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Your #1 Ranked Team for Exposure and Safety Solutions!

Society of Toxicology Annual Meeting

March 28th, 2022

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Evolving Relationship Between M&S Solutions and R&D

Model "supported" (first questions 20 years ago):

Do you think modeling and simulation might help?

Model "based" (questions 5 years ago):

How can I maximize the value of modeling and simulation in my development program?

Model "informed" (questions today):

How do I change our R&D process to reflect the availability of in silico tools and techniques?

What a Great Time to be a PBPK Modeler!

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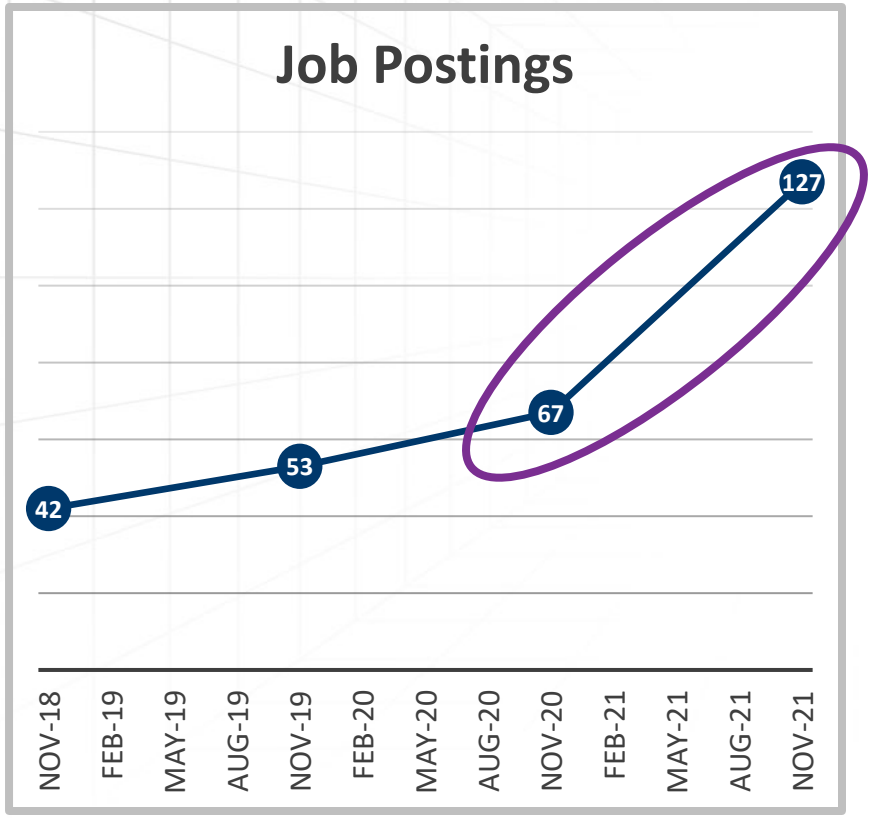
What pbpk modeling | Where City, state, zip code, or "remote" | Find jobs



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Session Game Plan

- Why is SLP a great teammate to have?
- ***Effective Use*** vs. ***Safety*** – Chemicals and Therapeutics
- How do we get on the court together?

We Put It All Together

Science

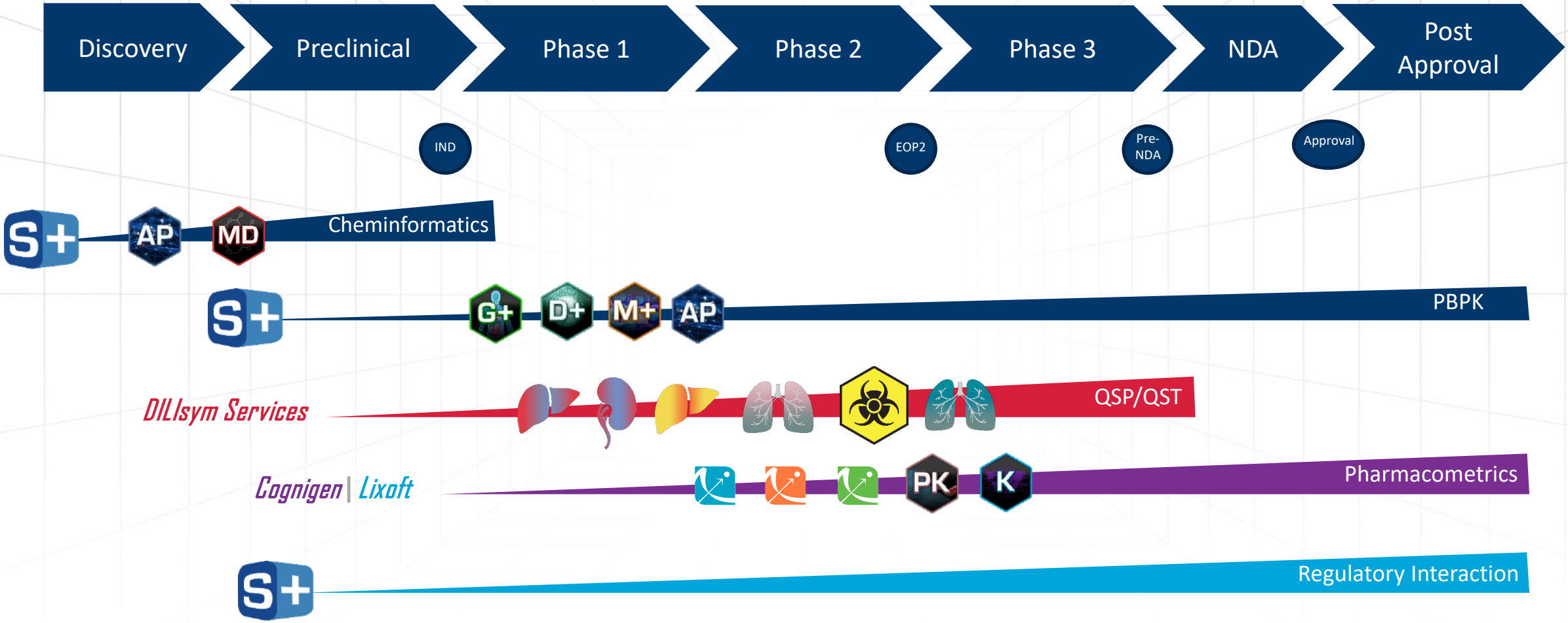
- Seamless collaboration
- Integrated, innovative solutions
- Customization options

Business

- Expert resources available
- One-stop shopping
- Flexible models

We have the *Solutions* and the *People* to Address *Your* R&D Questions!

Our Solutions Cover the Entire R&D Court



Pharmaceuticals/Chemicals/Consumer Goods

- Commercially maintained and validated software tools
- Experts to guide, manage, and support research and regulatory programs

CROs/Consultants

- Encourage onboarding our tools to support your clients
- Flexible business terms

Universities & Colleges

- Free (yes, free) access to our software for both teaching and research
- Internship & postdoc opportunities year-round

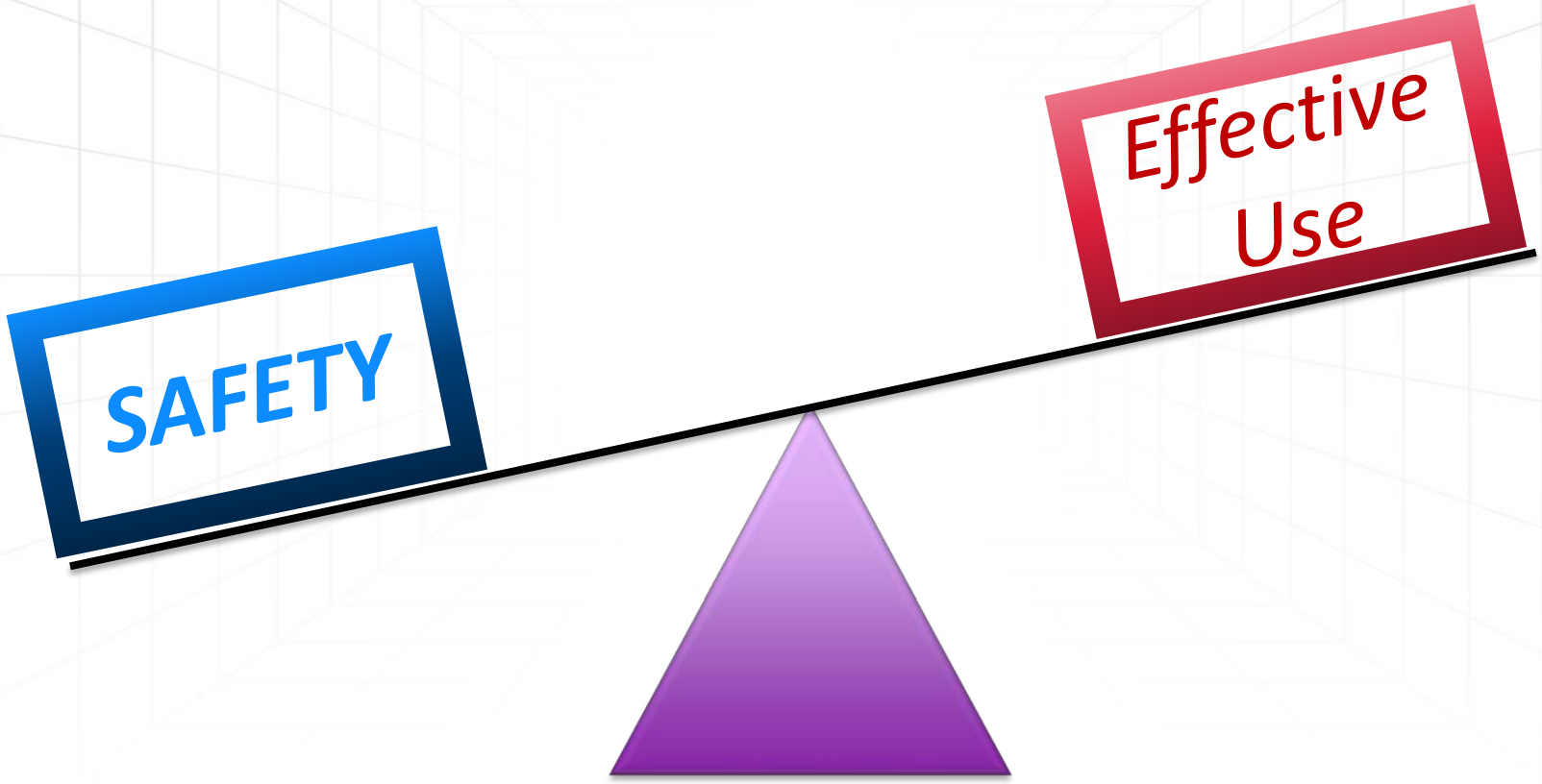
Government/Regulatory Agencies

- Heavily discounted (or free) access to our software
- Online and customized training opportunities

Session Game Plan

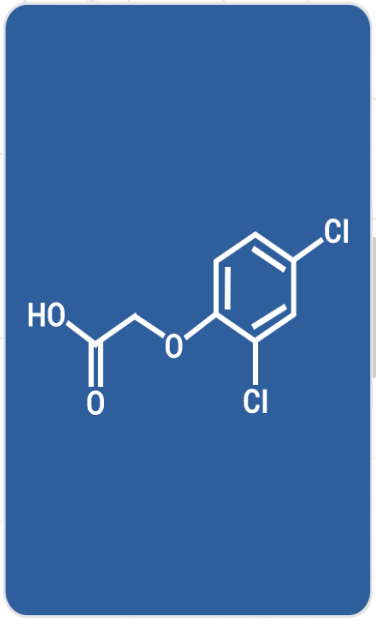
- Why is SLP a great teammate to have?
- ***Effective Use*** vs. ***Safety*** – Chemicals and Therapeutics
- How do we get on the court together?

Most of Your Organizations Focus on Designing Chemicals or Therapeutics for *Effective Use* – but How to Ensure the *Safety* of Those Products?

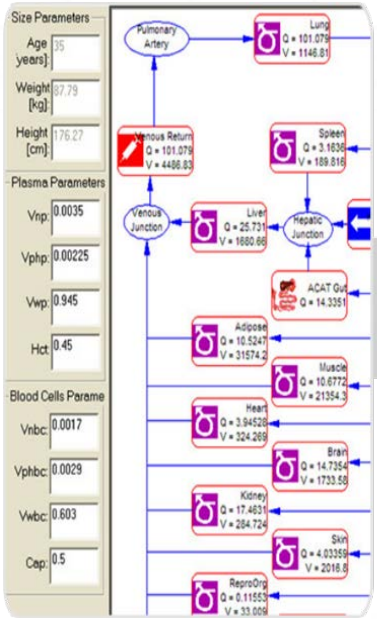


Your ‘Starting 5’ for Team *Safety*

Drawing Up the Play:
Machine Learning / PBPK / QST marriage



Permeability, solubility vs. pH, pKa(s), logD vs. pH, Fup, blood:plasma ratio, tissue Kps, CLint, CLfilt



Local/systemic exposure, distribution, parent/metabolite levels, patient variability

Quantitative Structure Activity Relationships (QSAR)

ADMET Predictor™

Physiologically-Based Pharmacokinetics (PBPK)

GastroPlus™

Quantitative Systems Pharmacology/Toxicology (QSP/QST)

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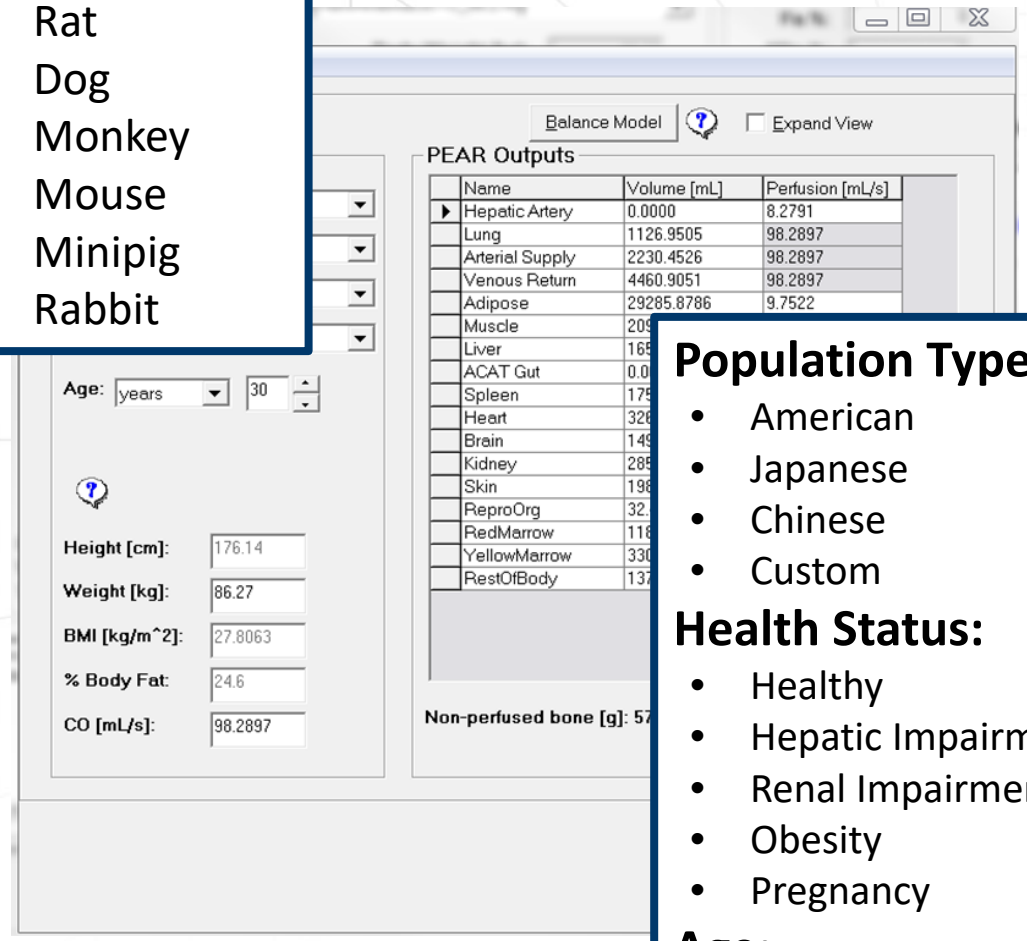
Validated System Models

Select Species:

- Human
- Rat
- Dog
- Monkey
- Mouse
- Minipig
- Rabbit



Population Types:

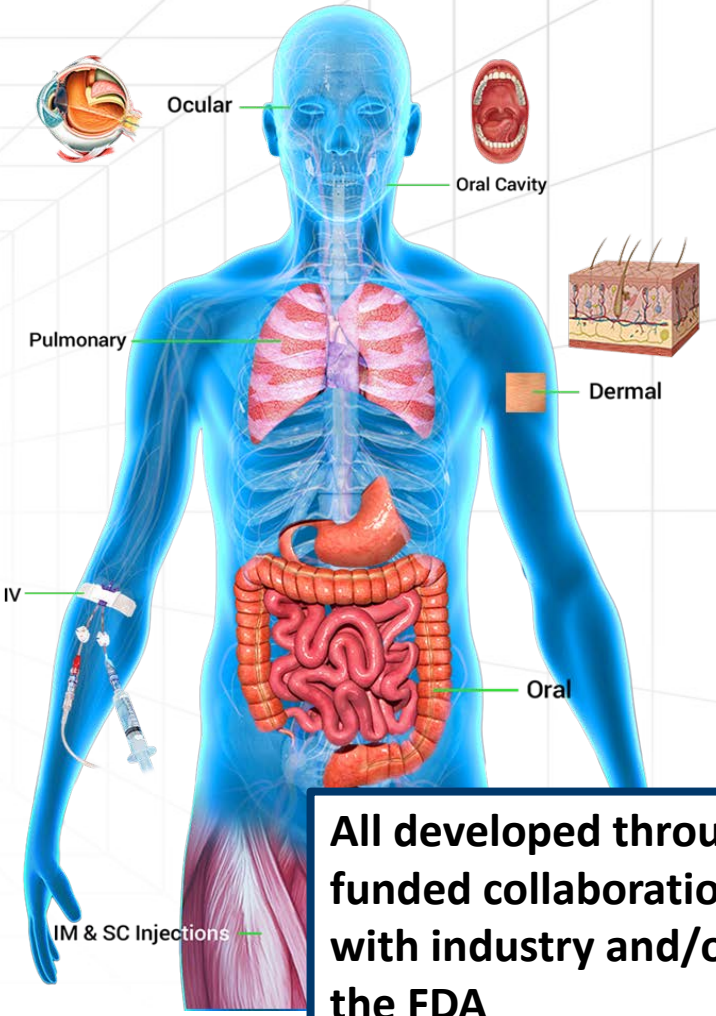
- American
- Japanese
- Chinese
- Custom

Health Status:

- Healthy
- Hepatic Impairment
- Renal Impairment
- Obesity
- Pregnancy

Age:

