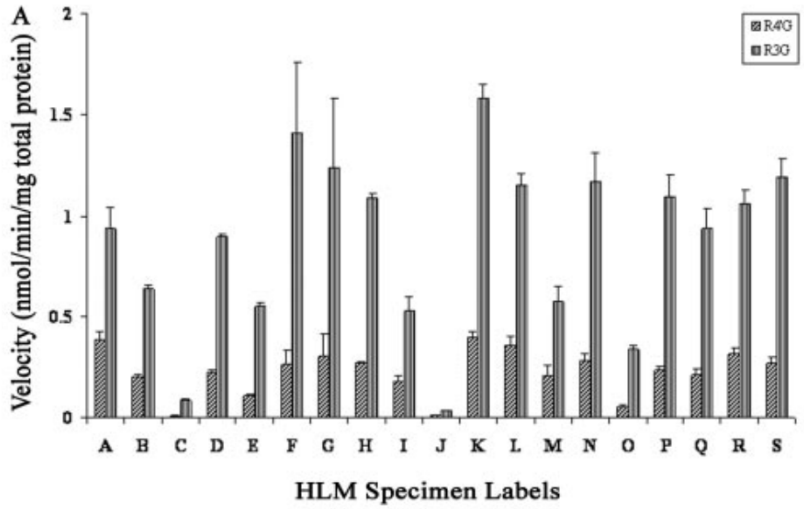
- Local tissue levels of active metabolites
  - Colorectal cancer chemoprevention
  - Lung cancer chemoprevention

RES induces UGT1A1 transcription

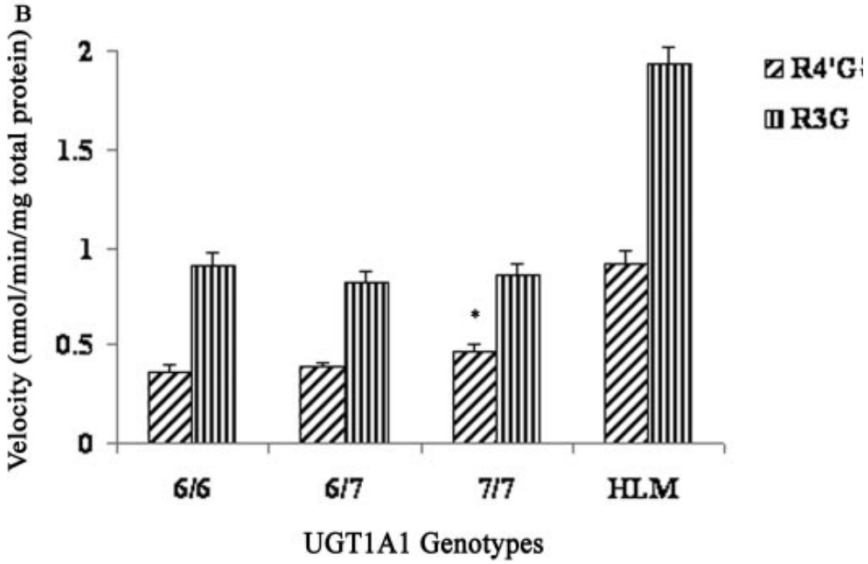


Reference: Iwuchukwu et al, Life Sci 2011, 88: 1047 – 54.

Human variability in RES glucuronidation



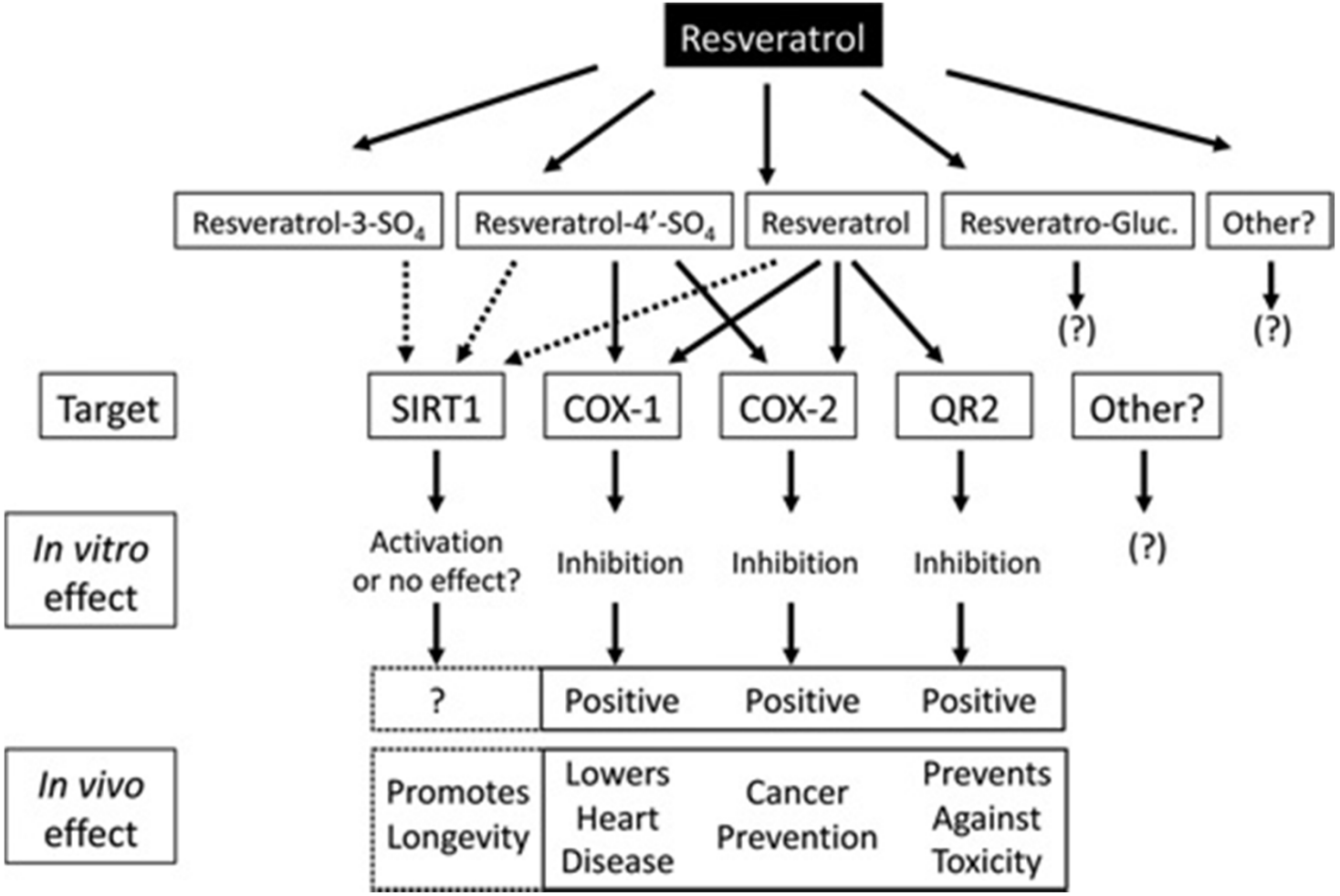
High variability in *trans*-resveratrol glucuronidation a human liver bank



Variability not explained by UGT1A1 TA repeat polymorphism

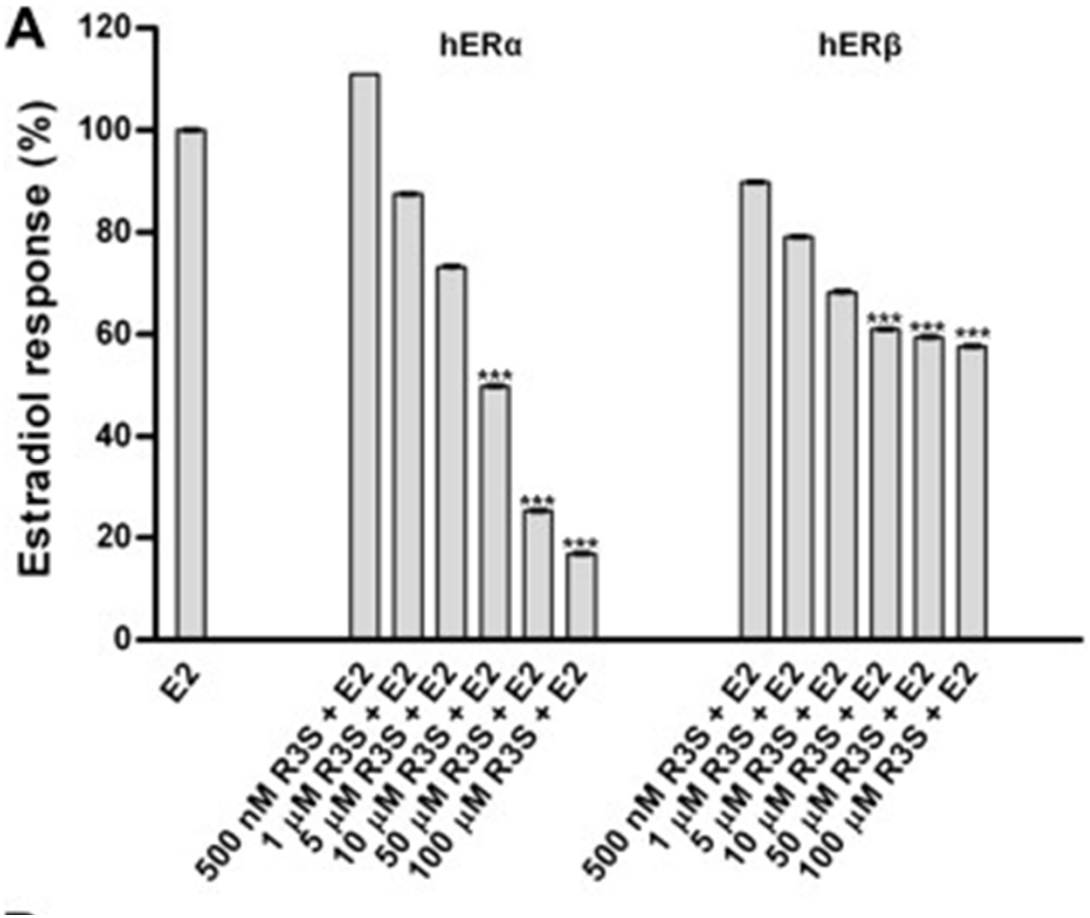
Reference: Iwuchukwu et al, DMD 2009; 37: 1726 - 1732

Active metabolites of RES



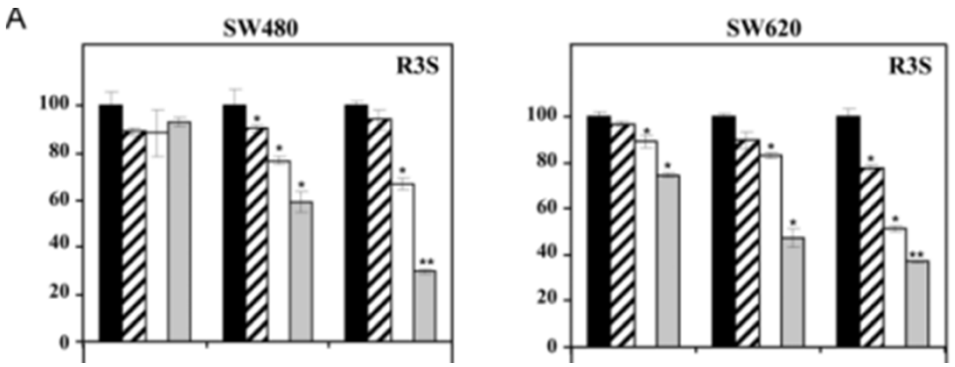
Reference: Calamini et al, Biochem J 2010; 429: 273-282.

Anti-estrogenic activity of R3S

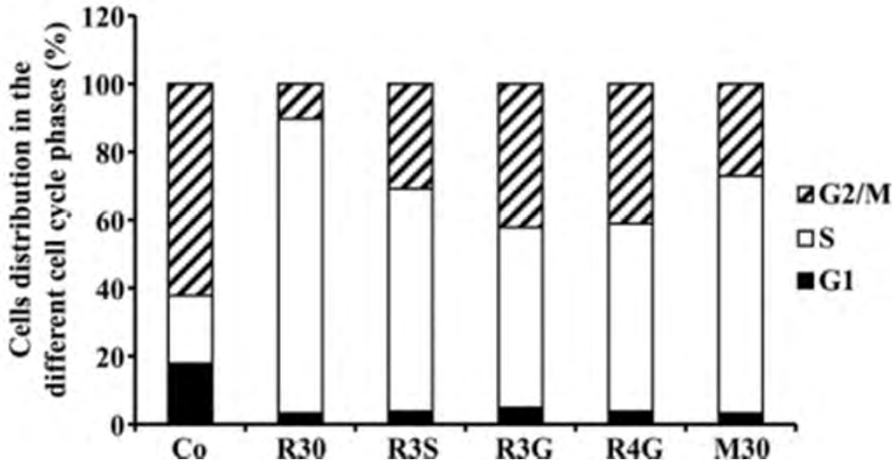


Reference: Ruotolo et al, Nutr Metab Cardiovasc Disease 2013; epub.