- Pediatrics/Adults/Geriatrics



All developed through funded collaborations with industry and/or the FDA

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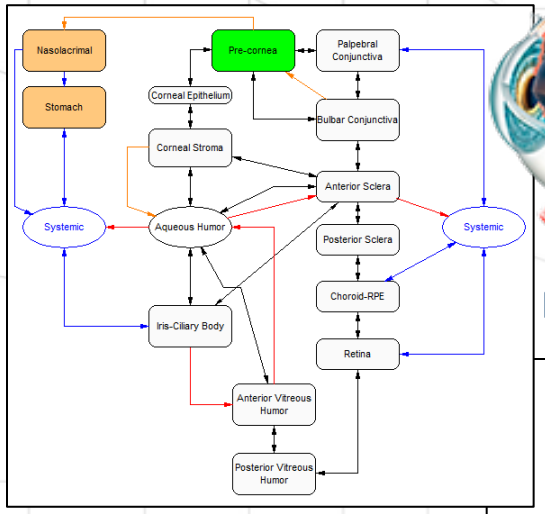
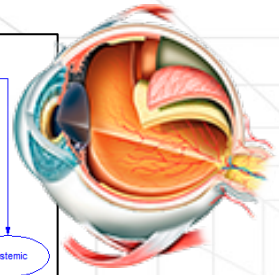
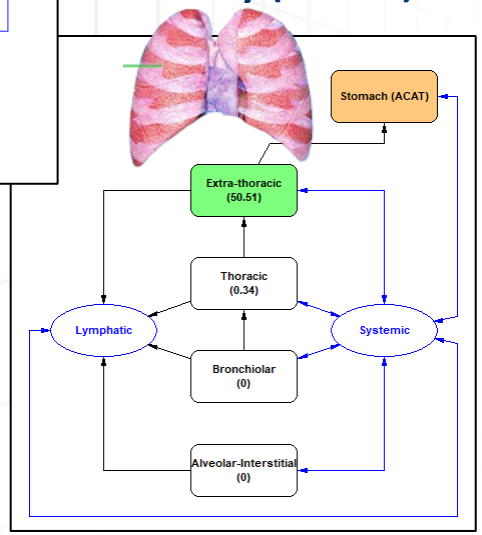
Pathways Beyond Oral Absorption…

Ocular (OCAT™)

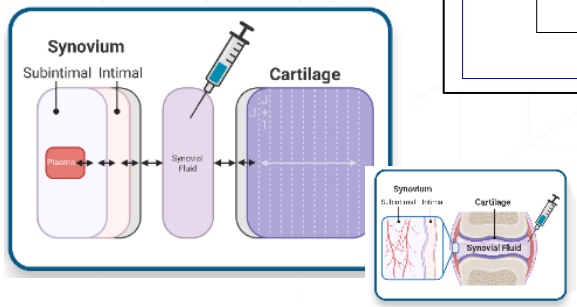


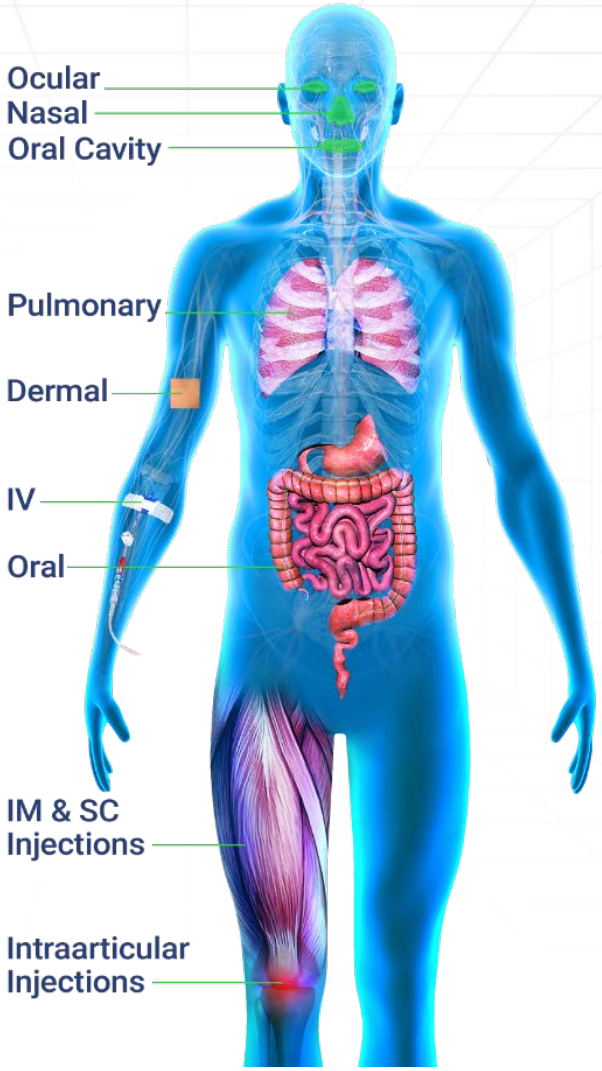


Pulmonary (PCAT™)

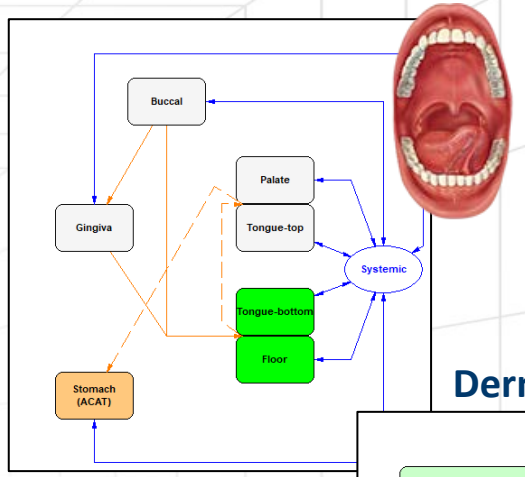


Intraarticular (ICAT™)

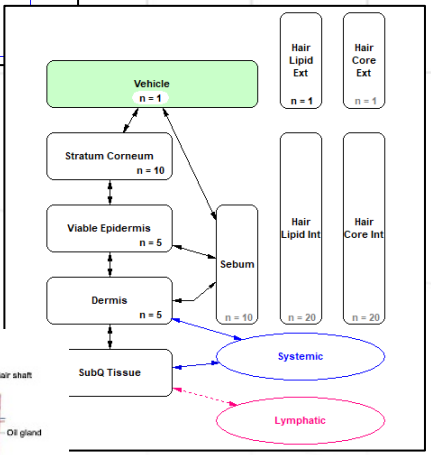


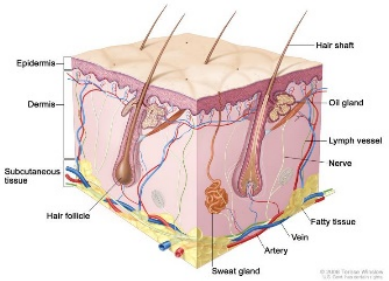


Oral Cavity (OCCAT™)



Dermal (TCAT™)





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ML/PBPK Modeling to Predict Toxicokinetics of Chemicals

- Dow Chemical performed an evaluation of the machine learning-PBPK marriage for several dosing routes:
  - Oral exposure
    - 88% predicted within 10-fold
  - Dermal exposure
    - 83% predicted within 10-fold
  - Inhaled exposure
    - 63% predicted within 10-fold
- Additional validation performed on key physicochemical inputs:
  - pKa(s)
  - logP
  - Henry's Law Constant
  - Intrinsic clearance
  - Plasma protein binding

SAR AND QSAR IN ENVIRONMENTAL RESEARCH
https://doi.org/10.1080/1062936X.2018.1518928

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Performance evaluation of the GastroPlus™ software tool for prediction of the toxicokinetic parameters of chemicals

F. Zhang[a], M. Bartels[b], A. Clark[a], T. Erskine[a], T. Auernhammer[a], B. Bhhatarai[c], D. Wilson[a] and S. Marty[a]

[a]The Dow Chemical Company, Midland, MI, USA; [b]ToxMetrics.com LLC, Midland, MI, USA; [c]Novartis Institute for Biomedical Research, Cambridge, MA, USA

ABSTRACT

The accurate prediction of toxicokinetic parameters arising from oral, dermal and inhalation routes of chemical exposure is a key element in chemical safety assessments. In this research, the phy-

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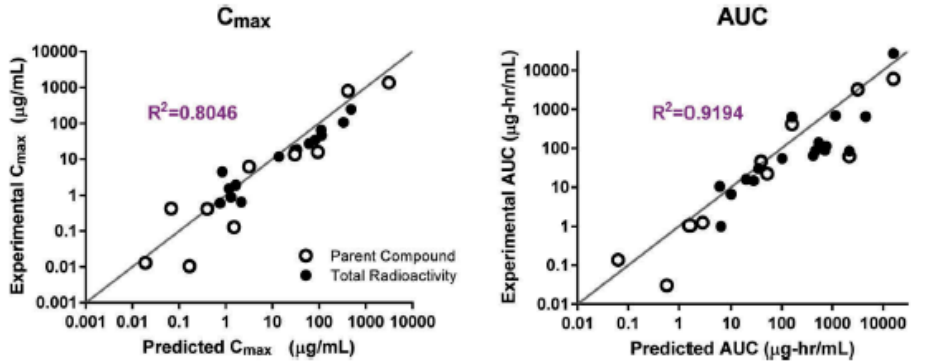

Total radioactivity: Total C_{max} or AUC of all radioactivity after dosing
Parent compound: Total C_{max} or AUC of parent compound after dosing

Figure 4. Correlation of predicted C_{max} and AUC versus empirical data following oral exposure.

Zhang et al. SAR QSAR Environ Res 2018

ML/PBPK Modeling to Predict Toxicokinetics of Chemicals

Oral exposure

Table 2. Experimental and predicted C_{max} and AUC from oral exposure.

Compound name	D.L. (mg)	Species	Emp. data sources (ref.)	Pre. C_{max} (μg/mL)	Pre. AUC_{0-t} (ug-h/mL)	Expt. C_{max}	Expt. C_{max} based on the parent	Expt. C_{max} based on the total rad.	Expt. AUC_{0-t}	Expt. AUC_{0-t} based on the parent	Expt. AUC_{0-t} based on rad.	C_{max} Ratio (pre. value/ expt. value)	C_{max} Ratio (expt. value/ pre. value)	C_{max} fold diff.	AUC_{0-t} ratio (pre. value/ expt. value)	AUC_{0-t} ratio (expt. value/ pre. value)	AUC_{0-t} fold diff.
Pomalidomide	2	Human (70kg)	[44]	0.01903	0.0631	0.013	0.013	NA	0.137	0.137	NA	1.46	0.68	1 to 3	0.46	2.17	1 to 3
Mirabegron	160	Human (70kg)	[50]	1.2898	5.9	0.879	NA	0.879	10.443	NA	10.443	1.47	0.68	1 to 3	0.56	1.77	1 to 3
Brivanib Alaninate	800	Human (70kg)	[41]	3.15	38.794	6.1	6.1	NA	45.9	45.9	NA	0.52	1.94	1 to 3	0.85	1.18	1 to 3
triamcinolone acetonide	5	Human (70kg)	[38]	0.167	0.57532	0.0105	0.0105	NA	0.0304	0.0304	NA	15.90	0.06	>10	18.93	0.05	>10
Lenalidomide	25	Human (70kg)	[35]	0.40274	2.74	0.413	0.413	NA	1.248	1.248	NA	0.98	1.03	1 to 3	2.20	0.46	1 to 3
Beclometasone Dipropioate	4.0	Human (70kg)	[37]	0.01629	0.17284	0.000703	0.000703	NA	0.010158	NA	NA	23.17	0.04	>10	17.02	0.06	>10
Setipirant	1000	Human (86.5kg)	[43]	94.053	2139.4	15.1	15.6	15.1	83.9	61.1	83.9	6.23	0.16	3 to 10	25.50	0.04	>10
Bisphenol A	7	Human (70kg)	[51]	0.0678	1.58	0.423	0.423	NA	1.05	1.05	NA	0.16	6.24	3 to 10	1.50	0.66	1 to 3
Vandetanib	300	Human (70kg)	[47]	1.503	51.365	0.129	0.129	NA	22.03	22.03	NA	11.65	0.09	>10	2.33	0.43	1 to 3
AMP	4.5	Rat (0.25 kg)	[48]	0.8336	34.548	4.42	NA	4.42	29.64	NA	29.64	0.19	5.30	3 to 10	1.17	0.86	1 to 3
TBBPA-DBPE	5	Rat (0.25 kg)	[46]	0.749	19.738	0.6	NA	0.6	15.85	NA	15.85	1.25	0.80	1 to 3	1.25	0.80	1 to 3
MethylParaben	25	Rat (0.25 kg)	[32]	113	757.11	45.92	NA	45.92	112.891	NA	112.891	2.46	0.41	1 to 3	6.71	0.15	3 to 10
PropylParaben	25	Rat (0.25 kg)	[32]	60.713	710.5	26.85	NA	26.85	88.249	NA	88.249	2.26	0.44	1 to 3	8.05	0.12	3 to 10
ButylParaben	25	Rat (0.25 kg)	[32]	33.424	462.5	18.13	NA	18.13	86.46	NA	86.46	1.84	0.54	1 to 3	5.35	0.19	3 to 10
Cyclohexene oxide	25	Rat (0.25 kg)	[49]	81.68	373.03	34	NA	34	NA	NA	NA	2.40	0.42	1 to 3	NA	NA	NA
Dihydrocapsiate	2.5	Rat (0.25 kg)	[34]	1.634	9.96	1.87	NA	1.87	6.745	NA	6.745	0.87	1.14	1 to 3	1.48	0.68	1 to 3
SQ109	3.25	Rat (0.25 kg)	[45]	2.139	6.277	0.644	NA	0.644	0.992	NA	0.992	3.32	0.30	3 to 10	6.33	0.16	3 to 10
Perfluorohexanoate	25	Rat (0.25 kg)	[40]	482.63	4516.7	246	NA	246	650	NA	650	1.96	0.51	1 to 3	6.95	0.14	3 to 10
Ethylene glycol	2.5	Rat (0.25 kg)	[39]	30.01	158.6	17.3	13.4	17.3	636.6	413	636.6	1.73	0.58	1 to 3	0.25	4.01	3 to 10
Ethylene glycol	250	Rat (0.25 kg)	[39]	3104.2	15860	1235	1350	1235	27282	6041	27282	2.51	0.40	1 to 3	0.58	1.72	1 to 3
Phenoxyethanol	0.75	Rat (0.25 kg)	[33]	29.92	101.77	13.96	NA	13.96	54.5	NA	54.5	2.14	0.47	1 to 3	1.87	0.54	1 to 3
Phenoxyethanol	75	Rat (0.25 kg)	[33]	330.24	1151.8	105	NA	105	684	NA	684	3.15	0.32	3 to 10	1.68	0.59	1 to 3
Phenol	37.5	Rat (0.25 kg)	[36]	110.76	415.49	63.93	NA	63.93	64.64	NA	64.64	1.73	0.58	1 to 3	6.43	0.16	3 to 10
Propylene glycol	7	Human (70kg)	[52]	416.12	3137.7	800	800	NA	3230	3230	NA	0.52	1.92	1 to 3	0.97	1.03	1 to 3
4-Nonylphenol	2.5	Rat (0.25 kg)	[42]	1.172	27.568	1.498	NA	1.498	14.65	NA	14.65	0.78	1.28	1 to 3	1.88	0.53	1 to 3
4-Nonylphenol	25	Rat (0.25 kg)	[42]	13.774	533.75	11.7	NA	11.7	139.8	NA	139.8	1.18	0.85	1 to 3	3.82	0.26	3 to 10

NA: data not available; C_{max}: Maximum plasma concentration of parent or total radioactivity; AUC_{0-t}: Area under the curve from time 0 to the last measurable concentration; Fold difference was based on the ratio of experimental value over predicted value or predicted value over experimental value.
Expt.: Experimental; Emp.: Empirical; Spe.: Species; D.L.: Dose level; Pre.: Predicted; Expt.: Experimental; Rad.: Radioactivity; Ref.: references; Diff.: difference.

Inhaled exposure

Table 3. Experimental and predicted C_{max} and AUC from inhalation exposure.

Compound name	D. (m	Spe.	Emp. data sources (ref.)	Pre. C_{max} (μg/mL)	Pre. AUC_{0-t} (μg-h/mL)	Expt. C_{max}	Expt. C_{max} based on the parent	Cmax ratio (Pre. value/ Expt. value)	Cmax ratio (Expt. value/ Pre. value)	C_{max} fold diff.	AUC_{0-t} ratio (pre. value/ expt. value)	AUC_{0-t} ratio (expt. value/ pre. value)	AUC_{0-t} fold diff.
CS-8958	5	Human (70kg)	[55]	0.02869	0.04456	0.0128	0.0453	2.24	0.45	1 to 3	0.984	1.017	1 to 3
CS-8958	10	Human (70kg)	[55]	0.05547	0.0866	0.0290	0.108	1.91	0.52	1 to 3	0.802	1.247	1 to 3
CS-8958	12	Human (70kg)	[55]	0.35034	0.6164	0.423	1.57	0.828	1.21	1 to 3	0.393	2.542	1 to 3
triamcinolone acetonide	5	Human (70kg)	[54]	0.0014	0.00517	0.00200	0.0119	0.700	1.43	1 to 3	0.434	2.302	1 to 3
Amiloride	4.5	Human (70kg)	[56]	0.03171	0.22225	0.00157	0.0144	20.2	0.05	>10	15.43	0.065	>10
Beclometasone Dipropionate	1.0	Human (70kg)	[37]	0.00452	0.03058	0.000319	0.000151	14.2	0.07	>10	203	0.005	>10
Albuterol	0.1	Human (76kg)	[53]	0.000181	0.00412	0.000001469	0.00000427	123	0.01	>10	965	0.001	>10
Tobramycin	80	Human (76kg)	[57]	0.1464	0.8978	0.570	4.37	0.257	3.89	3 to 10	0.205	4.871	3 to 10

NA: data not available; Cmax: Maximum plasma concentration of parent or total radioactivity; AUC_{0-t}: Area under the curve from time 0 to the last measurable concentration; Fold difference was based on the ratio of experimental value over predicted value or predicted value over experimental value.
Expt.: Experimental; Emp.: Empirical; Spe.: Species; D.L.: Dose level; Pre.: Predicted; Expt.: Experimental; Rad.: Radioactivity; Ref.: references; Diff.: difference.

Dermal exposure

Table 4. Experimental and predicted C_{max} from dermal exposure.