Colorectal cell death by RES metabolites



Cell antiproliferative activity of R3S

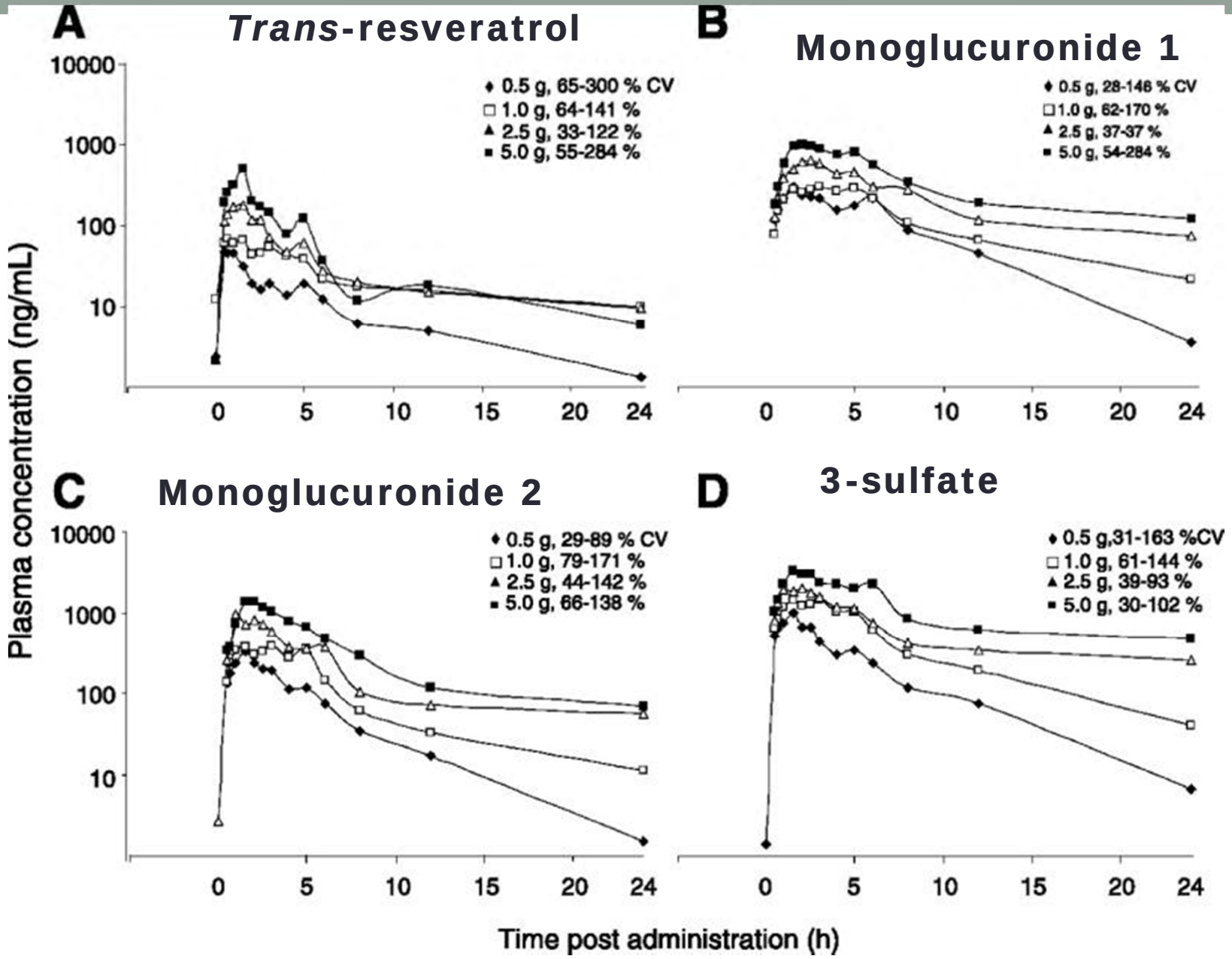


S-phase arrest

Reference: Aires et al, Mol Nutr Food Res 2013; epub.

Outline

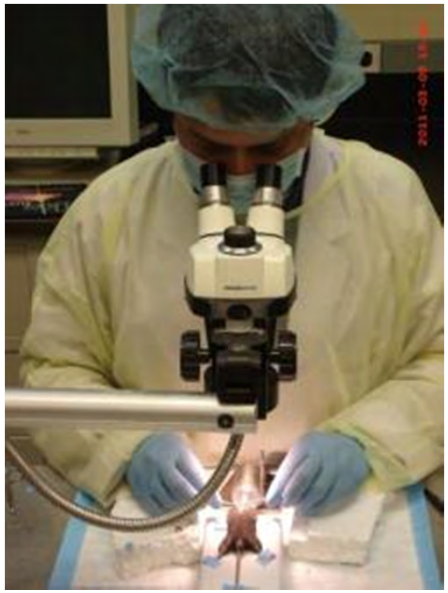
- A renewed interest in xenobiotic conjugation
  - FDA guidelines
  - Poor IVIVE of conjugation and prediction of DDI
- Resveratrol as a model substrate
  - Tissue-specific glucuronidation and sulfation
  - Possibly active metabolites, pharmacogenetics
  - Auto-induction of glucuronidation
- Pharmacokinetics of resveratrol and its conjugates
  - Simple compartmental modeling
  - Incorporation of transporters