Compound name	Dermal exposure format	D.L. (mg)	Species	Emp. data sources (ref.)	Pre. C_{max} (μg/mL)	Pre. AUC_{0-t} (μg-h/mL)	Expt. C_{max} (μg/mL)	Expt. C_{max} based on the parent (μg/mL)	Expt. C_{max} based on the total rad. (μg/mL)	C_{max} Ratio (pre. value/ expt. value)	C_{max} Ratio (expt. value/ pre. value)	C_{max} Fold Diff.
Coumarin	solution	1.8	Human (70kg)	[60]	0.01502	0.3557	0.025	NA	0.025	0.601	1.664	1 to 3
D-limonene	solution	12	Human (70kg)	[58]	0.01551	2.2595	0.12	NA	0.12	0.129	7.737	3 to 10
N,N-Diethyl-*m*-tolumide	solution	12	Human (70kg)	[66]	0.02905	0.39219	0.002204	NA	0.002204	13.2	0.076	>10
OPP	solution	0.4	Human (70kg)	[67]	0.00128	0.1222	0.015	NA	0.015	0.085	11.719	>10
Rivastigmine	1 patch 10cm^2 (24hr)	18	Human (70kg)	[63]	0.0074	0.12285	0.0068	0.0068	NA	1.09	0.919	1 to 3
Testosterone	2 patch (2x2.5) 96hr	100	Human (80kg)	[59]	0.005618	0.1109	0.00739	0.00739	NA	0.760	1.315	1 to 3
Estradiol	solution	340	Human (70kg)	[68]	0.0002	0.02932	NA	NA	NA	NA	NA	NA
Methyl salicylate	2 patch (2X74.88)	599	Human (70kg)	[64]	0.02261	0.28014	0.0086	0.0086	NA	2.63	0.380	1 to 3
Menthol	2 patch (2X74.88)	299.5	Human (70kg)	[64]	0.04551	0.82774	0.0076	0.0076	NA	5.99	0.167	3 to 10
Camphor	2 patch (2X74.88)	93.6	Human (70kg)	[64]	0.07287	0.96066	0.0135	0.0135	NA	5.40	0.185	3 to 10
Clonidine	1 patch 10cm^2 (72hr)	6.0	Human (70kg)	[61]	0.000231	0.02139	0.00016	0.00016	NA	1.44	0.693	1 to 3
DonePezil	1patch 12.5 cm^2 (72hr)	43.75	Human (70kg)	[62]	0.02183	2.9193	0.00524	0.00524	NA	4.17	0.240	3 to 10
Triprolidine	1patch 10 cm^2 (34hr)	5	Human (70kg)	[65]	0.00599	0.13015	0.002	0.002	NA	3.00	0.334	1 to 3

NA: data not available; Cmax: Maximum plasma concentration of parent or total radioactivity; Fold difference was based on the ratio of experimental value over predicted value or predicted value over experimental value.
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ML/PBPK Modeling to Predict Toxicokinetics of Chemicals

- Evaluation of the machine learning-PBPK marriage to predict systemic exposure for MDI and derivatives following inhaled and dermal administration:
  - Model developed and validated from data collected on 3 MDI monomers
  - Non-monomeric MDI constituents predicted to have lower relative uptake
- The ML/PBPK simulations should be useful for category-based, worst-case, Read-Across assessments

Bartels et al. Reg Toxicology Pharmacology 2022

Regulatory Toxicology and Pharmacology 129 (2022) 105117

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

In silico predictions of absorption of MDI substances after dermal or inhalation exposures to support a category based read-across assessment

Michael Bartels [a,*], William van Osdol [b], Maxime Le Merdy [b], Anne Chappelle [c], Adam Kuhl [d], Robert West [c]

[a] ToxMetrics.com LLC, Midland, Michigan, USA
[b] Simulations Plus, Lancaster, CA, USA
[c] International Isocyanate Institute, Mountain Lakes, NJ, USA
[d] Huntsman LLC, The Woodlands, Texas, USA

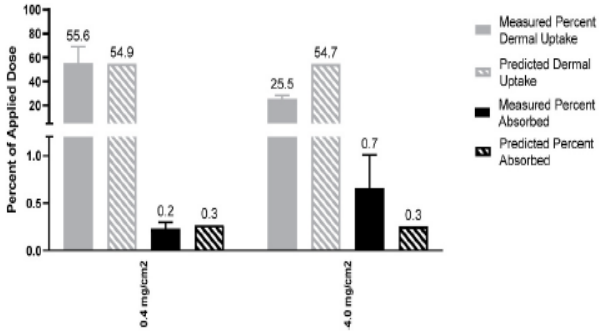


Fig. 3. Measured and Predicted Dermal Uptake and Absorption of 4,4′-MDI in the Rat (8 h exposure to 10 cm^2 rat dorsal of 0.4 or 4.0 mg/10 μL vehicle/cm^2 of 4,4′-MDI in acetone vehicle (occluded)), sampling/predictions conducted at 24 h post-dosing, as per Leibold et al., 1999). Note: empirical measurements based on total radioactivity (parent + metabolites) while model results based on parent compound only.

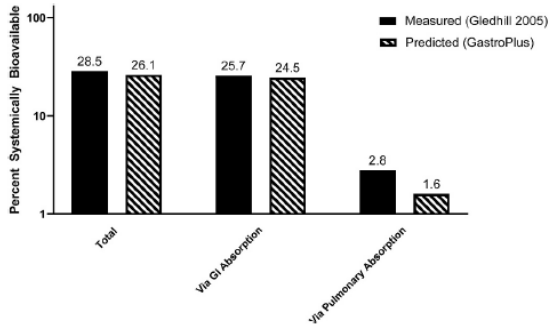


Fig. 7. Measured and Predicted Fractional Absorption of Inhaled 4,4′-MDI *via* the Pulmonary Tissues or GI Tract in the Rat (6 h nose-only exposure to 3.79 ppm ^{14}C-4,4′-MDI, test material intake assumed to occur *via* inhalation route (38.5% of received dose) and oral route (25.5% and 36% of received dose, while systemic bioavailability of absorbed test material assumed to occur primarily *via* oral route (90%) with 10% *via* pulmonary tissues) (*in vivo* data from Gledhill et al., 2005). Note: empirical measurements based on total radioactivity (parent + metabolites) while model results based on parent compound only.

ML/PBPK Modeling to Predict Skin Penetration

- Cosmetics Europe consortium evaluated the predictive performance of different *in silico* skin penetration models:
  - Machine learning predictions or *in vitro* data used as input into models to predict dermal delivery for 24 chemicals
- The ML/PBPK simulations from GastroPlus® were ranked #1 and can be utilized to rank substances on their ability to pass through the skin
  - From this work, a collaboration with Cosmetics Europe was initiated to improve the GastroPlus® TCAT™ model

Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.sciencedirect.com/journal/computational-toxicology

Cosmetics Europe evaluation of 6 *in silico* skin penetration models

Sébastien Grégoire [a,*], Ian Sorrell [b,1], Daniela Lange [c], Abdulkarim Najjar [c], Andreas Schepky [c], Corie Ellison [d], John Troutman [d], Eric Fabian [e], Hélène Duplan [f], Camille Genies [f], Carine Jacques-Jamin [f], Martina Klaric [g,2], Nicola J. Hewitt [g]

[a] L'Oreal Research & Innovation, Aulnay-Sous-Bois, France
[b] Unilever, Sharnbrook, Bedfordshire, UK
[c] Beiersdorf AG, Hamburg, Germany
[d] The Procter & Gamble Company, Cincinnati, USA
[e] BASF, Ludwigshafen, Germany
[f] Pierre Fabre Dermo-Cosmétique, Toulouse, France
[g] Cosmetics Europe, Brussels, Belgium

Table 3
Impact of measured and QSAR $K_{SC/buffer}$ and Dsc values on correlation coefficients (R^2) between predicted and measured values of DD of 24 chemicals applied in PBS as a preliminary criterion of performance.

Model	Condition (Table 2)	R^2 using $K_{SC/buffer}$ and D_{sc}	
		QSAR	Measured
TCAT	T1	0.80	0.53
Surrey	Su2 (2D Model)	0.29	-
	Su4 (1D Model)	-	0.28
DSkin	D2	0.60	0.14
SimCyp	SC1	0.23	0.58

Gregoire et al. Computational Toxicology 2021

ML/PBPK Modeling for Dermally Applied Consumer Products

Toxicology in Vitro 63 (2020) 104746

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products

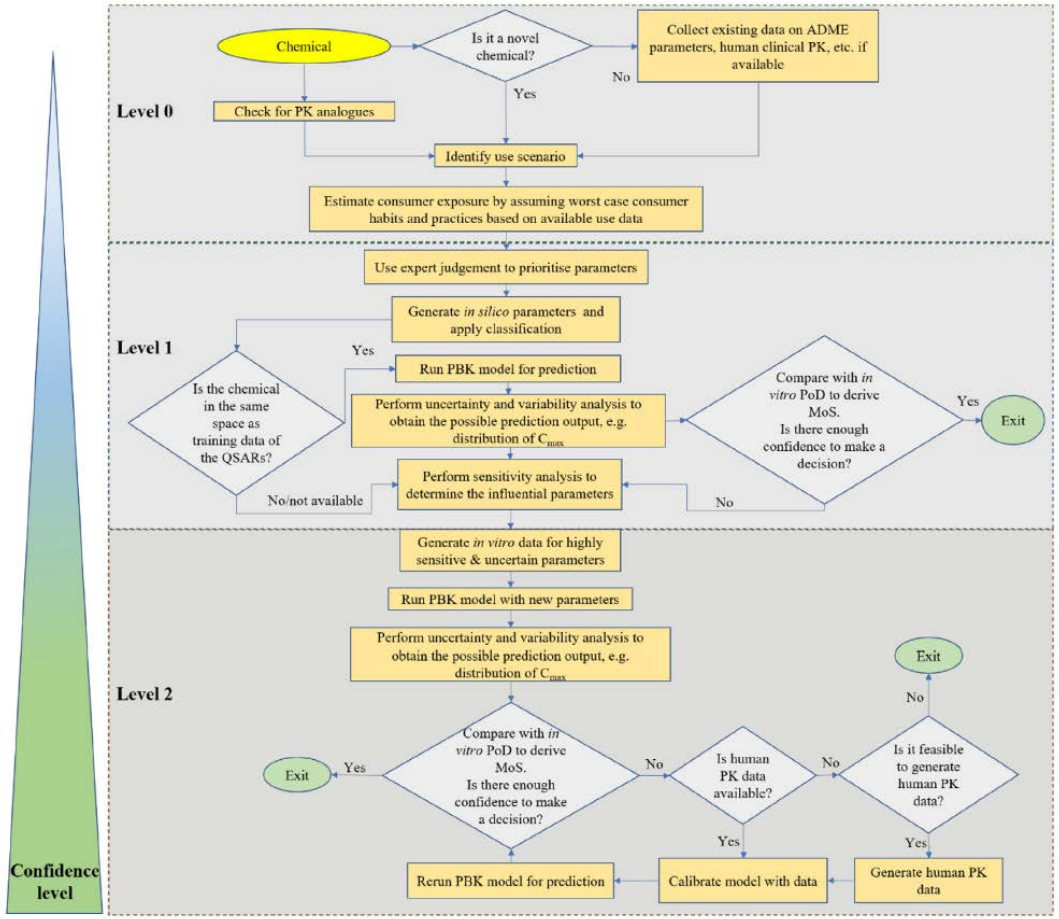
Thomas E. Moxon*, Hequn Li*, Mi-Young Lee, Przemyslaw Piechota, Beate Nicol, Juliette Pickles, Ruth Pendlington, Ian Sorrell, Maria Teresa Baltazar

Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Shambrook, Bedfordshire MK44 1LQ, UK

ABSTRACT

Next Generation Risk Assessment (NGRA) is a procedure that integrates new approach methodologies (NAMs) to assure safety of a product without generating data from animal testing. One of the major challenges in the application of NGRA to consumer products is how to extrapolate from the *in vitro* points of departure (PoDs) to the human exposure level associated with product use. To bridge the gap, physiologically based kinetic (PBK) modelling is routinely used to predict systemic exposure (C_{max} or *AUC*) from external exposures.

A novel framework was developed for assessing the exposure of new ingredients in dermally applied products based on the construction of PBK models describing consumer habits and practices, formulation type, and ADME (absorption, distribution, metabolism and excretion) properties exclusively obtained from NAMs. This framework aims to quantify and reduce the uncertainty in predictions and is closely related to the risk assessment process (*i.e.*, is the margin of safety sufficient to cover the uncertainties in the extrapolation between the *in vitro* and *in vivo* toxicodynamics and toxicokinetics?). Coumarin, caffeine, and sulforaphane in four product types (kitchen cleaner liquid, face cream, shampoo, and body lotion) were selected to exemplify how this framework could be used in practise. Our work shows initial levels of the framework provide a conservative estimate of C_{max} in most cases which can be refined using sensitivity analysis to inform the choice of follow-up *in vitro* experiments. These case studies show the framework can increase confidence in use of PBK predictions for safety assessment.



Moxon et al. (2020) Toxicology in Vitro 63:104746

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SimulationsPlus
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Levels 0 & 1: Description/Inputs

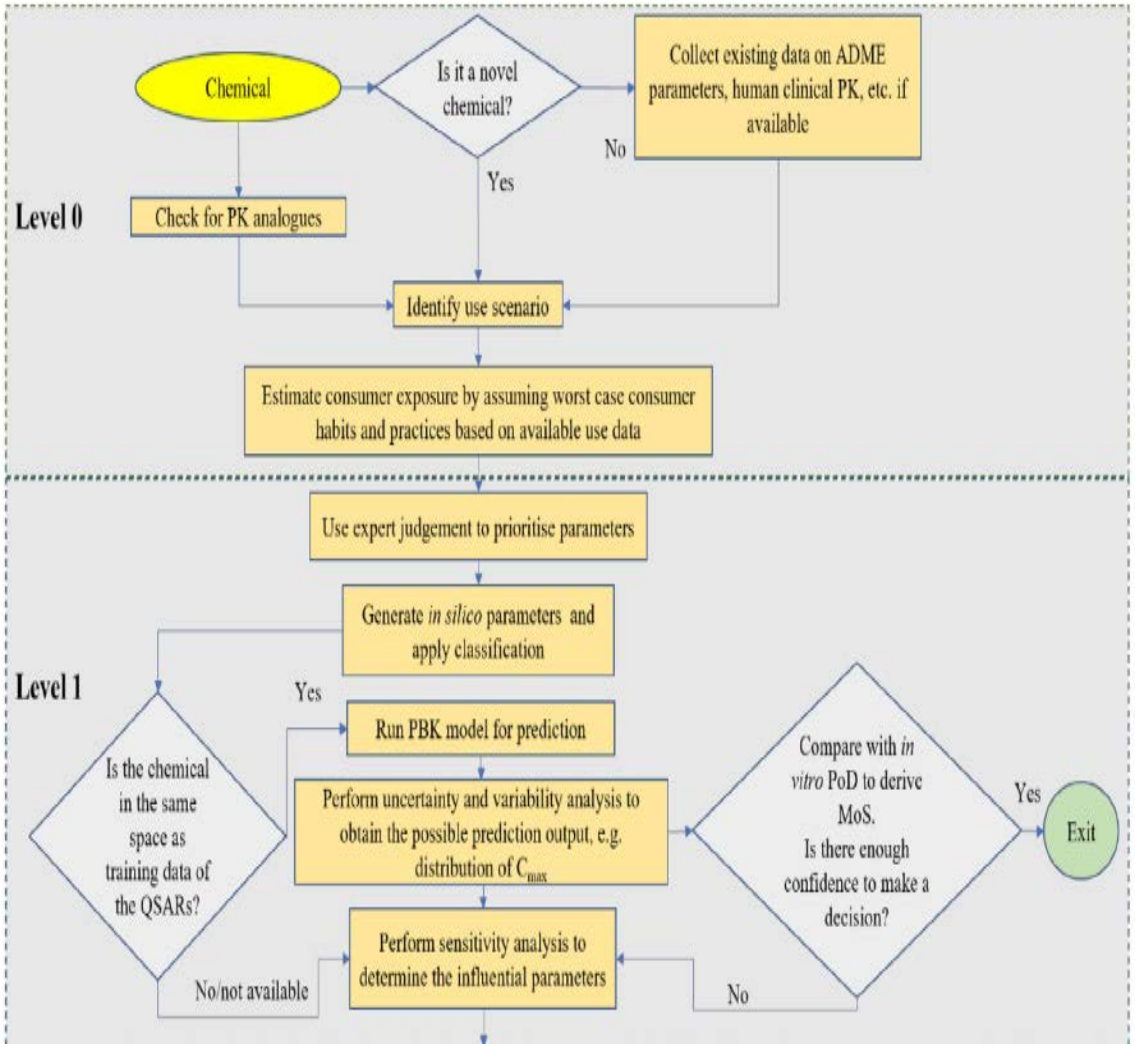


Table 1
Four product types and their typical use scenarios.

Product types	Face cream	Body lotion	Shampoo	Kitchen cleaner liquid
Amount of product used per day (g/day) using 90th percentile	1.54 (Hall et al., 2007)	7.82 (Hall et al., 2007)	10.46 (Hall et al., 2007)	4.24[a]
Frequency of use	2 times/day [b] (Bernauer et al., 2018)	2 times/day [c] (Bernauer et al., 2018)	1 time/day (Bernauer et al., 2018)	1 time/day (Johnson and Lucica, 2012)
Amount of product in contact with skin per occasion (mg)	770	3910	10,460	4240
Hypothetical Ingredient inclusion level	0.1%	0.1%	0.1%	0.1%
Application site	Face[d]	Whole body (excluding head)	Scalp	Palm of 1 hand
Skin surface area (cm^2)	565 (Bernauer et al., 2018)	15,670 [e] (Bernauer et al., 2018)	1440 (Bernauer et al., 2018)	212.25 [f]
Leave on or rinse off	Leave on	Leave on	rinse off	Leave on
Exposure duration per occasion	12 h	12 h	24 h	20 min (HERA, 2005)
For rinse off product, retention factor of finished product on skin [g]	n.a.	n.a.	0.01 (Hall et al., 2007)	n.a.
Amount of ingredient in contact with skin per occasion (mg)	0.77	3.91	0.105	4.24
Local dermal exposure per occasion ($\mu g/cm^2$)	1.36	0.25	0.073	19.98

[a] Sponge water uniformly distributed between 0.022 and 0.1331 L (Garcia-Hidalgo et al., 2017), Cleaner amount assumed to be triangular distribution with mean of 60 g per task, min of 30 g max of 110 g (HERA, 2005). From this the 90th percentile of the concentration is calculated as 2000 g/L. Film thickness on hands is 0.01 cm (HERA, 2005) (product in contact), multiplied by contact surface area (212.25 cm^2) gives 2.12 mL of product in contact with palm of hand, from which the product amount of 4.24 g in contact with the skin is calculated.
[b] rounded from 2.14 times/day (Bernauer et al., 2018).
[c] rounded from 2.28 times/day (Bernauer et al., 2018).
[d] Assuming hands are washed immediately after application and no absorption takes place from the palms.
[e] specified as Leg region in GastroPlus.
[f] Weight and heights for British women (Ruston et al., 2004) were used to calculate the whole area of the hands using equations from (Anderson et al., 1985) and divided by 4 to get the area of the palm.
[g] The retention factor was introduced by the SCCNFP to take into account rinsing off and dilution of finished products by application on wet skin or hair (e.g. shower gels, shampoos) (Hall et al., 2007).

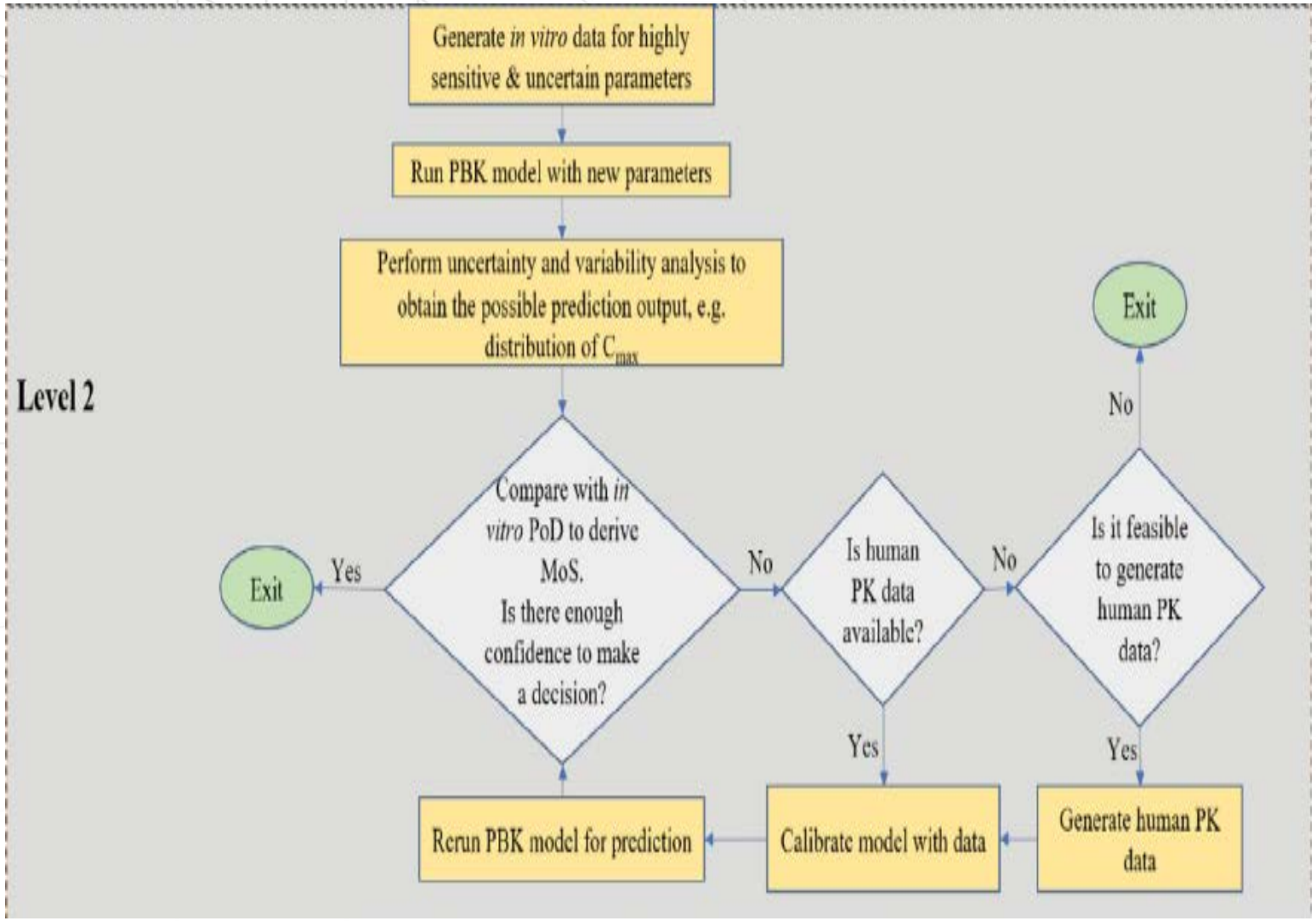
Table 2
ADME parameters (obtained from both *in silico* and *in vitro* data) for coumarin.

	In silico predictions		*In vitro* measurements	
	Value	Source	Value	Source
Molecular weight	146.1 g/mol			
log P	1.89	ADMET predictor	1.39	Measured (Hansch et al., 1995)
Water solubility	0.37 mg/mL at pH 7	ADMET predictor	0.96 mg/mL in phosphate buffer (pH 7.4)	Measured
Unbound fraction in plasma (f_{ub})	0.24	ADMET predictor	0.31	Measured
Blood: plasma ratio	1.18	ADMET predictor	0.7	Measured
Hepatic intrinsic clearance (L/h)	98.57	ADMET predictor with total HLM (converted hepatic clearance is 18.5 L/h)	1844.8	> 554 µL/min/mg protein (from half-life < 5 min) pooled Human Microsomes Measured
			929[a]	105 µL/min/million cells (half-life 13 min) in human cryopreserved hepatocytes Measured
MDCK permeability ($\times 10^{-6}$ cm/s)	93.3	ADMET Predictor		
Ionization	Neutral			
ECCS Class	Class 2 (Metabolism)			
Renal excretion	0[b]			
Stratum corneum/water partition coefficient	5.73	GastroPlus suggested default value (WKN)	8	Fitted against experimental skin penetration data
Stratum corneum diffusivity (cm^2/s)	1.33×10^{-9}	GastroPlus suggested default value (WKN)	3.0×10^{-10}	Fitted against skin pen data
Epidermis/water partition coefficient	0.7	GastroPlus suggested default value (Kretsos)	0.7	Fitted against skin pen data
Epidermis diffusivity (cm^2/s)	2.7×10^{-6}	GastroPlus suggested default value (Kretsos)	2.7×10^{-6}	Fitted against skin pen data
Dermis/water partition coefficient	0.7	GastroPlus suggested default value (Kretsos)	0.7	Fitted against skin pen data
Dermis diffusivity (cm^2/s)	2.7×10^{-6}	GastroPlus suggested default value (Kretsos)	2.7×10^{-6}	Fitted against skin pen data

[a] *in vitro* intrinsic clearance value used for making PBK predictions in Level 2.
[b] Based on the ECCS (extended clearance classification system) coumarin is predicted to be cleared mainly by metabolism and so renal clearance was assumed to be insignificant and therefore renal clearance rate was set to zero (Varma et al., 2015).

Moxon et al. (2020) Toxicology in Vitro 63:104746

Level 2: IVIVE



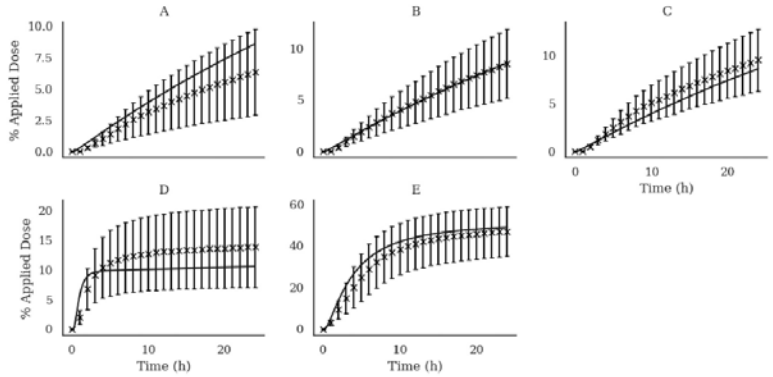


Fig. 4. Plot of percentage of Coumarin dose absorbed through dermatomed skin, with simulated results (solid line) and experimental results (x with standard deviation). Five different vehicles are plotted: A) ethanol:DEP with 20% coumarin, occluded (24 h exposure) B) ethanol:DEP with 7.5% coumarin, occluded (24 h exposure), C) ethanol:DEP with 1% coumarin, occluded (24 h exposure), D) laundry hand wash formulation, 1% non-occluded (0.5 h exposure), and E) standard lotion formulation, 1% non-occluded (24 h exposure).

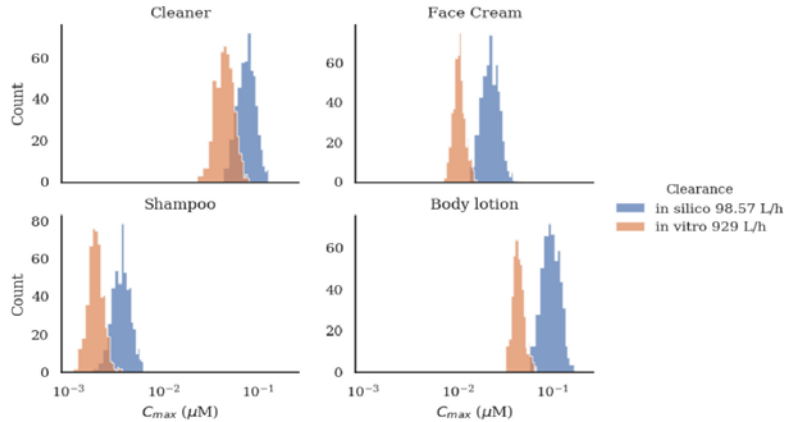


Fig. 5. Distribution of C_{max} values after performing Monte Carlo simulation on 4 different formulation of 0.1% coumarin. The different colour distributions represent use of different clearance values as the mean of the prior distribution.

Moxon et al. (2020) Toxicology in Vitro 63:104746

Common Theme of Recent Work…

- Simulations Plus continues to lead in the areas of ML/PBPK modeling for oral and non-oral delivery routes to support regulatory submissions and alternatives to animal testing

Physiologically Based Pharmacokinetic Analyses — Format and Content

Guidance for Industry

The Use of Physiologically Based Pharmacokinetic Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls

Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact Paul Seo at 301-796-4874.

Evaluation of Gastric pH-Dependent Drug Interactions With Acid-Reducing Agents: Study Design, Data Analysis, and Clinical Implications

Guidance for Industry

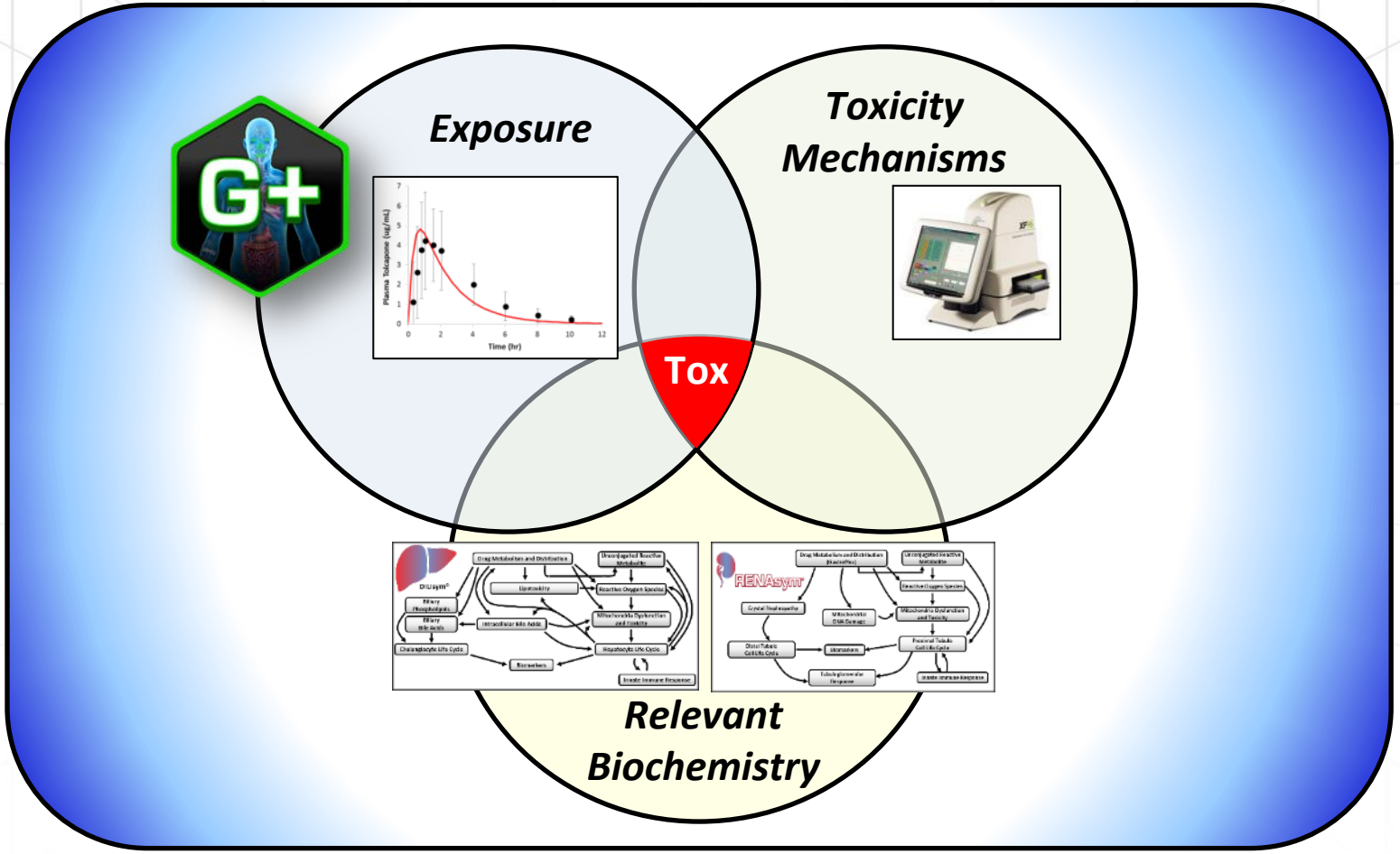
DRAFT GUIDANCE

https://www.fda.gov/media/144026/download

Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes

Series on Testing and Assessment
No. 331

QST Predicts Toxicity via the Intersection Between Exposure, Mechanisms, and Inter-Patient Variability



The DILI-sim and RENAsym Consortia are Partnerships Between DILIsym Services and Pharmaceutical Companies to Minimize Organ Injury

Excellent Scientific Advisory Boards

Current DILI-sim / RENAsym Members

For a comprehensive review of progress, see *Watkins 2020, Current Opinion in Toxicology (23-24:67-73)*

- *Overall Goals*
  - *Improve patient safety*
  - *Reduce the need for animal testing*
  - *Reduce the costs and time necessary to develop new drugs*
- *History*
  - *Officially started in 2011*
  - *21 major pharmaceutical companies have participated*
  - *Members have provided compounds, data, and conducted experiments to support effort*
  - *Over $10 million invested in project*
- ***At least 30 cases of use for regulatory purposes***
- ***Over 30 publications***

DILIsym Services QST Software Aids Decisions

- Predicts drug-induced liver disease
- v8A released Q1 2019
- Includes mechanistic representation of normal hepatic biochemistry
- Evaluated >80 compounds with 40+ companies

So how can DILIsym help my organization?

- Predict DILI liabilities beforehand and save **$$$**
- Choose the lead candidate ***most likely to succeed*** from a DILI standpoint
- Communicate with regulators on safety issues with information they have requested from others numerous times and from a platform they license (FDA)
- ***Keep patients safer….***

DILIsym Utilizes Various Data Types to Inform Decisions

DMPK and Exposure Data

PBPK Modeling

- **Compound Properties**
  - Tissue partition coefficients
- **Tissue penetration studies**
  - *Liver to blood ratio*
- **Pharmacokinetic data**
  - *Absorption, extra-hepatic clearance, metabolites*
- ***in vitro* data**
  - *Metabolite synthesis, active uptake*

In vitro Mechanistic DILI Data

Data Collected for Quantitative DILI Mechanism Info

- **Oxidative stress** *(high content imaging)*
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity** *(XF Analyzer)*
  - *ETC inhibition*
  - *Uncoupling*
- **Bile acid / phospholipid transporter inhibition**
  - *BSEP, MRP3 and 4, NTCP, MDR3*
- **Bilirubin transport/metabolism**
  - *OATP1B1, OATP1B3, UGT1A1, MRP2, MRP3*

Modeling & Simulation

Simulations and Assays inform:

- **Prediction of DILI risk**
- **Participating DILI mechanisms**
- **Characteristics of patients at risk for DILI**
- **Drug dosing paradigms**
- **DILI monitoring strategies**

Clinical Data / Information

- **Dosing Protocols, fasting/fed state, meal times**
- **Patient types (NHV, disease, etc.)**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**

Relevant Recent DILIsym News / Publications

U.S. FDA Renews Annual DILIsym Software Licenses

FDA Maintains Access to Leading Liver Injury Software Program

May 06, 2020 08:30 AM Eastern Daylight Time

RESEARCH TRIANGLE PARK, N.C.--(BUSINESS WIRE)--DILIsym Services, Inc., a Simulations Plus company (Nasdaq: SLP) and a leading provider of simulation and modeling software for pharmaceutical safety and efficacy, today announced that the U.S. Food and

First Approved Cancer Treatment for TGCT Included DILIsym Simulations in FDA Review

FDA Review Cites DILIsym Results as Part of Turalio® Submission

October 27, 2020 08:30 AM Eastern Daylight Time

RESEARCH TRIANGLE PARK, N.C.--(BUSINESS WIRE)--DILIsym Services, Inc., a Simulations Plus company (Nasdaq: SLP) and a leading provider of modeling and simulation software for pharmaceutical safety and efficacy, today announced that simulations using their DILIsym® software were noted in a U.S. Food and Drug Administration (FDA) review of the New Drug

Application of the DILIsym® Quantitative Systems Toxicology drug-induced liver injury model to evaluate the carcinogenic hazard potential of acetaminophen

Gary Eichenbaum[a,*], Kyunghee Yang[b], Yeshitila Gebremichael[b], Brett A. Howell[b], F. Jay Murray[c], David Jacobson-Kram[d], Hartmut Jaeschke[e], Edwin Kuffner[a], Cathy K. Gelotte[f], John C.K. La

[a] Johnson & Johnson
[b] DILIsym Services In
[c] Murray & Associate

Pharm Res (2020) 37:24
https://doi.org/10.1007/s11095-019-2726-0

RESEARCH PAPER

Comparison of the Hepatotoxic Potential of Two Treatments for Autosomal-Dominant Polycystic Kidney Disease Using Quantitative Systems Toxicology Modeling

J. L. Woodhead[1] • L. Pellegrini[2] • L. K. M. Shoda[1] • B. A. Howell[1]

Current Opinion in Toxicology

ELSEVIER

Clinical Pharmacology & Therapeutics

Article

Quantitative Systems Toxicology Modeling Predicts that Reduced Biliary Efflux Contributes to Tolvaptan Hepatotoxicity

James J. Beaudoin, William J. Brock, Paul B. Watkins, Kim L. R. Brouwer

First published: 03 August 2020 | https://doi.org/10.1002/cpt.2007

Research Article

DILIsym: Quantitative systems toxicology impacting drug development

Paul B. Watkins

CrossMark

OXFORD

SO

academic.oup.com/toxsci

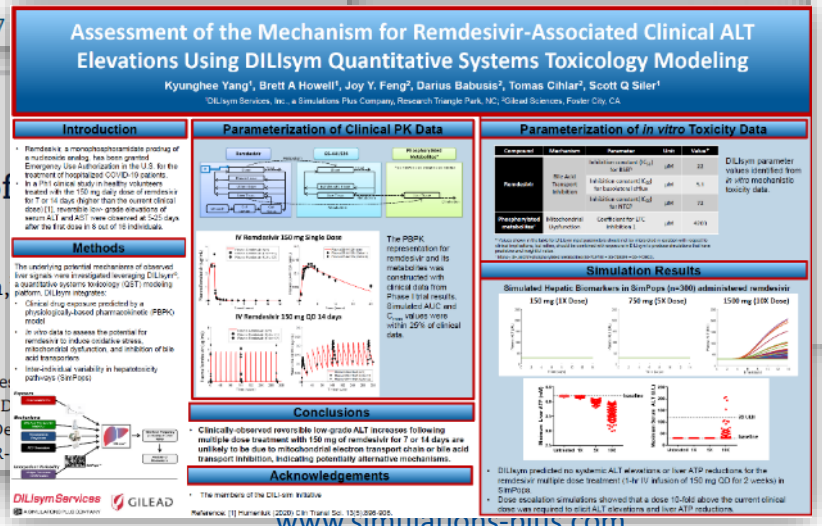
Analyzing the Mechanisms Behind Macrolide Antibiotic-Induced Liver Injury Using Quantitative Systems Toxicology Modeling

Jeffrey L. Woodhead[1] • Kyunghee Yang[1] • David Oldach[2] • Chris MacLauchlin[2] • Prabhavathi Fernandes[2] • Paul B. Watkins[3] • Scott Q. Siler[1] • Brett A. Howell[1]

Assessment of the Mechanism for Remdesivir-Associated Clinical ALT Elevations Using DILIsym Quantitative Systems Toxicology Modeling

Kyunghee Yang[1], Brett A Howell[1], Joy Y. Feng[2], Darius Babusis[2], Tomas Cihlar[2], Scott Q Siler[1]

[1]DILIsym Services, Inc., a Simulations Plus Company, Research Triangle Park, NC; [2]Gilead Sciences, Foster City, CA



Mechanistic Investigations Support Liver Safety of Ubrogepant

Brenda Smith,* Josh Rowe,*,[1] Paul B. Watkins,[†] Messoud Ashina, Jeffrey L. Woodhead,[§] Frank D. Sistare,[¶] and Peter J. Goadsby[‖]

*Allergan plc, Irvine, California; [†]Eshelman School of Pharmacy and Institute for Drug Safety Science University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [‡]Department of Neurology, D Headache Center, Faculty of Health and Medical Sciences, University of Copenhagen, København, De [§]DILIsym Services, Durham, North Carolina; [¶]Merck & Co., Inc., West Point, Pennsylvania and [‖]NIHR-

Quantitative systems toxicology (QST) reproduces species differences in PF-04895162 liver safety due to combined mitochondrial and bile acid toxicity

Grant Generaux[1] | Vinal V. Lakhani[1] | Yuching Yang[1] | Sashi Nadanaciva[2] | Luping Qiu[3] | Keith Riccardi[4] | Li Di[4] | Brett A. Howell[1] | Scott Q. Siler[1] | Paul B. Watkins[5,6] | Hugh A. Barton[7] | Michael D. Aleo[3] | Lisl K. M. Shoda[1]