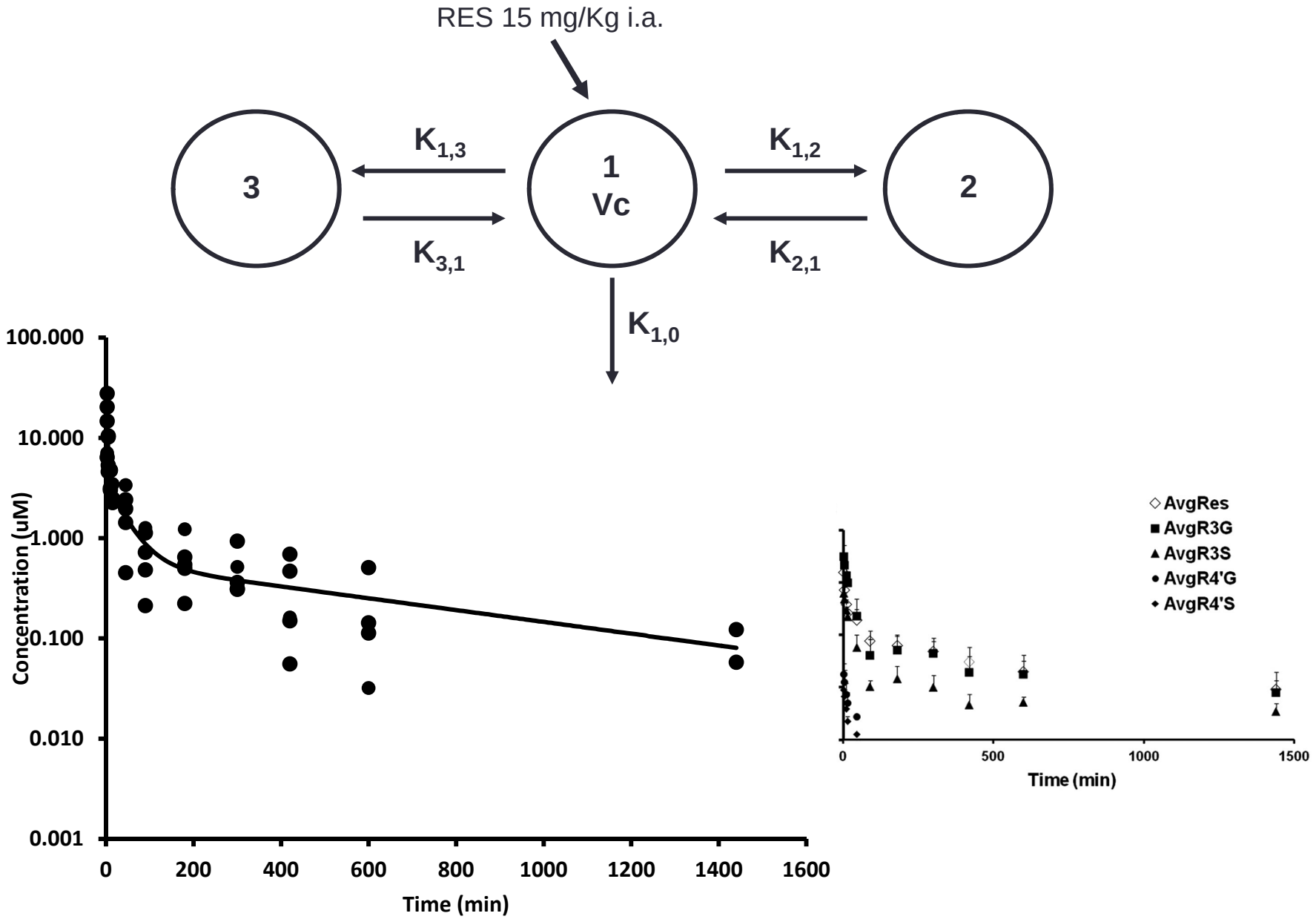


Reference: Boocock et al, Cancer Epidemiol Biomarkers Prev 2007; 16: 1246 – 1252

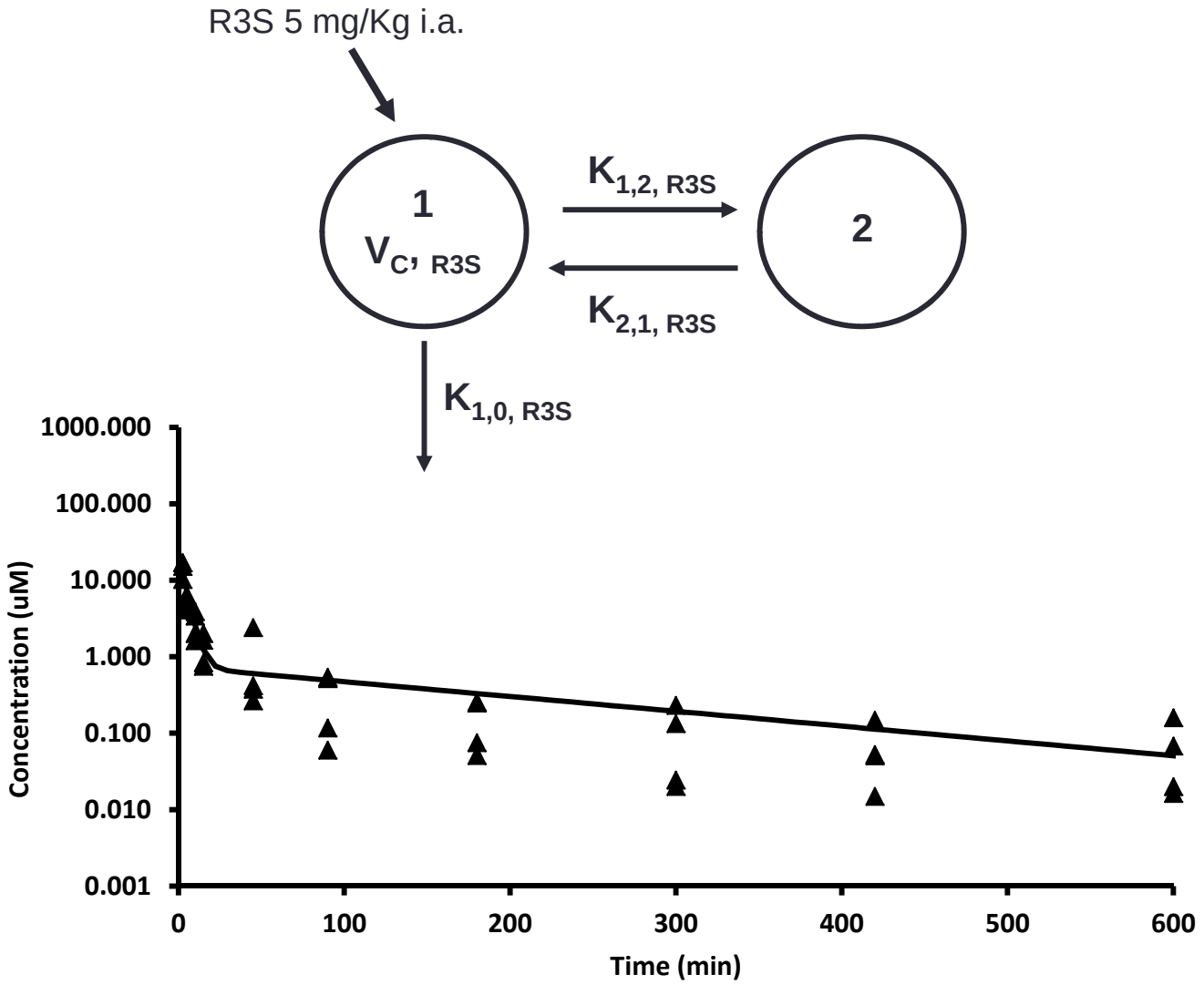
Mouse PK study design

- C57BL/6J black male mouse
- Right carotid artery was cannulated and 20 uL blood serially sampled
- Sampling time points 2.5, 5, 10, 15, 45, 90, 180, 300, 420, 600 min & 24 hrs
- Oral, intravenous (I.V.) and intra arterial (I.A.) dosing was performed
- Blood samples were centrifuged at 14000 rpm for 2 minutes and plasma samples analyzed with LC-MS^n

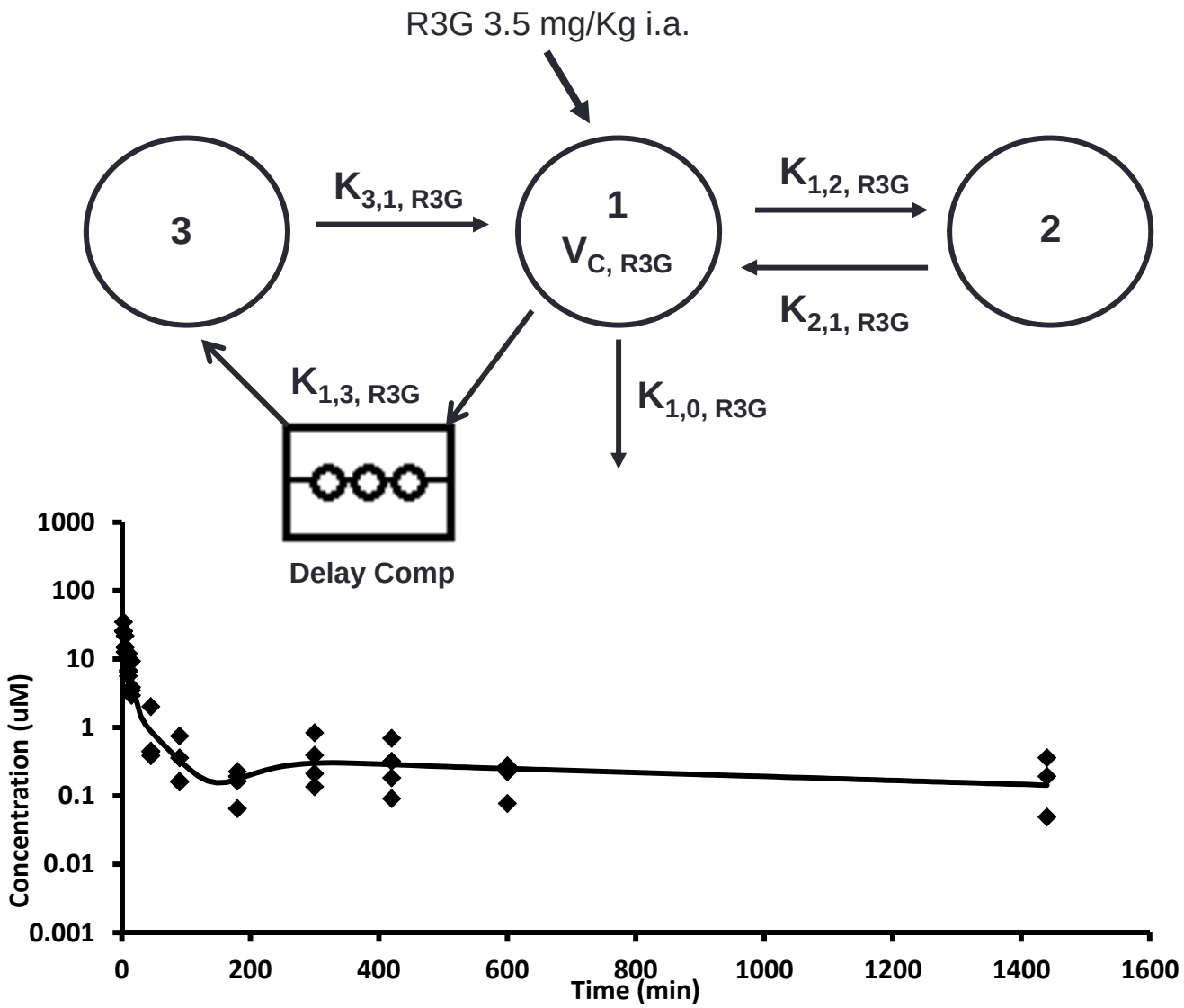




Reference: Sharan et al, DMD 2012, 40: 1993-2001.