[1]DILIsym Services Inc., Research Triangle Park, North Carolina
[2]Compound Safety Prediction, Worldwide Medicinal Chemistry, Pfizer Inc., Groton, Connecticut
[3]Investigative Toxicology, Drug Safety Research and Development, Pfizer Inc., Groton, Connecticut

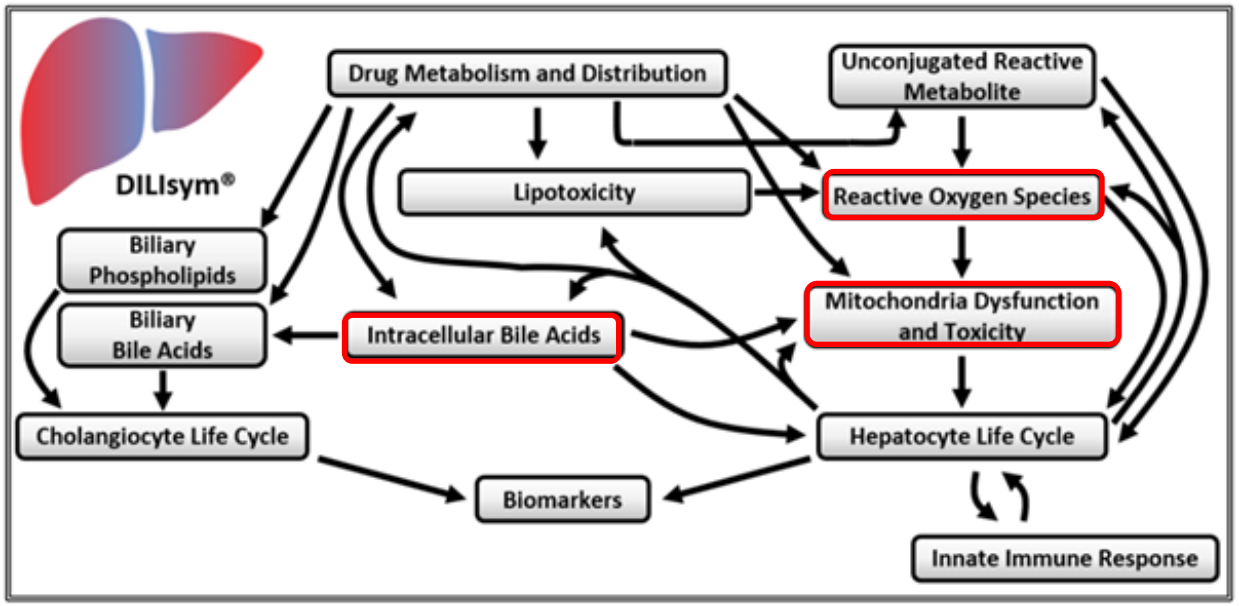
Backup versus Lead Application: Internal Decision Making Support

- Company Z observed liver enzyme elevations in an early clinical trial, raising serious doubts about the development potential of Compound Z1
  1. DILIsym was first utilized to test Compound Z1 for validation that predictions would match the clinical data to a reasonable degree
  2. Next, DILIsym was applied to a backup candidate, Compound Z2, to compare to Z1

Experimental Data Indicate that Comp Z1 and Comp Z1M1 Elicit *In Vitro* Signals for Various Liver Toxicity Mechanisms

Comp Z1

- DILIsym represents 3 distinct mechanisms of toxicity
- Comp Z1 and Comp Z1M1 experimental data were gathered to evaluate effects related to all 3 mechanisms
- Mild bile acid transporter signals observed for Comp Z1 and Comp Z1M1
- Mild mitochondrial dysfunction signal observed for Comp Z1
- Oxidative stress signal observed for Comp Z1

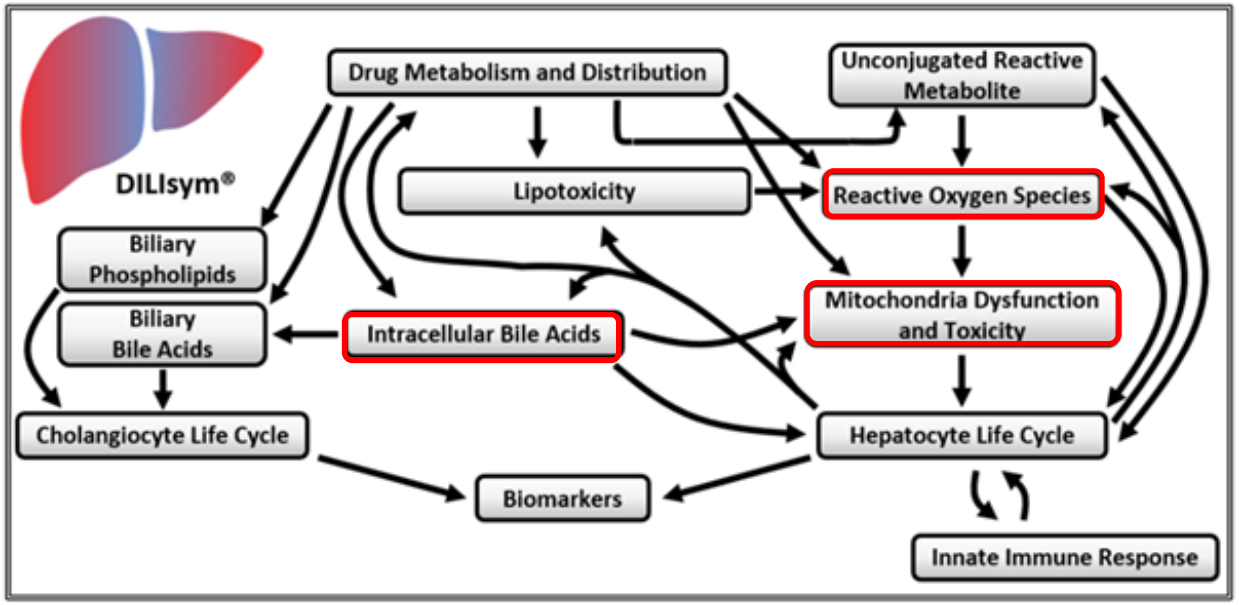


Compounds	BA Transport signals	Mitochondrial dysfunction signals	Oxidative stress signals
Comp Z1	Yes	Yes	Yes
Comp Z1M1	Yes	No	No

Experimental Data Indicate that Comp Z2, Comp Z2M1, and Comp Z2M2 Elicit *In Vitro* Signals for Various Liver Toxicity Mechanisms

Comp Z2

- DILIsym represents 3 distinct mechanisms of toxicity
- Comp Z2, Comp Z2M1 and Comp Z2M2 experimental data were gathered to evaluate effects related to all 3 mechanisms
- Mild bile acid transporter signals observed for Comp Z2, Comp Z2M1 and Comp Z2M2
- Mild mitochondrial dysfunction signal observed for Comp Z2, Comp Z2M1 and Comp Z2M2
- Oxidative stress signal observed for Comp Z2



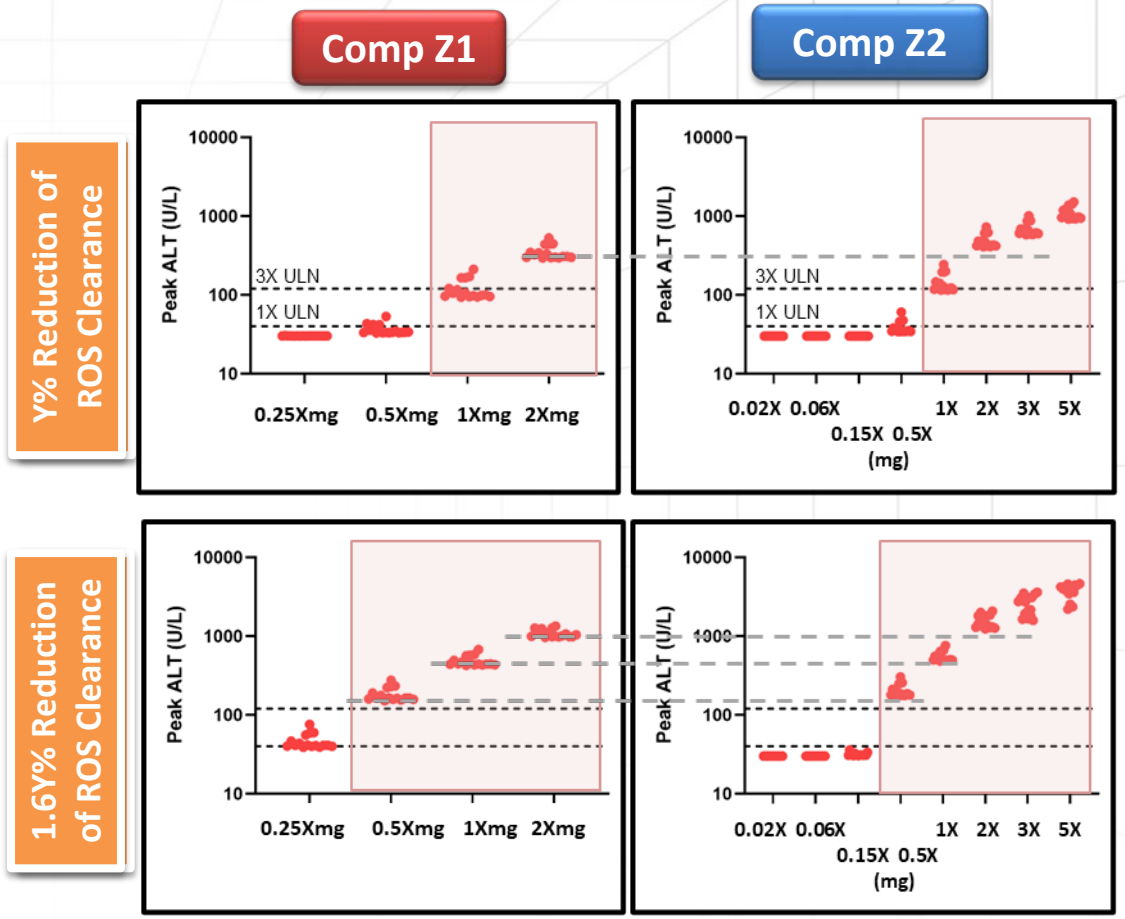
Compounds	BA Transport signals	Mitochondrial dysfunction signals	Oxidative stress signals
Comp Z2	Yes	Yes	Yes*
Comp Z2M1	Yes	Yes	No
Comp Z2M2	Yes	Yes	No

*Oxidative stress observed in HepaRG spheroids, but not in HepG2 cells, suggesting that oxidative stress is attributed to unknown metabolite(s)

Comparison of Dose-ALT Response in Exploratory Simulations with Reduced ROS Clearance

- Exploratory simulations with reduced ROS clearance **predicted greater ALT increases for Comp Z2 compared to Comp Z1 at similar doses**

- Combined with clinical ALT elevations observed for Comp Z1, this is concerning for Comp Z2



Comps Z1/Z2 Backup versus Lead Application: Internal Decision Making Support

- Company Z observed liver enzyme elevations in an early clinical trial, raising serious doubts about the development potential of Compound Z1
- Company Z incorporated DILIsym into their key internal presentations and communications as part of their decision-making process for Compounds Z1 and Z2
  - ***DILIsym generally recapitulated the clinical liver safety signals seen with Compound Z1***
  - ***DILIsym predicted as much or more liver injury with Compound Z2***
  - ***However, Compound Z2 may be much more potent and thus require a much lower dose for efficacy (maybe dose could be lowered) - TBD***

Lixivaptan DILIsym Project

DILI Background

- An approved compound in the same class had no DILI signals in hyponatremia, but signals were observed in ADPKD patients
- Lixivaptan had no DILI signals in hyponatremia

Question

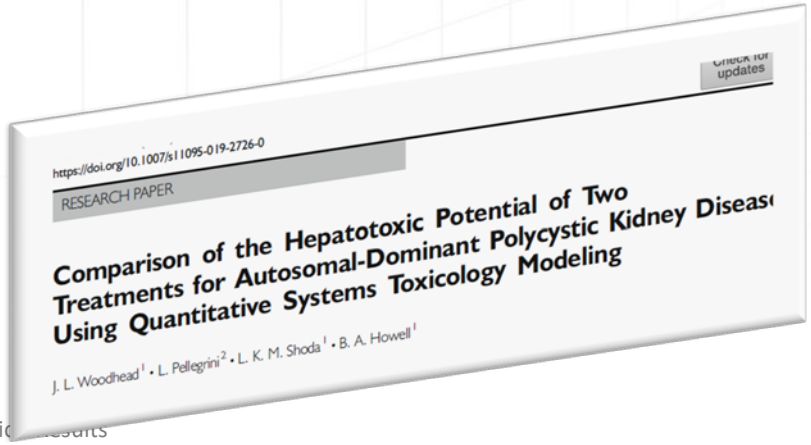
- Will lixivaptan experience similar DILI liability as the competitor in ADPKD patients?

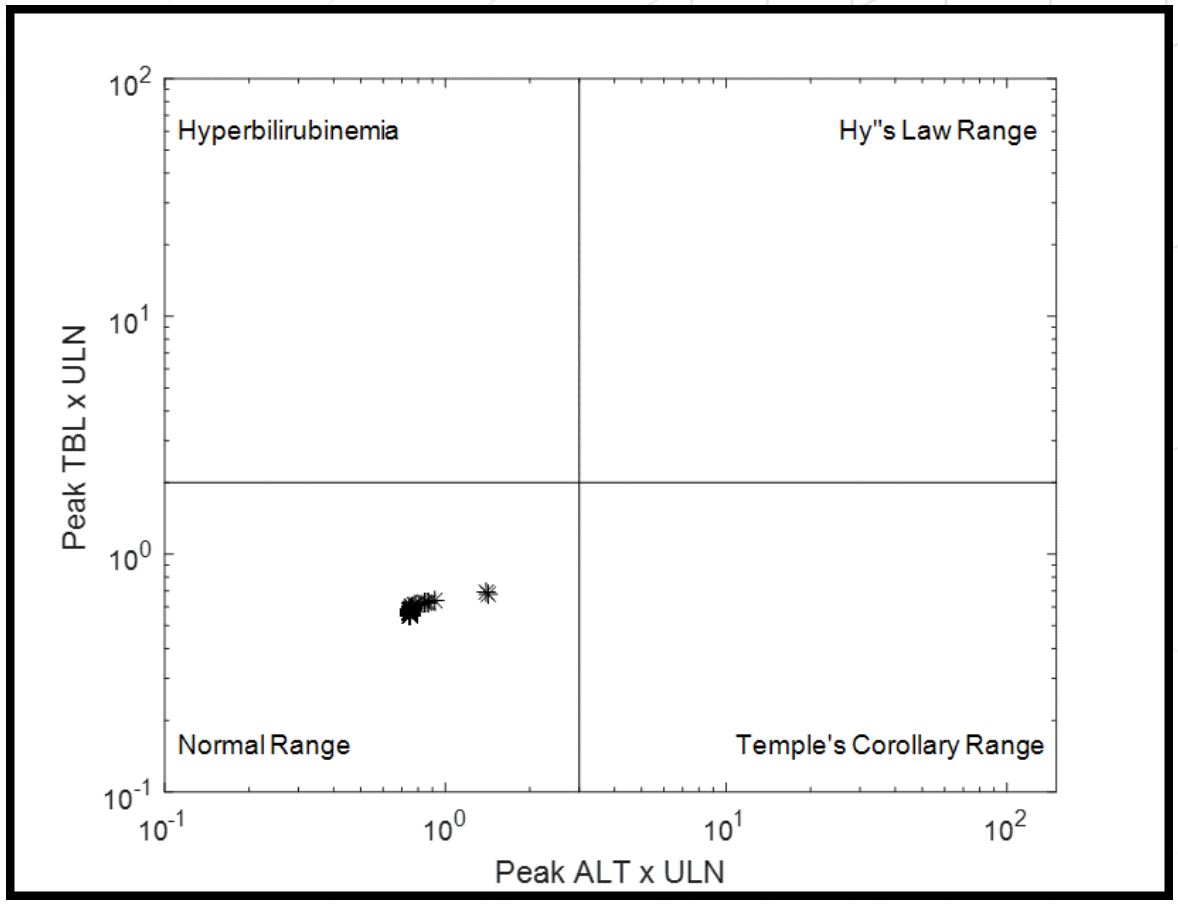
Approach

- Develop a mechanistic representation of lixivaptan in DILIsym, a QST model of drug-induced liver injury (DILI), to assess the potential for liver toxicity with the intended dosing for lixivaptan

Lixivaptan Simulations Predicted Minimal ALT Elevations at 200/100 mg BID

- Lixivaptan simulated in SimPops of N = 285
- No ALT elevations simulated in 100 mg BID 60-day simulation
  - Consistent with observed clinical similarity to placebo (validation)
- **No ALT elevations simulated in 200/100 split daily dosing scenario for 12 weeks**
  - **Maximum intended clinical dosing for ADPKD**





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Palladio Biosciences Receives FDA IND Clearance to Begin the ELiSA Study, a Phase 2 Clinical Trial with Lixivaptan in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)

May 8, 2018

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Palladio Biosciences Website

Key development milestones achieved to date include:

DILIsym Liver Safety Evaluation of Lixivaptan

Prior to administering lixivaptan to ADPKD patients, Palladio studied lixivaptan's liver safety profile in DILIsym, a state-of-the art, predictive, quantitative systems toxicology modeling tool, and compared it to the liver safety profile of tolvaptan. Full details of this study have been published in a peer-reviewed journal. Briefly, results suggest that lixivaptan may not cause liver transaminase elevations, an indication of liver toxicity, and therefore it may have a favorable liver safety profile.

Orphan Drug Designation

The U.S. FDA, through its Office of Orphan Drug Products, designated lixivaptan as an orphan drug for treating ADPKD. This designation provides eligibility for certain benefits and confers seven years of market exclusivity following receipt of regulatory approval.

irst study of lixivaptan in ADPKD

the vasopressin antagonist,
other chemistry tests over a year of

assess liver safety of lixivaptan

Centessa Pharmaceuticals Initiates Global Phase 3 ACTION Study of Lixivaptan in Autosomal Dominant Polycystic Kidney Disease, Reports Initial Positive Safety Data from ALERT Study, and Announces Notice of Allowance for Key Lixivaptan U.S. Patent Application

December 14, 2021 | PDF Version Go Back

~ Initiation of registrational Phase 3 ACTION clinical study with lixivaptan is an important milestone to bring this potential new treatment option to ADPKD patients ~

~ All four subjects in the ALERT Study who previously discontinued JYNARQUE® due to liver toxicity successfully titrated to maintenance dose of lixivaptan; no subjects met pre-specified stopping criteria; no cases of suspected drug-induced liver injury (DILI) ~

~ Issuance of new patent would cover use of lixivaptan in ADPKD through at least 2038 ~

BOSTON and LONDON, Dec. 14, 2021 (GLOBE NEWSWIRE) -- Centessa Pharmaceuticals plc ("Company") (Nasdaq: CNTA), together with subsidiary Palladio Biosciences, Inc. ("Palladio"), today announced the initiation of active recruitment of the global ACTION Study, a pivotal Phase 3 clinical trial evaluating lixivaptan as a potential treatment for Autosomal Dominant Polycystic Kidney Disease (ADPKD). Additionally, the Company reported initial safety data from four subjects who participated in the ongoing open-label ALERT Study of ADPKD subjects who previously discontinued JYNARQUE® (tolvaptan) due to liver toxicity and announced the Notice of Allowance for a U.S. Patent application covering use of lixivaptan in ADPKD.