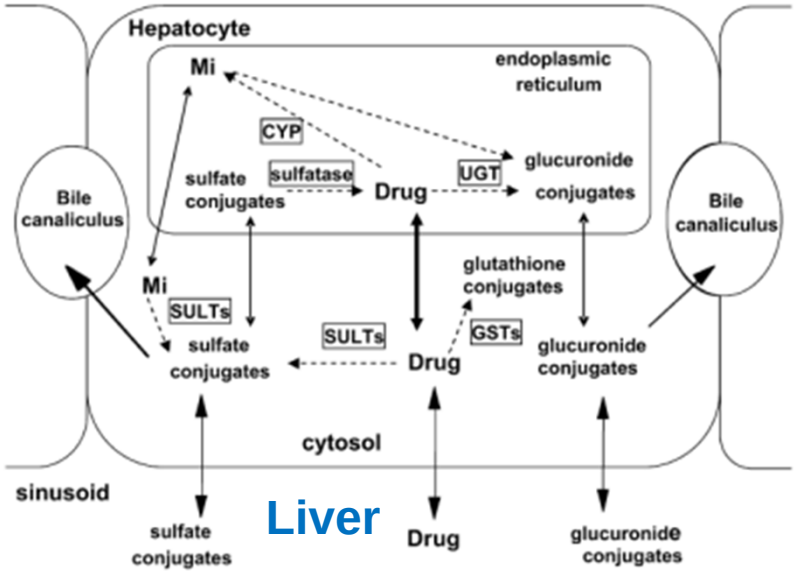
Reference: Sharan et al, DMD 2012, 40: 1993-2001.

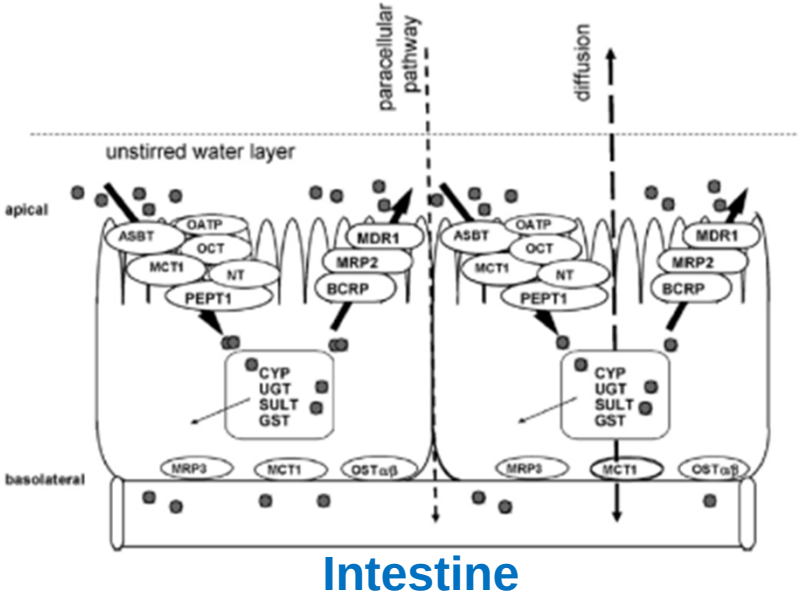


Reference: Sharan et al, DMD 2012, 40: 1993-2001.

In vivo formed versus preformed metabolites

- Different barriers to tissue access
- Zonal expression of metabolizing enzymes in the organ (not every organ is well-stirred)
- Complex metabolism (e.g. sequential metabolism)
- Different exposure to drug transporters