Advancing Calcitonin Gene-Related Peptide Receptor Antagonists Using Quantitative Systems Toxicology Modeling to Characterize Next-in-Class Compounds Compared to the Hepatotoxic First in Class Telcagepant

Woodhead, Jeffrey L. (1); Siler, Scott Q. (1); Howell, Brett A. (1); Conway, Charles M. (3); Watkins, Paul B (2)

1. DILIsym Services, Inc., a Simulations Plus company, Research Triangle Park, NC, USA; 2. Institute for Drug Safety Sciences, UNC-Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA; 3. Biohaven Pharmaceuticals, Inc., New Haven, CT, USA

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INTRODUCTION

While CGRP receptor antagonists have demonstrated efficacy in the acute and preventive treatment of migraine, two early CGRP signal-blocking compounds (gepants) showed liver injury signals in clinical trials. During clinical development of next-in-class gepants, confidence in compound safety was needed given the prior experience.

AIM

Biohaven enlisted DILIsym Services, Inc. (DSSI) to use DILIsym to independently assess the potential for liver toxicity to compare four next-in-class gepant compounds in clinical development to the hepatotoxic agent telcagepant.

MATERIAL & METHODS

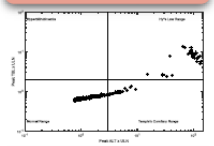
Models for telcagepant and four novel CGRP receptor antagonists (rimegepant, zavegepant, ubrogepant, and atogepant) were constructed in DILIsym v6A, a quantitative systems toxicology (QST) model of drug-induced liver injury. *In vitro* experiments were performed to measure the potential for each compound to inhibit bile acid transporters, produce oxidative stress, and cause mitochondrial dysfunction; physiologically-based pharmacokinetic (PBPK) models were produced for each compound to estimate liver exposure. Compounds were simulated at and above respective clinical dose regimens.

RESULTS

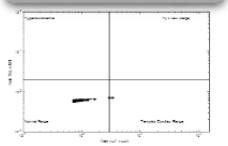
Telcagepant showed liver safety signals including: a) dose-dependent decrease in oxygen consumption rate (OCR) consistent with electron transport chain (ETC) inhibition, b) noncompetitive BSEP inhibition and c) liver exposure accumulation greater than in plasma resulting in an eDISH profile falling into Hy's Law range (see plots). Model-based elimination to identify the impact of contributors suggested synergy between bile acid accumulation and ETC inhibition as contributing to telcagepant toxicity. None of the other 4 novel gepants showed eDISH signals in Hy's Law range (see plots) and none showed simulated signals >1% frequency for ALT > 3X upper limit of normal (ULN) at clinical doses (see table). When clinical doses were exceeded only atogepant and ubrogepant showed simulated signals ≥10% frequency for ALT > 3X ULN. Simulations predicted rimegepant, zavegepant, atogepant, and ubrogepant would be safe at clinical doses.

RESULTS (cont'd)

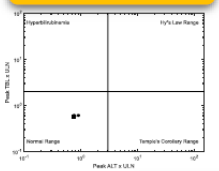
Telcagepant; 140 mg BID, 12 weeks, high ETCi



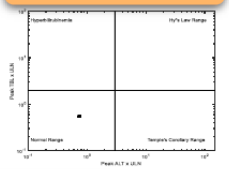
Rimegepant; 75 mg QD, alternate day dosing, 14 total doses over 28 days



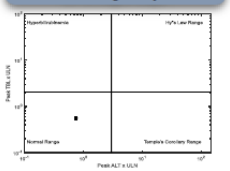
Atogepant; 60 mg BID, 12 weeks



Ubrogepant; 100 mg QD, 25 straight days



Zavegepant; 20 mg IN or 750 mg PO or 7.5 mg IV, 25 straight days



Compound	Oral Dosing Protocol	Simulated* ALT > 3X ULN	Observed ALT > 3X ULN in Clinic
Telcagepant – Original ETC	140 mg BID, 12 weeks	17.5% (50/285)	1.9% (5/263)
	280 mg BID, 12 weeks	76.1% (217/285)	3.2% (8/265)
Telcagepant – Alternate ETC	140 mg BID, 12 weeks	0.0% (0/285)	1.9% (5/263)
	280 mg BID, 12 weeks	7.72% (22/285)	3.2% (8/265)
Rimegepant	75 mg QD, alternate day dosing, 14 total doses	0.35% (1/285)	--
	75 mg QD, 5 days on, 1 day off, 25 total doses	0.7% (2/285)	--
	75 mg QD, daily dosing for 25 days, 25 total doses	1% (3/285)	--
Zavegepant	750 mg oral QD, 25 days, 25 total doses	0.0% (0/285)	
	7.5 mg IV QD, 25 days, 25 total doses	0.0% (0/285)	
Atogepant	60 mg BID, 12 weeks	0% (0/285)	
	300 mg BID, 12 weeks	0.3% (1/285)	
	600 mg BID, 12 weeks	10.2% (29/285)	
Ubrogepant	100 mg QD, 25 days	0% (0/285)	
	500 mg QD, 25 days	1.4% (4/285)	
	1000 mg QD, 25 days	11.6% (33/285)	

*In vitro experiments led to the parameterization of each of the five CGRP compounds within DILIsym, with an alternate parameterization for telcagepant based on uncertainty in the *in vitro* data.

CONCLUSION

DILIsym correctly predicted the DILI liability of the first generation compound telcagepant. The four next-in-class compounds did not show the same signal for liver safety concerns as telcagepant. Subsequent clinical trials have validated these results, with rimegepant, ubrogepant and atogepant all approved by the FDA with no black-box warning for hepatotoxicity. Zavegepant continues in late-stage development. This work demonstrates the potential for QST modeling to prospectively differentiate between hepatotoxic and non-hepatotoxic molecules within the same class.

ACKNOWLEDGEMENTS

The DILI-sim Initiative, a partnership between pharmaceutical companies and DILIsym Services, Inc., has funded the development of DILIsym.

REFERENCES

1. Ho TW, Ho AP, Ge YJ, Assaid C, Gottwald R, MacGregor EA, et al. Randomized controlled trial of the CGRP receptor antagonist telcagepant for prevention of headache in women with perimenstrual migraine. Cephalalgia Int J Headache. 2016 Feb;36(2):148–61.

DISCLOSURES

Drs. Woodhead, Siler, and Howell are employees of DILIsym Services, Inc., developers of DILIsym. Dr. Conway is employed by Biohaven, developers of rimegepant and zavegepant.

CONTACT INFORMATION

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Independent Ubrogepant Project Reached Same Conslusions

DILIsym modeling was part of the weight of evidence that *supported FDA approval* of Ubrogepant for the treatment of acute migraine headaches.

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

doi: 10.1093/toxsci/kfaa093
Advance Access Publication Date: 24 June 2020
Research Article

Mechanistic Investigations Support Liver Safety of Ubrogepant

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Allergan plc, Irvine, California; †Eshelman School of Pharmacy and Institute for Drug Safety Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; ‡Department of Neurology, Danish Headache Center, Faculty of Health and Medical Sciences, University of Copenhagen, København, Denmark

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

In the investigative hepatotoxicity assays using HepG2 (human hepatocellular carcinoma) cells and HepaRG spheroids (a metabolically active system) and a proprietary in silico analysis system, the effects of ubrogepant were compared to those of two other CGRP receptor antagonists, for which development was discontinued because of hepatotoxicity. The results indicated that ubrogepant inhibited bile acid transporters, inhibited HepG2 oxygen consumption rate in a concentration-dependent manner (suggesting the potential to induce mitochondrial toxicity), and exhibited "a modest induction of oxidative stress in HEPG2 cells," considered an effect of ubrogepant itself rather than metabolite(s). Based on "Eight different clinical protocols of ubrogepant…investigated in SimPops," the sponsor concluded that …despite in vitro results, no ALT elevations were predicted for any of the protocols tested…indicating that ubrogepant would be safe at doses up to 10-fold higher than the clinical dose in the hepatic safety clinical study (dosing 100 mg 2 days on, 2 days off for 56 days, 28 total doses)." The maximum recommended clinical dose for the proposed indication (acute migraine) is 200 mg/day, suggesting a 5-fold safety margin with a similar dosing regimen.

QUANTITATIVE SYSTEMS TOXICOLOGY (QST) TO INVESTIGATE MECHANISMS CONTRIBUTING TO CLINICAL BILIRUBIN ELEVATIONS

Christina Battista, Brett A Howell, Lisl KM Shoda

DILIsym Services, Inc., a Simulations Plus company, Research Triangle Park, NC

Some patients given Drug X experienced concomitant ALT and bilirubin elevations. Key questions addressed in this project include: Are the bilirubin elevations observed following Drug X administration due to severe hepatotoxicity? Can QST modeling (i.e., DILIsym software) address this question? Can QST modeling provide a mechanistic explanation that would otherwise explain the observed bilirubin elevations?

ABSTRACT

BACKGROUND: Some patients treated with Drug X experienced elevations in serum bilirubin with concomitant ALT elevations, potentially indicative of severe liver injury. However, Drug X directly alters bilirubin transporters and enzymes, potentially leading to bilirubin elevations absent liver injury. Distinguishing between these two possibilities is critical to inform drug development decisions. DILIsym®, a QST platform of drug induced liver injury (DILI), was used to investigate the interpretation of putative Drug X-related elevations in liver biomarkers.

METHODS: The initial investigation estimated hepatocyte loss by approximating the clinical ALT profiles through imposed hepatocyte death[1], then checked for concomitant bilirubin elevations[2]. Then, the potential for Drug X mediated altered bilirubin disposition to account for observed bilirubin elevations was investigated[3]. Simulations combined Drug X exposure predictions from a PBPK model with mechanistic bilirubin inhibition parameters derived from the *in vitro* assays in a simulated population (SimPops®).

RESULTS: Simulated hepatocyte loss that resulted in ALT profiles mimicking clinical data were not sufficient to yield clinically significant bilirubin elevations, suggesting ALT and bilirubin elevations were decoupled and thus did not reflect severe liver injury.

Simulation results combining Drug X exposure and the mechanistic interaction of Drug X with bilirubin transporters and enzymes were consistent with timing, but underestimated magnitude, of clinical bilirubin elevations, suggesting that altered bilirubin disposition had the potential to cause clinically observed bilirubin elevations but a mechanism might be missing. Inclusion of newer data on MRP2 expression allowed simulations to account for observed serum bilirubin elevations.

CONCLUSIONS: DILIsym investigations suggested that observed bilirubin elevations did not reflect serious liver injury and might be a result of altered bilirubin disposition..

INTRODUCTION

- DILIsym software applies a quantitative systems toxicology (QST) approach to investigate dose-dependent DILI by integrating *in vitro* mechanistic toxicity data, *in vivo* predictions of dynamic drug exposure, known biochemistry, and intra-patient variability to predict hepatotoxic risk for novel therapeutics.
- Transaminase and bilirubin elevations were observed in multiple patients treated with Drug X. DILIsym was used to (a) investigate whether simultaneous elevations in ALT >3x ULN and bilirubin >2x ULN were consistent with severe liver injury as defined in Hy's Law cases, and (b) provide *in vivo* context by which mechanisms for altered bilirubin disposition might account for clinical observations.

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2. Longo, Diane M., et al. Refining liver safety risk assessment: application of mechanistic modeling and serum biomarkers to cimaglermin alfa (GGF2) clinical trials. Clin. Pharmacol. Ther. 102.6, 961-969 (2017).
3. Yang, K. *et al.* Systems pharmacology modeling of drug-induced hyperbilirubinemia: Differentiating hepatotoxicity and inhibition of enzymes/ transporters. *Clin. Pharmacol. Ther.* 101, 501–509 (2017).

ACKNOWLEDGEMENTS

We gratefully acknowledge the sponsor for their willingness to share the results of these analyses and the DILI-sim Initiative for their ongoing support of DILIsym software development.

DISCLOSURES

CB, BH, and LS are employees of DILIsym Services, Inc.

METHODS

Estimating hepatocyte loss based on clinical ALT profiles

- Apply hepatocyte death via direct apoptosis (no specific mechanism) to replicate observed ALT profiles from two clinical patients as previously described[1] (Figure 1a)
- Compare resulting simulated bilirubin profiles to clinical bilirubin elevations

Figure 1. Simulations increase hepatocyte apoptotic flux leading to ALT release from dying hepatocytes , and simulated ALT elevation.

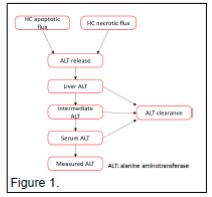

Figure 1.

Mechanistic simulations of Drug X

- Physiologically-based pharmacokinetic (PBPK) model for Drug X: Drug X liver exposure predicted in GastroPlus® using available *in vivo* and *in vitro* pharmacokinetic data
- Mechanisms of altered bilirubin disposition: Simulations apply IC_{50} values from *in vitro* assays to describe interaction of Drug X with bilirubin transporters and enzymes (Figure 2)
- Bilirubin SimPops®: Simulations conducted in N=285 individuals with variability in bilirubin biochemistry
- Software customization: DILIsym equations modified to include Drug X mediated inhibition of MRP2 expression based on newer data; creation of new SimCohorts of N=16 individuals most sensitive to altered bilirubin disposition based on the SimPops results

Figure 2. Simulated Drug X modulates bilirubin transporters and enzymes (*in grey italics*) according to the IC_{50} values determined from *in vitro* data. Simulations provide *in vivo* context for altered bilirubin disposition based on direct drug effects.

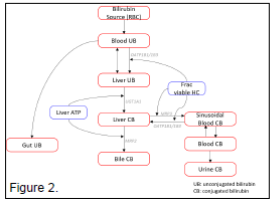

Figure 2.

RESULTS

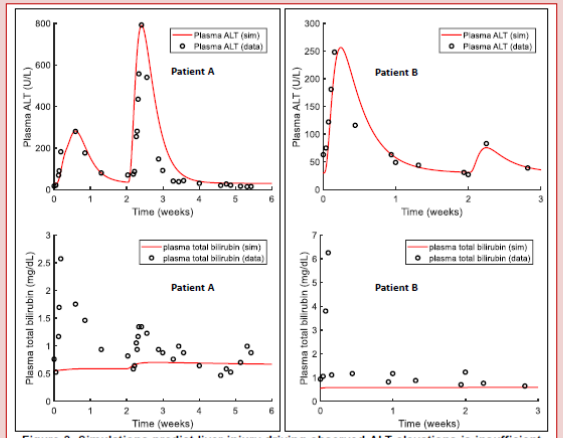

Figure 3. Simulations predict liver injury driving observed ALT elevations is insufficient to account for observed bilirubin elevations. Hepatocyte apoptosis was optimized to result in simulated ALT profiles that align with data from Patient A (top left) and Patient B (top right). The same simulation results were then used to compare how hepatocyte death (indicated by ALT elevations) impacted bilirubin elevations. Simulations that reproduce ALT elevations failed to yield clinically significant bilirubin elevations, suggesting that clinically-observed bilirubin elevations are not a result of severe liver injury.

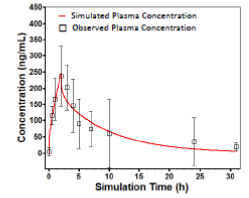

Figure 4. Simulated plasma profile following single dose of Drug X in the baseline human. Simulated plasma profile (red) was optimized to observed plasma profiles (black boxes). Training and validation data sets were evaluated.

Drug X *In vitro* IC_{50} values		
Bilirubin Transporter/Enzyme	Units	DILIsym parameter value
OATP1B1 IC_{50}	µM	1.4*
OATP1B3 IC_{50}	µM	18.9
MRP2 IC_{50}	µM	314
MRP3 IC_{50}	µM	39.85
UGT1A1 IC_{50}	µM	15.3

*Used for OATP inhibition constant in DILIsym (a conservative approach)

Table 1. *In vitro* assessment of Drug X on bilirubin transporters and enzymes. Experimental data characterizing Drug X inhibition of bilirubin transporters and enzymes were directly translated as IC_{50} values within DILIsym.

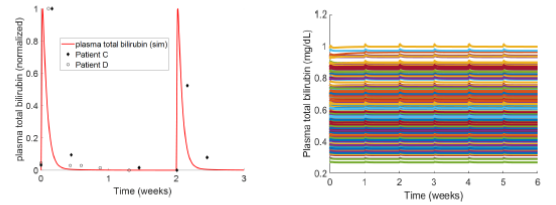

Figure 5. Simulations predict Drug X *in vivo* exposure and effects on bilirubin transporters and enzymes recapitulate timing but not magnitude of observed bilirubin elevations. Using predicted exposure and measured IC_{50} values for the effect of Drug X on bilirubin transporters and enzymes, DILIsym predicted minimal changes in bilirubin. When normalized to the maximum value (left), the simulated timing for bilirubin elevations (red line, one simulated individual) was consistent with the timing observed in clinic (shown in 2 patients: black diamond, black open circle). SimPops results (right) supported the argument that altered bilirubin disposition might plausibly account for bilirubin elevations, but additional considerations not yet included would be required to reproduce the magnitude of observed bilirubin elevations.

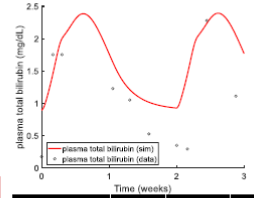

Figure 6. Simulations predict changes in Drug X mediated MRP2 expression combined with inhibition of transporters and enzymes can account for clinically observed bilirubin elevations. With inclusion of Drug X mediated inhibition of MRP2 expression, DILIsym predicted bilirubin elevations that could exceed 2 mg/dL. Illustrative results from one simulated individual shown. Both timing and magnitude of simulated bilirubin elevations were consistent with clinical data.

Reduction in MRP2 expression	TBIL > 2x ULN	TBIL > 1.5x ULN	TBIL > 2x baseline	TBIL > 1.5x baseline
10%	0/16	0/16	0/16	0/16
20%	0/16	0/16	0/16	0/16
30%	0/16	0/16	0/16	0/16
40%	0/16	0/16	0/16	0/16
50%	0/16	0/16	0/16	6/16
60%	0/16	3/16	2/16	15/16
70%	2/16	11/16	11/16	16/16
80%	9/16	13/16	13/16	16/16
90%	12/16	15/16	15/16	16/16

Table 2. Use of simulations to evaluate MRP2 required for clinical bilirubin elevations. Uncertainty in the relationship between *in vitro* and *in vivo* inhibition of MRP2 expression was addressed by evaluating 10-90% inhibition. Results illustrate that with >50% MRP2 reduction, bilirubin canalicular efflux was compromised, resulting in clinically relevant bilirubin elevations.

CONCLUSIONS

- Using hepatocyte loss to reproduce ALT elevations under-predicted clinically observed bilirubin elevations, suggesting clinical observations following administration of Drug X do not reflect severe liver injury
- Simulation results combining Drug X exposure and mechanistic interaction with bilirubin transporters and/or metabolism are consistent with the timing, but under-predict magnitude, of bilirubin elevations
- Simulations predict that Drug X-mediated reduction in MRP2 expression in conjunction with inhibition of bilirubin transporters and enzymes by Drug X can account for observed bilirubin elevations

DILI-sim Initiative

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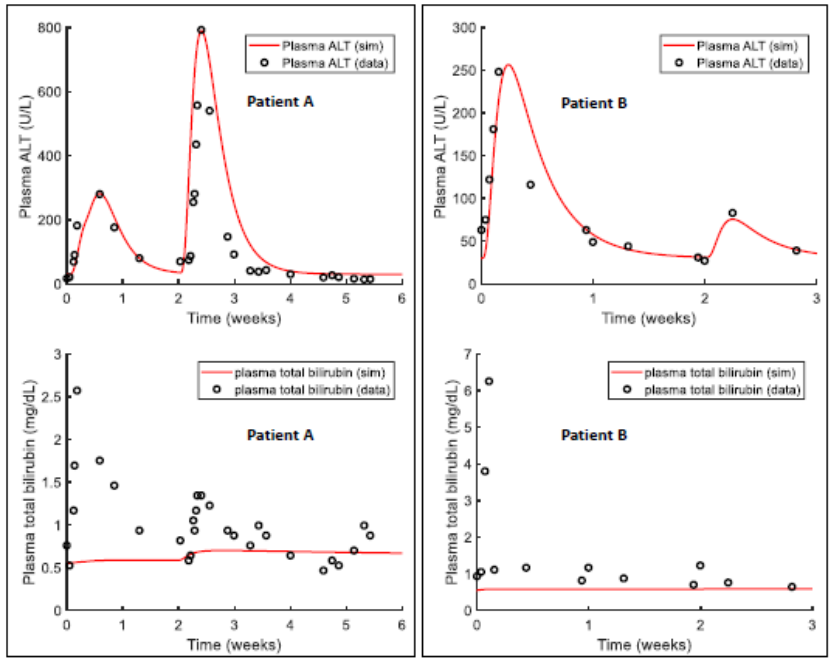
RESULTS



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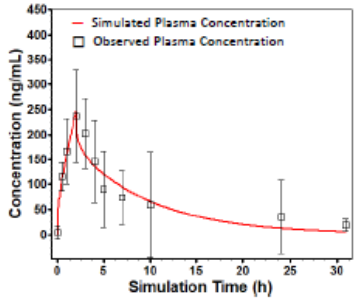

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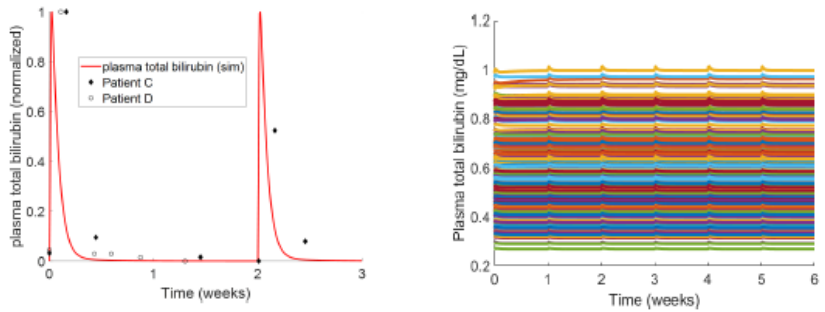


Figure 5. Simulations predict Drug X *in vivo* exposure and effects on bilirubin transporters and enzymes recapitulate timing but not magnitude of observed bilirubin elevations. Using predicted exposure and measured IC_{50} values for the effect of Drug X on bilirubin transporters and enzymes, DILIsym predicted minimal changes in bilirubin. When normalized to the maximum value (left), the simulated timing for bilirubin elevations (red line, one simulated individual) was consistent with the timing observed in clinic (shown in 2 patients: black diamond, black open circle). SimPops results (right) supported the argument that altered bilirubin disposition might plausibly account for bilirubin elevations, but additional considerations not yet included would be required to reproduce the magnitude of observed bilirubin elevations.

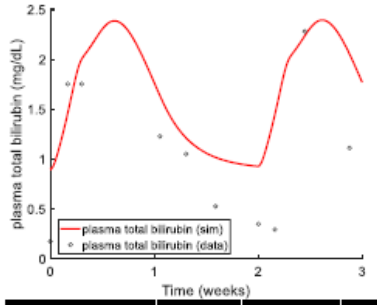


Figure 6. Simulations predict changes in Drug X mediated MRP2 expression combined with inhibition of transporters and enzymes can account for clinically observed bilirubin elevations. With inclusion of Drug X mediated inhibition of MRP2 expression, DILIsym predicted bilirubin elevations that could exceed 2 mg/dL. Illustrative results from one simulated individual shown. Both timing and magnitude of simulated bilirubin elevations were consistent with clinical data.

Reduction in MRP2 expression	TBIL > 2x ULN	TBIL > 1.5x ULN	TBIL > 2x baseline	TBIL > 1.5x baseline
10%	0/16	0/16	0/16	0/16
20%	0/16	0/16	0/16	0/16
30%	0/16	0/16	0/16	0/16
40%	0/16	0/16	0/16	0/16
50%	0/16	0/16	0/16	6/16
60%	0/16	3/16	2/16	15/16
70%	2/16	11/16	11/16	16/16
80%	9/16	13/16	13/16	16/16
90%	12/16	15/16	15/16	16/16

Table 2. Use of simulations to evaluate MRP2 required for clinical bilirubin elevations. Uncertainty in the relationship between *in vitro* and *in vivo* inhibition of MRP2 expression was addressed by evaluating 10-90% inhibition. Results illustrate that with >50% MRP2 reduction, bilirubin canalicular efflux was compromised, resulting in clinically relevant bilirubin elevations.

CONCLUSIONS

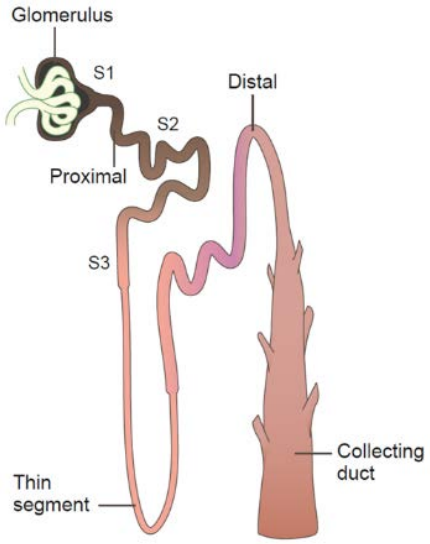
- Using hepatocyte loss to reproduce ALT elevations under-predicted clinically observed bilirubin elevations, suggesting clinical observations following administration of Drug X do not reflect severe liver injury
- Simulation results combining Drug X exposure and mechanistic interaction with bilirubin transporters and/or metabolism are consistent with the timing, but under-predict magnitude, of bilirubin elevations
- Simulations predict that Drug X-mediated reduction in MRP2 expression in conjunction with inhibition of bilirubin transporters and enzymes by Drug X can account for observed bilirubin elevations

DILI-sim Initiative

DILIsym Services
A SIMULATIONS PLUS COMPANY

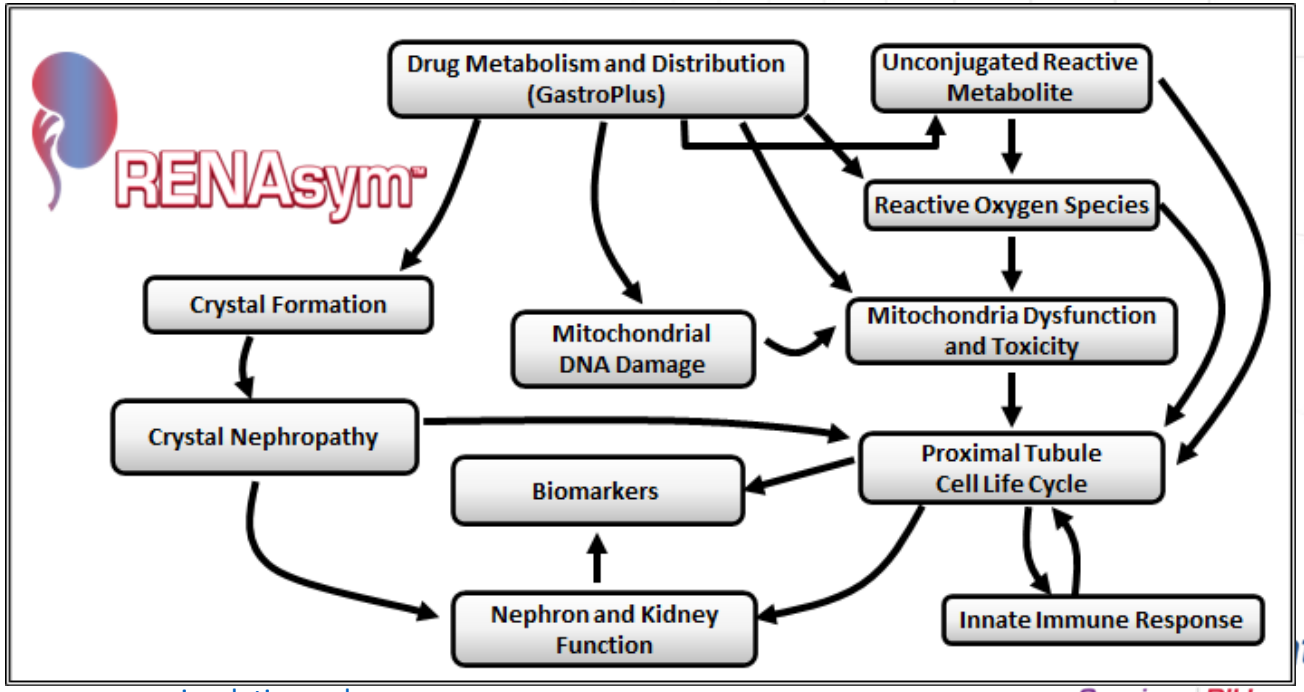
RENAsym Software Overview

- **Species: human and rat**
  - Population variability
- **Primary focus is nephron proximal tubules**
- **Multiscale biology**
- **Proximal tubule cells (PTC)**
  - Cellular energy balance
  - Apoptosis and necrosis, and proliferation
  - GSH depletion
  - Mitochondrial dysfunction, toxicity, DNA depletion
  - Crystal nephropathy
  - Inflammatory response
  - Neutrophils, macrophages, DCs
  - HMGB1, TNF-α, IL-1β, IL-6, IL-10, IL-18, HGF
- **Biomarkers**
  - Biomarkers of cell death and function (alpha GST, KIM-1)
  - Emerging biomarkers (uIL-18)
  - GFR, creatinine, RBF
- **Renal function**
  - Hemodynamics
  - Na+, Water reabsorption
  - RAAS modulation



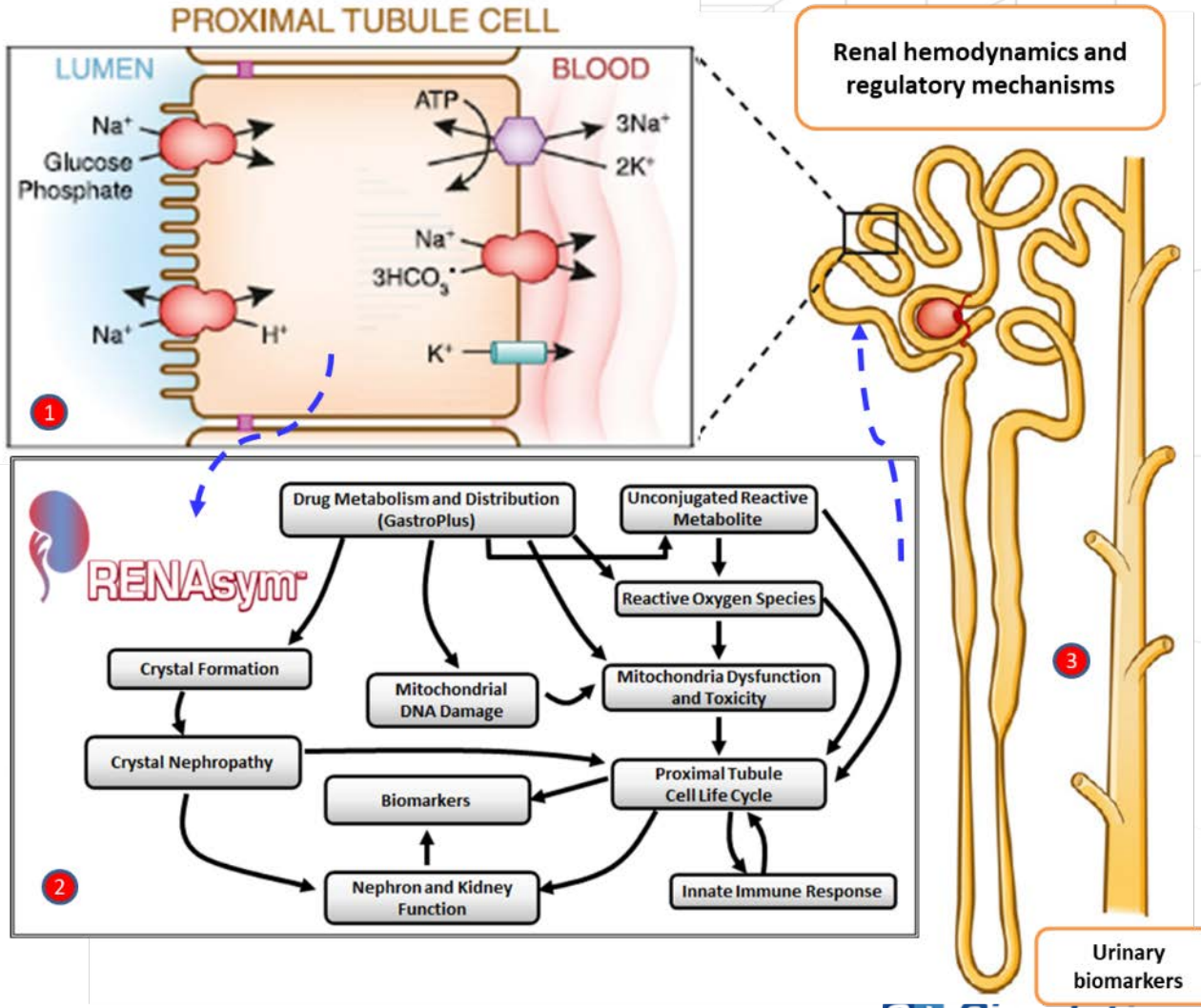
Ghezzi et al., Diabetologia 2018

- **Drugs**
  - Cisplatin
  - Gentamicin
  - Piroxicam
  - Cyclosporin A
  - Acyclovir
  - Indinavir
  - Valproate



RENAsym Represents Two Major Dynamically Interacting Sub-models

- RENAsym consists of two major sub-models:
  - Drug induced renal proximal tubule epithelial cell injury (PTC injury) sub-model
  - Renal pathophysiology systems (cardio-renal) sub-model
- The two sub-models interact with each other dynamically, where cellular level effects alter renal hemodynamic responses, and vice versa



RENAsym Utilizes Various Data Types to Inform Decisions

DMPK and Exposure Data

PBPK Modeling

- **Compound Properties**
  - Tissue partition coefficients
- **Tissue penetration studies**
  - *Kidney to blood ratio*
- **Pharmacokinetic data**
  - *Absorption, extra-hepatic clearance, metabolites*
- ***in vitro* data**
  - *Metabolite synthesis, active uptake*

In vitro Mechanistic AKI Data

Data Collected for Quantitative DILI Mechanism Info

- **Oxidative stress** *(high content imaging)*
  - *Direct and reactive metabolite-mediated*
- **Mitochondrial toxicity** *(XF Analyzer)*
  - *ETC inhibition*
  - *Uncoupling*
- **Phys/chem properties related to solubility**
- **Other assays to be added**

RENAsym® ***Modeling & Simulation***

Simulations and Assays inform:

- **Prediction of kidney injury risk**
- **Participating injury mechanisms**
- **Characteristics of patients at risk for injury**
- **Drug dosing paradigms**
- **Biomarker monitoring strategies**

Clinical Data / Information

- **Dosing Protocols, fasting/fed state, meal times**
- **Patient types (NHV, disease, etc.)**
- **Anthropometric data**
  - **Body weight, age, ethnicity**
- **Pharmacokinetic data**
  - **Absorption, extra-hepatic clearance, metabolites**

Mechanistic Modeling of Cyclosporine A-induced Acute Kidney Injury with RENAsym®

Pallavi Bhargava[a], Christina Battista[a], Viera Lukacova[b], Jeffrey L. Woodhead[a]

[a]DILIsym Services, Inc., Research Triangle Park, NC; [b]Simulations Plus Inc., Lancaster, CA

ABSTRACT

OBJECTIVES: The use of Cyclosporine A (CsA) can cause tubular damage leading to a decline in renal function as determined by decreases in serum creatinine levels, glomerular filtration rate (GFR), and ATP[1]. This work uses RENAsym®, a quantitative systems toxicology (QST) model of acute kidney injury (AKI), to recapitulate clinical outcomes following CsA administration in humans.

METHODS: The effects of CsA on mitochondrial function and reactive oxygen species (ROS) production were assessed to define the potential for CsA-induced kidney injury. Human renal proximal tubule epithelial cells (RPTECs) were treated with CsA and its effects on mitochondrial respiration as well as ROS production were measured. Seahorse XF96 Analyzer was used to measure mitochondrial respiration. High content screening was used to measure ROS production after RPTECs were exposed to dihydroethidium staining. These *in vitro* data were used to define kidney toxicity parameters, and together with PBPK simulations of clinical CsA exposure created in GastroPlus®, kidney injury was predicted in RENAsym.

RESULTS: CsA inhibited the mitochondrial election transport chain flux (ETC inhibition coefficient=1458.33 µmol/L) and induced ROS production (V_{max}=0.049 1/hr, K_m =13.075 µmol/L). RENAsym predicted CsA-induced kidney injury such as a decrease in kidney average ATP as shown in Figure 4. RENAsym was further utilized to perform a mechanistic analysis to determine the main driver in simulated CsA nephrotoxicity. The mechanistic analysis indicated that CsA-induced kidney injury is primarily driven by inhibiting mitochondrial function via inhibition of the electron transport chain.

CONCLUSION: Using *in vitro* data to determine toxicity parameters, RENAsym accurately predicted CsA-induced nephrotoxicity in humans, consistent with observations from clinical studies

INTRODUCTION

Cyclosporine A (CsA) is an immunosuppressant known for inhibiting T-lymphocyte driven immune responses. CsA is commonly used following organ transplant to prevent organ rejection and in other diseases such rheumatoid arthritis, atopic dermatitis, and psoriasis. However, the use of CsA in humans, at doses range from 3 to 10 mg/kg, can cause nephrotoxicity. CsA can cause renal tubular damage subsequently leading to a decrease in renal function, indicated by an increase in serum creatinine levels and more importantly, a decrease in glomerular filtration rate (GFR)[2]. Here we use *in vitro* data and RENAsym®, a quantitative systems toxicology (QST) model of acute kidney injury (AKI), to recapitulate clinical outcomes following short-term CsA administration in humans.

RESULTS

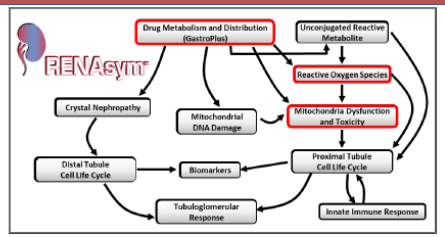


Figure 1: RENAsym is comprised of submodels that interact with one another to predict kidney injury outcomes. RENAsym combines data from *in vitro* toxicity studies, predictions of metabolism and distribution, as well as inner workings of kidney physiology to predict the potential for a given drug to induce acute kidney injury or cause nephrotoxicity.

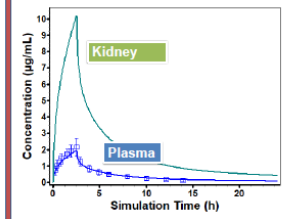


Figure 2: Simulated a single intravenous dose of CsA, 4 mg/kg, for 24 hours resulting in a plasma concentration in line with data[3] and predicted kidney concentration.

Using GastroPlus 9.7® we simulated a single IV dose of 4 mg/kg CsA for 24 hours. The ADMET Predictor module within GastroPlus predicted the kidney to plasma partition coefficient, Kp, to be 5.23. At a peak of 2.5 hours, the plasma is 1.96 µg/ml and in the kidney it is 10.2 µg/ml.