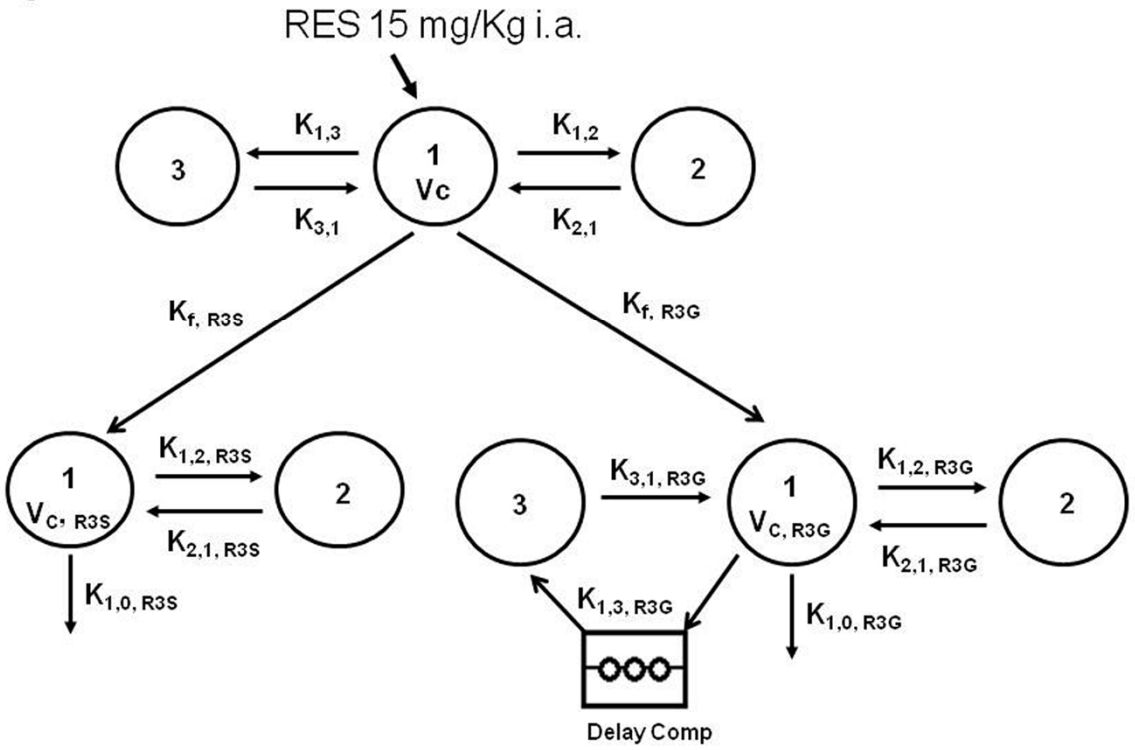


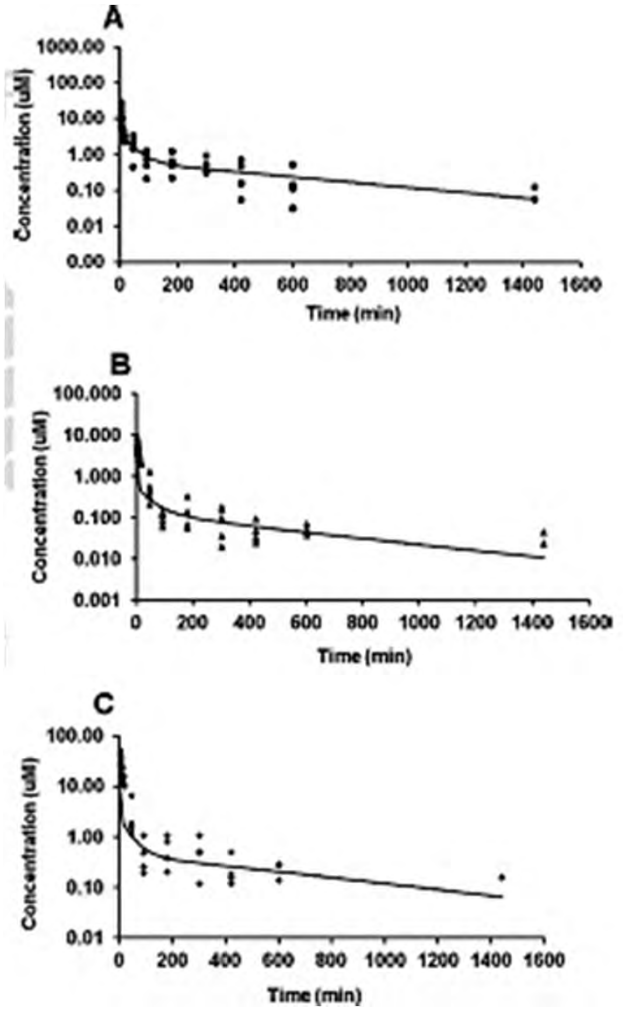


Reference: Pang KS, Chem Biol Interact. 2009,179:45-59.

Model 4

Fig 4



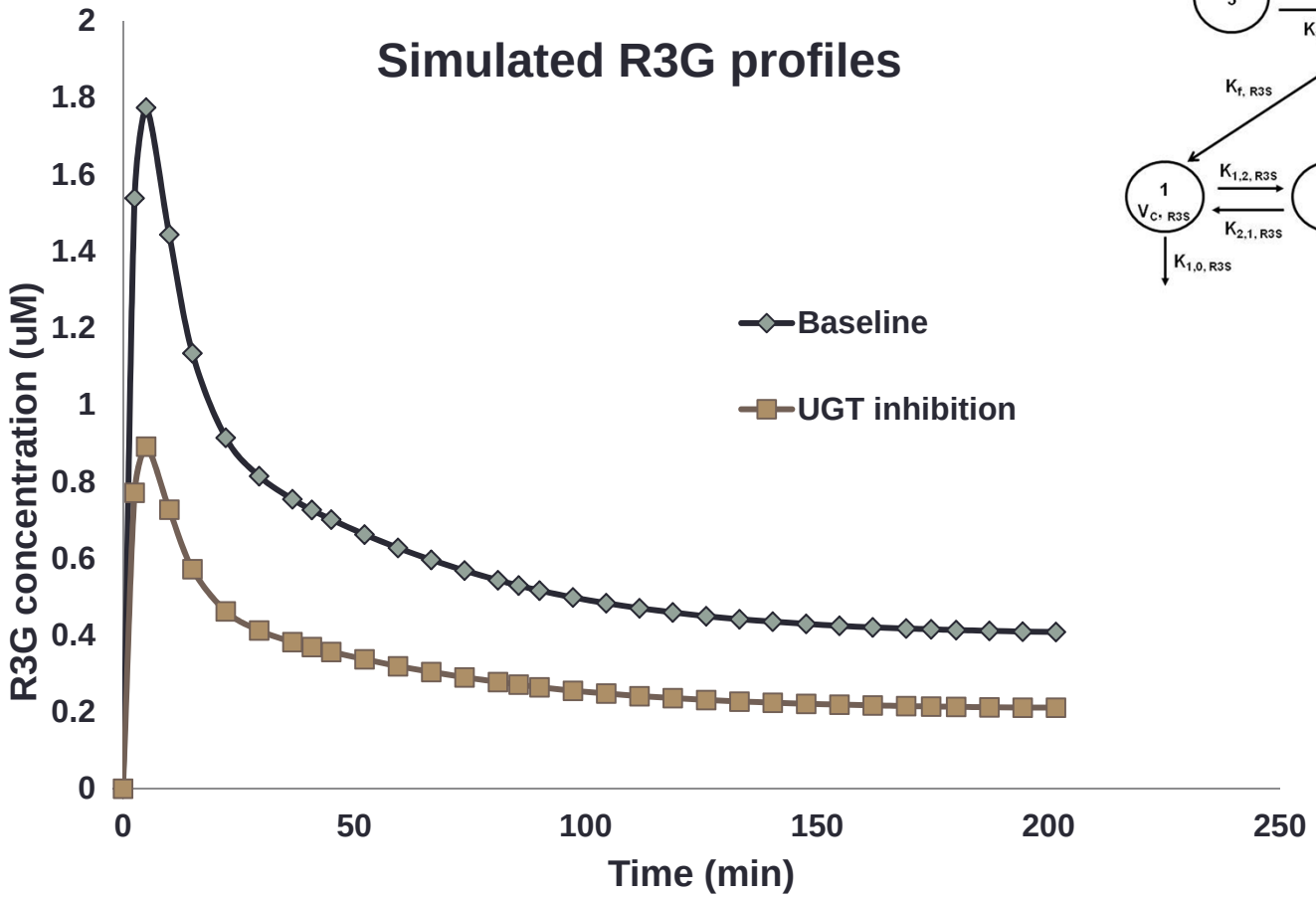


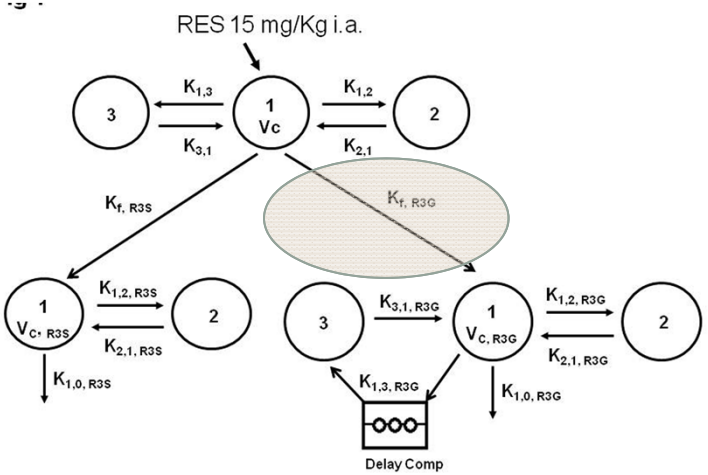
Reference: Sharan et al, DMD 2012, 40: 1993-2001.