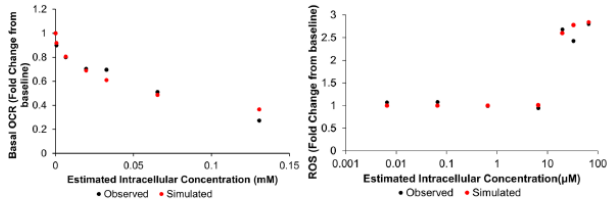


Figure 3: Simulated (red) and observed (black) basal oxygen consumption rate (OCR) (left) and reactive oxygen species (ROS) production (right) in RPTECs administered with 0.0125 to 25 µM of CsA.

CsA-induced electron transport chain (ETC) inhibition was simulated in MITOsym, and ROS production in RENAsym, against observed mitochondrial respiration and ROS production from *in vitro* studies. CsA inhibited the mitochondrial election transport chain flux (Non-saturable coefficient=1458.33 µmol/L, Saturable K_m=1.0488 µmol/L, Saturable V_{max}=0.3878 1/hr) and induced ROS production (V_{max}=0.049 1/hr, K_m =13.075 µmol/L)

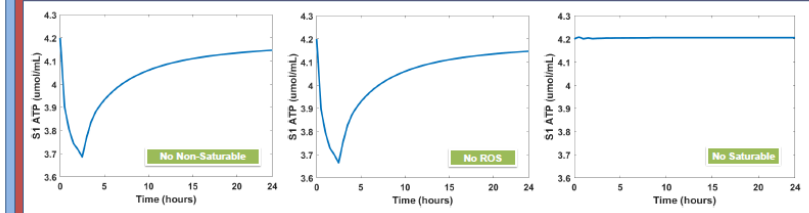


Figure 4: A mechanistic analysis was performed to predict which mechanism of toxicity was the primary driver in predicted CsA-induced nephrotoxicity. Simulations with no effect on non-saturable inhibition (left), no effect on ROS production (center), and no effect on saturable inhibition (right) were performed using RENAsym.

A single IV dose of CsA at 4 mg/kg was simulated in RENAsym with ROS and mitochondrial toxicity parameters. Each type of ETC inhibition, non-saturable or saturable, and ROS production was removed individually and then simulated in RENAsym. S1 ATP was used as a metric to compare contribution of each mechanism to simulated nephrotoxicity. When saturable inhibition was eliminated from simulations (right), the decrease in S1 ATP levels was predicted whereas, removing the other type of ETC inhibition and ROS production did not remove simulated nephrotoxicity. This suggests the effect of CsA on ETC is the primary driver in CsA-driven nephrotoxicity.

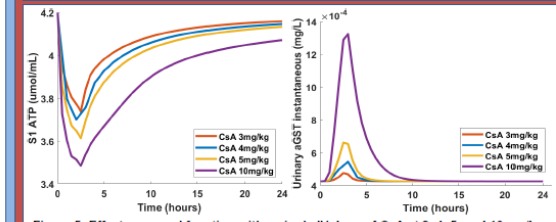


Figure 5: Effects on renal function with a single IV dose of CsA at 3, 4, 5, and 10 mg/kg for 24 hours include ATP levels (left) and instantaneous urinary αGST (right).

RENAsym was used to simulate a dose response of CsA exposure for 24 hours with a single IV dose of 3, 4, 5, or 10 mg/kg in humans. ATP levels decreased accordingly with an increase in dose. With a single dose of 10 mg/kg CsA, ATP levels dropped at 3 hours to 3.48 µmol/mL and did not return to baseline by 24 hours. An increase in urinary αGST, α-glutathione S-transferases, is a measure of tubular damage. Urinary αGST peaks at 3 hours for all four doses and levels return to baseline. Particularly, a single dose of 10 mg/kg peaks at 3 hours to 0.0013 mg/L and then returns to baseline.

METHODS

- Human renal proximal tubule epithelial cells (RPTECs) were treated with doses of CsA ranging from 0.01 to 25 µM.
- Mitochondrial respiration was measured using a Seahorse XFe96 Analyzer.
- Reactive oxygen species (ROS) production was measured using high content screening to quantify dihydroethidium staining following CsA exposure.
- PBPK simulations of a single dose of CsA was simulated in GastroPlus 9.7® for 3, 4, 5, 10 mg/kg.
- The kidney Kp for CsA was used to estimate intracellular concentration in toxicity assays, and toxicity parameterizations were based on intracellular kidney concentration.
- MITOsym® was used to parameterize ETC inhibition to *in vitro* mitochondrial respiration studies of CsA. ROS parameterization was performed in RENAsym.
- Simulations predicting kidney function and mechanistic analysis for CsA-induced nephrotoxicity were performed using RENAsym.

CONCLUSION

- Mechanistic analysis using RENAsym showed that inhibition of the mitochondrial electron transport chain is the primary mechanism responsible for the predicted decrease in kidney function.
- A dose response of CsA showed correlating decrease in renal ATP and an increase in urinary αGST.

REFERENCES

1. Schaft, J., Zuilen, A., Deinum, J., Bruijnzeel-Koomen, C. & Bruin-Weller, M. Serum Creatinine Levels During and After Long-term Treatment with Cyclosporine A in Patients with Severe Atopic Dermatitis. *Acta Derm. Venereol.* 95, 963–967 (2015)
2. Bagnis, C., Du Montcel S, Beaufils H, Jouanneau C, Jaudon M, Maksud P, Mallet A, Lehoang P, Deray G. Long-term Renal effect of Low-Dose Cyclosporine in Uveitis-treated Patients: Follow-up Study. JASN. 13, 2962-2968 (2002)
3. Kawait R., Mathew D, Tanaka C, & Rowland M. Physiologically Based Pharmacokinetics of Cyclosporine A: Extension to Tissue Distribution Kinetics in Rats and Scale-up to Human. *JPET.* 287 (2) 457-568 (1998)

ACKNOWLEDGEMENTS

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DILI-sim Initiative

DILIsym® Services
USING MECHANISTIC MODELING TO SOLVE LIVER TOXICITY PROBLEMS

SimulationsPlus
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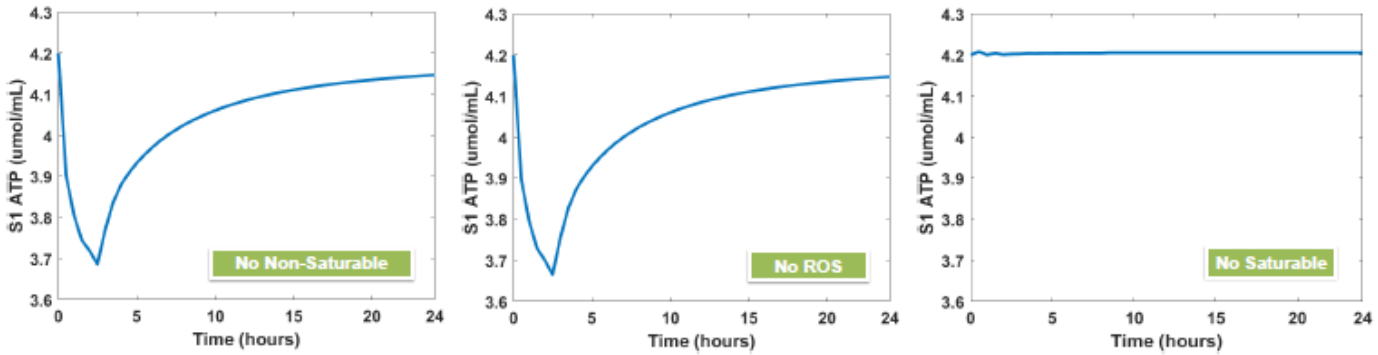
Mechanistic Modeling of Cyclosporine A-induced Acute Kidney Injury with RENAsym®

Pallavi Bhargava[a], Christina Battista[a], Viera Lukacova[b], Jeffrey L. Woodhead[a]
[a]DILIsym Services, Inc., Research Triangle Park, NC; [b]Simulations Plus Inc., Lancaster, CA



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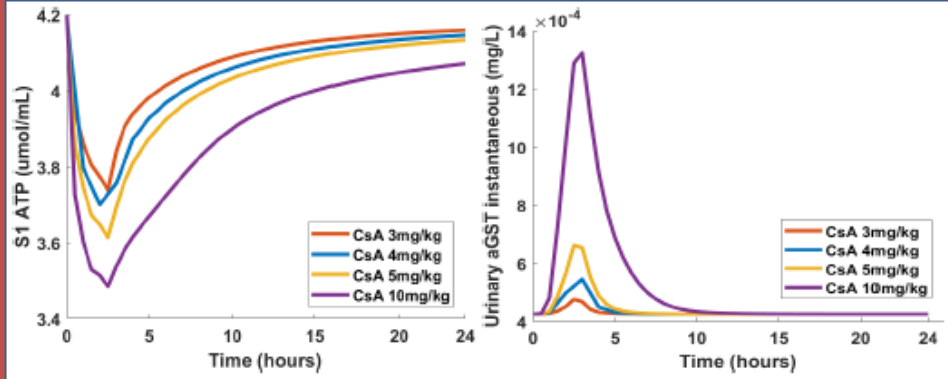

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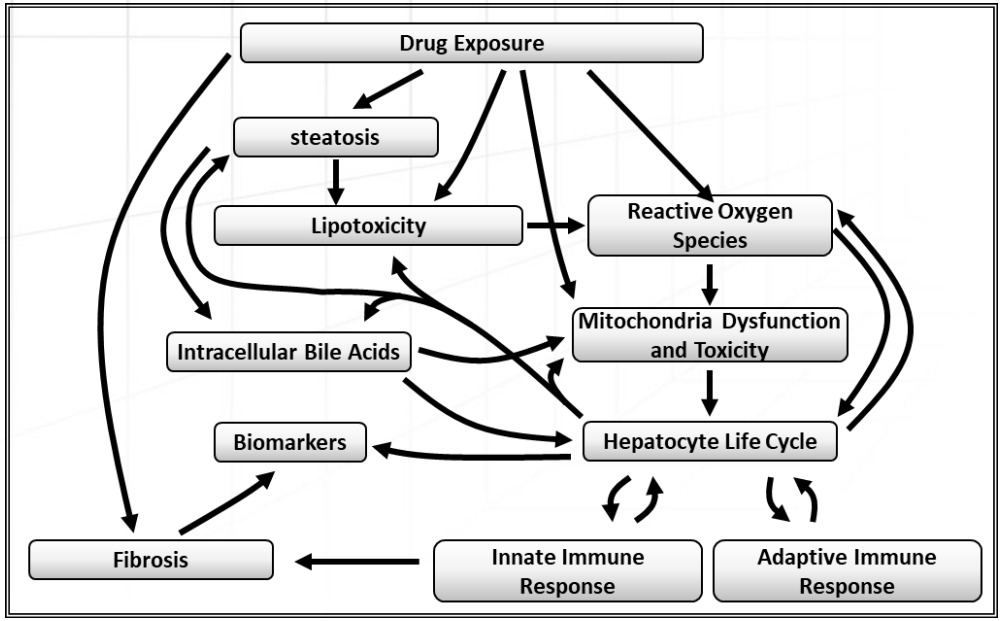
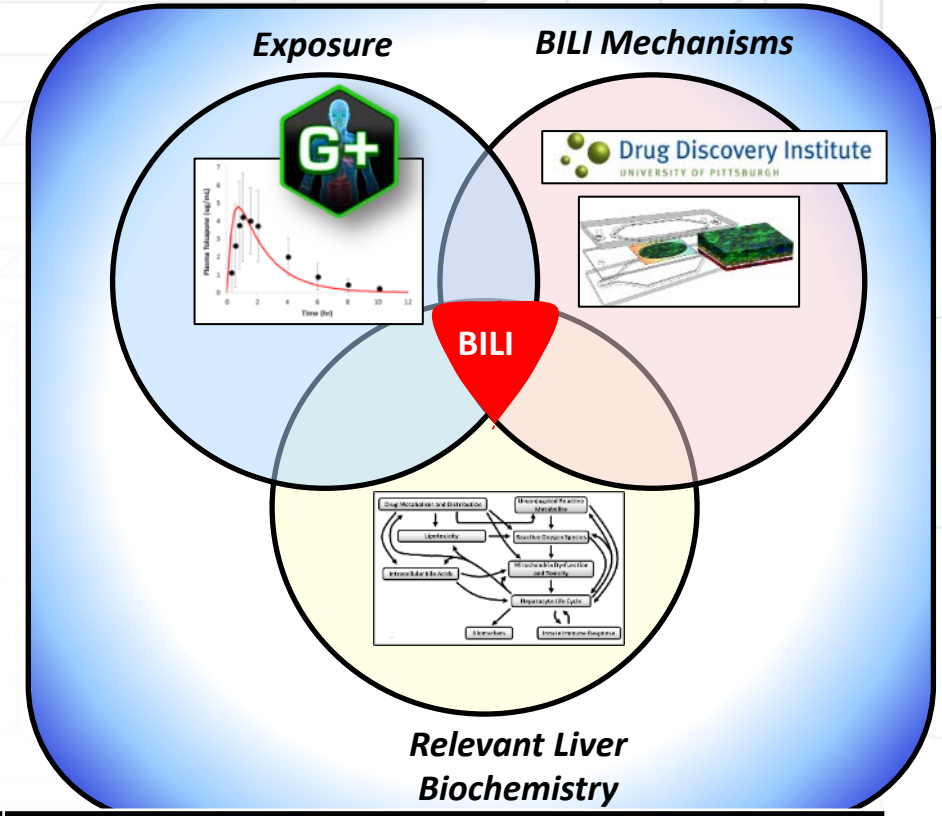
- A dose response of CsA showed correlating decrease in renal ATP and an increase in urinary αGST.

NIH NCATS Phase 2 Grant Received for Continued Development of BIOLOGXsym™

- BIOLOGXsym is in development to identify biologics-induced liver injury ("BILI") in new macromolecule therapeutic candidates and predict clinical liver injury outcomes using liver-on-a-chip inputs
  - Represents mechanistic pathways specific to biologics such as ***receptor-mediated indirect responses and on-target effects***





Class	Exemplar compounds
Biologics	GGF2, tocilizumab, ipilimumab, nivolumab, infliximab, ENZ-4176, bevacizumab
Small molecules	Methotrexate, cabozantinib
DDIs	Tocilizumab + methotrexate, nivolumab + cabozantinib, ipilimumab + nivolumab
Controls	Acetaminophen, azithromycin, metformin

Session Game Plan

- Why is SLP a great teammate to have?
- ***Effective Use*** vs. ***Safety*** – Chemicals and Therapeutics
- How do we get on the court together?

Pharmaceuticals/Chemicals/Consumer Goods

- Commercially maintained and validated software tools
- Experts to guide, manage, and support research and regulatory programs

CROs/Consultants

- Encourage onboarding our tools to support your clients
- Flexible business terms

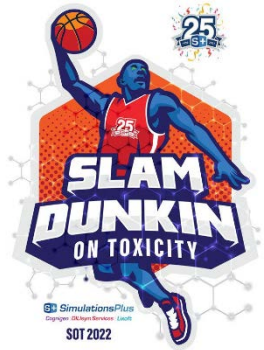
Universities & Colleges

- Free (yes, free) access to our software for both teaching and research
- Internship & postdoc opportunities year-round

Government/Regulatory Agencies

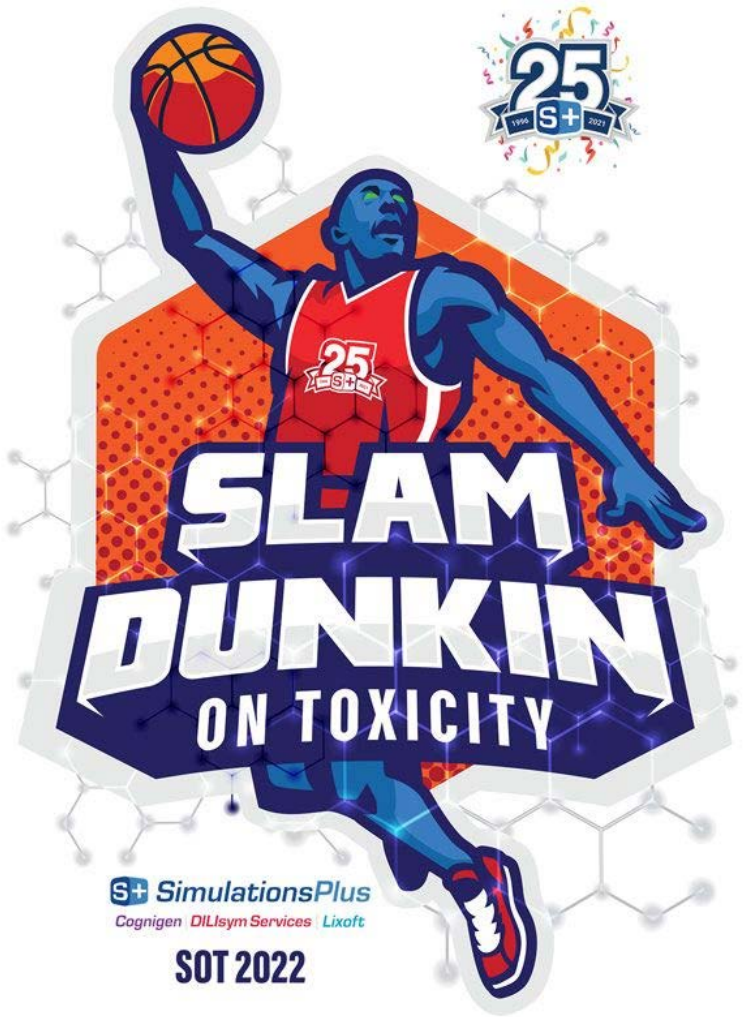
- Heavily discounted (or free) access to our software
- Online and customized training opportunities

Stop By Booth #1027 to Chat!



- **Michael Lawless**
  Physiologically Based Pharmacokinetic (PBPK) Simulations and Modeling of Botanical Constituents
  Session: Biological Modeling
  March 29 @ 10:45 AM – 12:30 PM (PT) CC Exhibit Hall (Hall B)
- **Lara Clemens**
  Mechanistic Modeling of Biologics-Induced Liver Injury (BILI) Predicts Hepatotoxicity of Tocilizumab through Both On- and Off-Target Effects
  Session Title: Biological Modeling
  March 29 @ 10:45 AM – 12:30 PM (PT) CC Exhibit Hall (Hall B)
- **Brett Howell**
  Quantitative Systems Toxicology (QST) Modeling ofCimaglermin Alfa (GGF2) Hepatotoxicity Shows the Potential of BIOLOGXsym to Predict Biologics-Induced Liver Injury (BILI)
  Session Title: Computational Toxicology II
  March 30 @ 10:45 AM – 12:30 PM (PT) CC Exhibit Hall (Hall B)
- **Jeff Woodhead**
  Modeling of Cyclosporine A-Induced Acute Kidney Injury with RENAsym
  Session Title: Kidney
  March 30 @ 10:45 AM – 12:30 PM (PT) CC Exhibit Hall (Hall B)
- **Pallavi Bhargava**
  Modeling of Indinavir-Induced Crystal Nephropathy in RENAsym
  Session Title: Kidney
  March 30 @ 10:45 AM – 12:30 PM (PT) CC Exhibit Hall (Hall B)
- **James J. Beaudoin**
  Simulating Multidrug Resistance Protein 3 (MDR3) Inhibition-Mediated Cholestatic Liver Injury Using DILIsym X, a Quantitative Systems Toxicology (QST) Modeling Platform
  Session Title: Systems Biology
  March 30 @ 2:30 – 4:15 PM (PT) CC Exhibit Hall (Hall B)
- **Nader Hamzavi**
  Evaluating the Nephrotoxicity of Cisplatin in Rats with RENAsym, a Mechanistic Model of Drug-Induced Acute Kidney Injury
  Session Title: Systems Biology
  March 30 @ 2:30 – 4:15 PM (PT) CC Exhibit Hall (Hall B)

THANK YOU!



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