Parameters	*In-vivo* formed metabolite	Preformed Metabolite
Cl,R3G (ml/min/Kg)	67.86	13.78 ± 5.75
Cl,R3S (ml/min/Kg)	313.08	76.29 ± 37.07
fm, R3G (%)	52.00	17.08
fm,R3S (%)	48.00	16.87

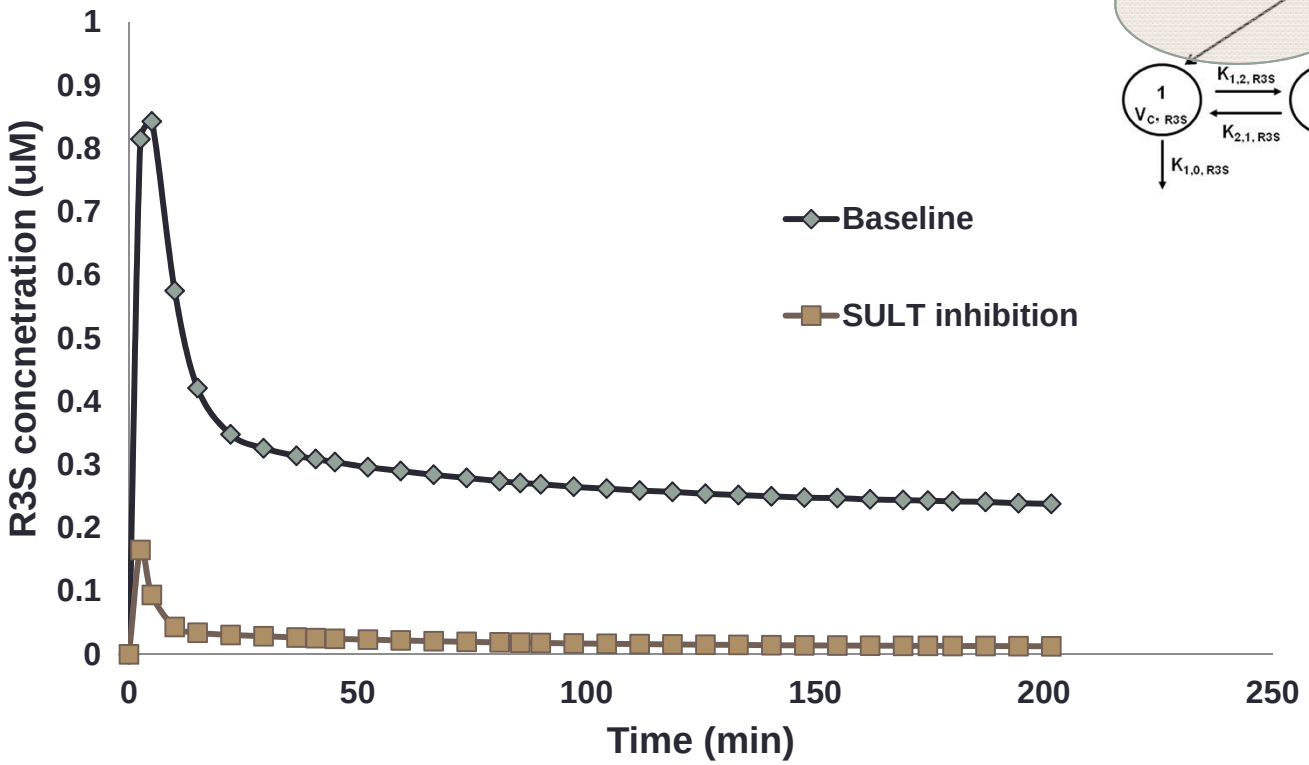
Reference: Sharan et al, DMD 2012, 40: 1993-2001.

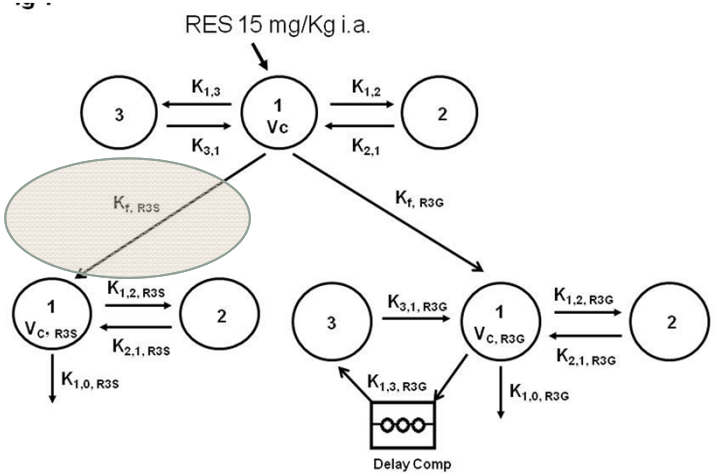
Simulating UGT inhibition



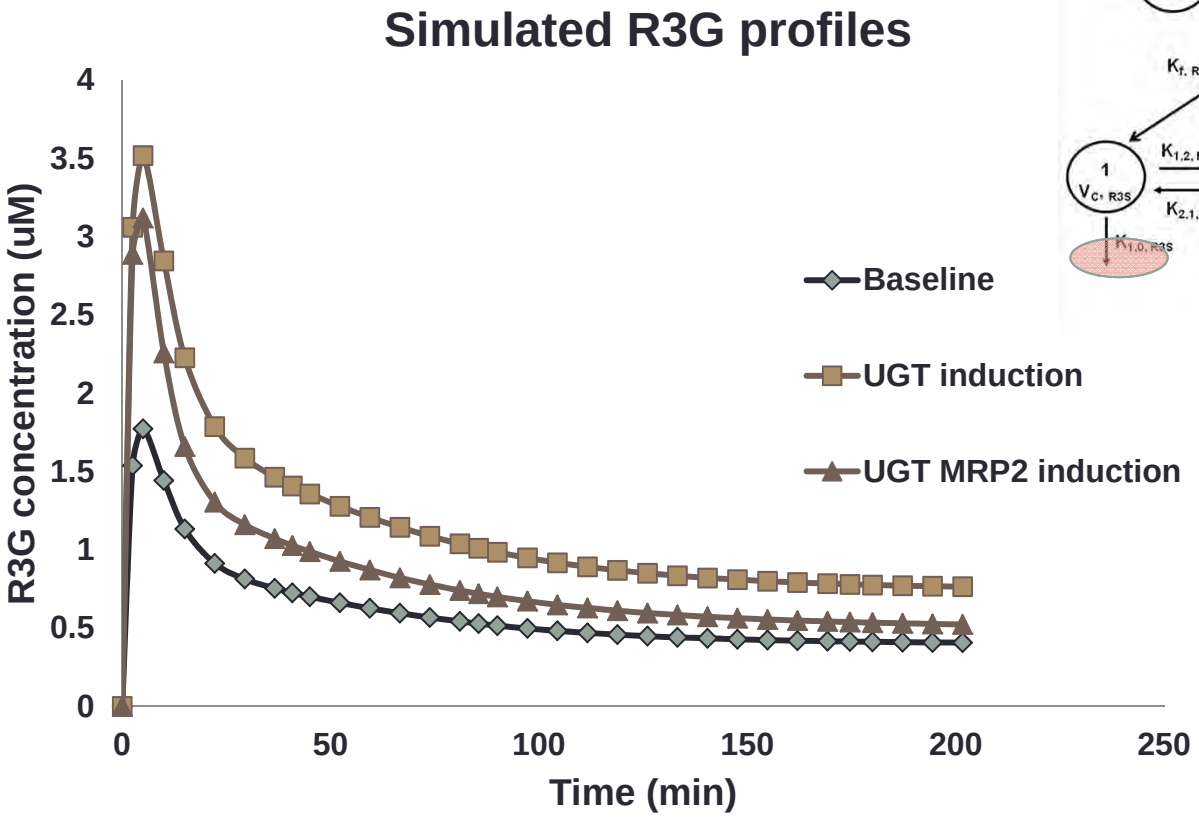


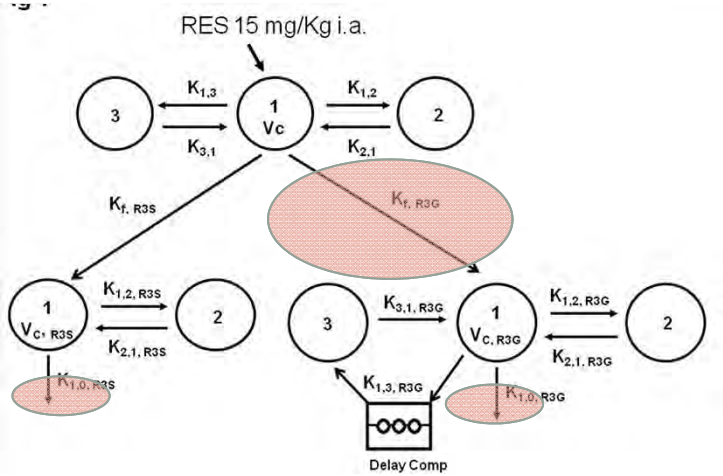
Simulating SULT inhibition



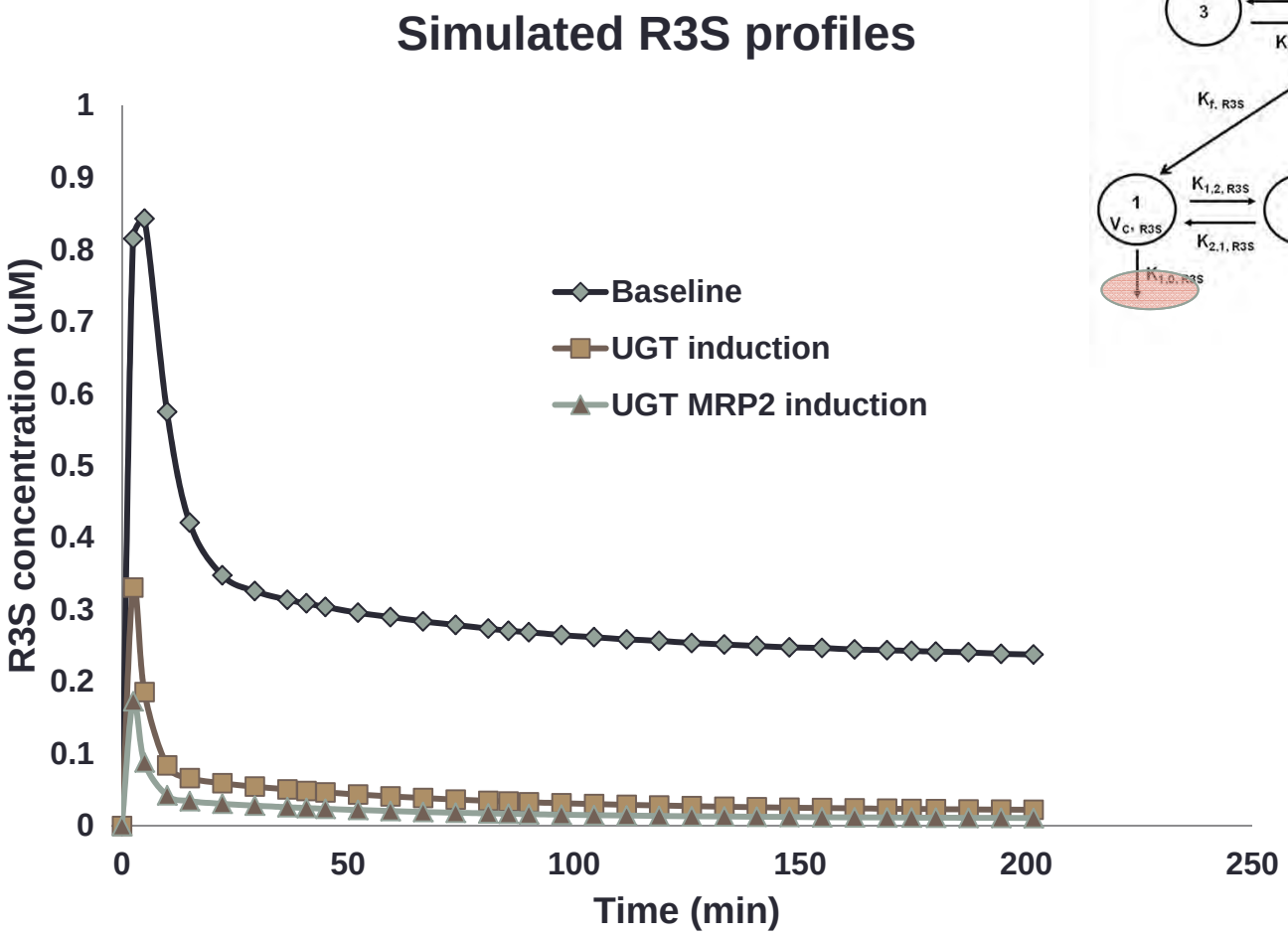


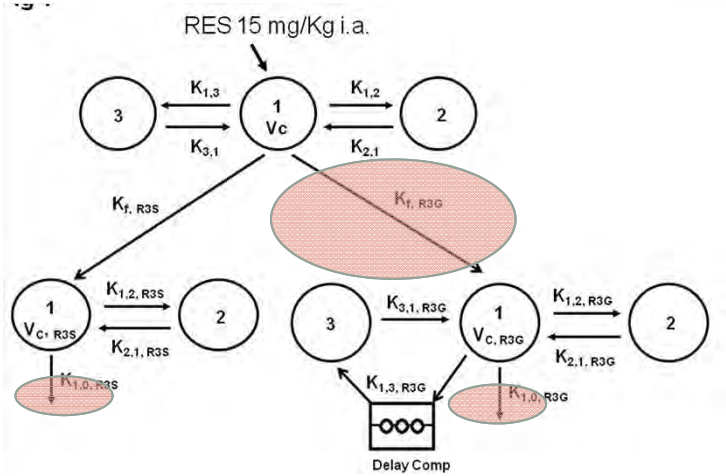
Simulating UGT induction (plus MRP2 induction)





Simulating UGT induction (plus MRP2 induction)





Summary

- Xenobiotic conjugation can be complicated by issues such as reversible metabolism, enterohepatic recycling, enzyme orientation in the cell, and interplay with transporters
- Standardized in vitro techniques, and tools like specific substrates/inhibitors/antibodies are needed
- Regulation of UGTs and genetic polymorphisms can alter the extent of clinical DDIs
- Interplay of conjugation and transport is important
- Compartmental modeling can provide a step toward predicting alterations in systemic drug and conjugated metabolite exposure

Future directions

- Single versus multiple dosing
  - Time-dependent UGT induction
- Combination dosing
  - Interaction between combinations of UGT substrates and inducers
  - Comparison between synthetic phytochemicals and dietary components
  - Prediction of food- and herb- drug interactions
- Transporter-UGT interactions
  - Clearance of conjugated metabolites
  - Common regulatory pathways

Acknowledgment

- Nagar Lab
  - Otito Iwuchukwu, PhD
  - Satish Sharan, PhD
  - Amir Youssef
  - Vaishnavi Ganti
  - Aneesh Argikar
  - Priyanka Kulkarni
- Collaborators
  - Upendra Argikar
  - Dan Canney
  - Ken Korzekwa
  - Henry Parkman
  - Ellen Walker
  - Absorption Systems
- Funding
  - NIH R03CA133943
  - NIH R03CA159389
  - NIH R01GM104178