Use of In Silico Mechanistic Models to Support Interspecies Extrapolation of Oral Bioavailability and Formulation Optimization: Model Example Using GastroPlus™

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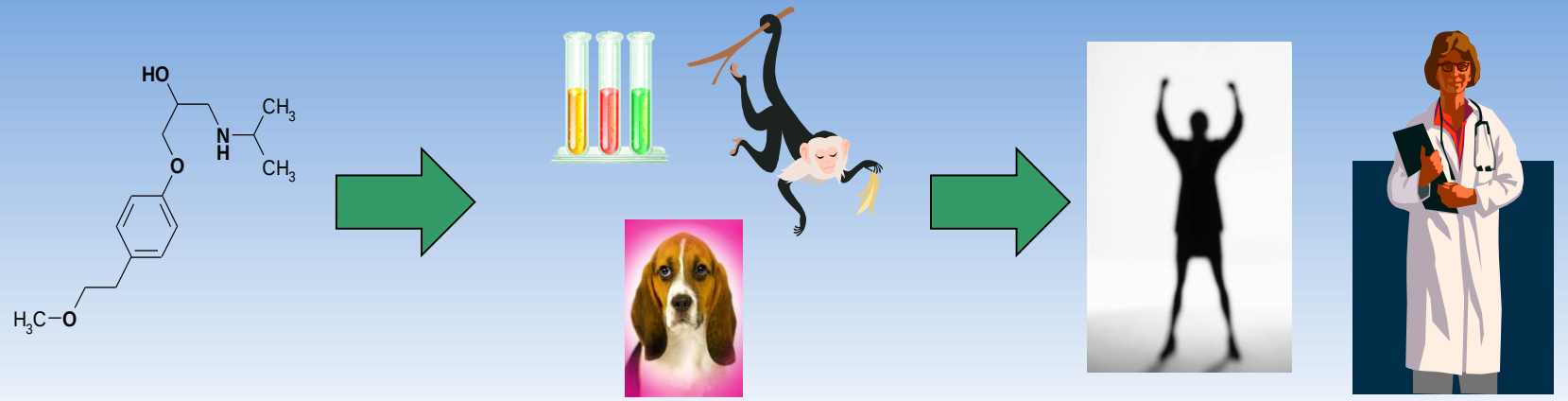
Outline

- Introduction to GastroPlus PBPK model
- Physiological considerations for interspecies extrapolation of oral absorption and bioavailability
- Examples:
  - Salt selection
    - Animal study was used to verify the *in silico* model prediction
  - Formulation development
    - Mechanistic model was used to understand the formulation behavior *in vivo* based on animal study
  - *in vitro – in vivo* dissolution extrapolation
    - Animal data was used to validate methodology for *in vitro - in vivo* dissolution extrapolation
  - Interspecies differences
    - Mechanistic model was used to investigate interindividual and interspecies differences in formulation behavior
- Summary

Discovery | Preclinical | Clinical



Discovery PK

Combine *in silico* technologies to screen compound libraries in animals or humans

Incorporate preclinical/*in vitro* data to extend FIH simulations to full *in vivo* outcomes (IVIVE)

Mechanistic predictions of hepatotoxicity through QSP

Clinical PK/Pharmacology

Simulate population behaviors (e.g., pediatrics, disease)

Build population PK/PD models

Predict DDIs

Pharmaceutical Development

Assess various strategies during formulation development

Assist with Quality by Design (QbD) implementation

Develop mechanistic *in vitro-in vivo* correlations (IVIVCs)

Understand food effects

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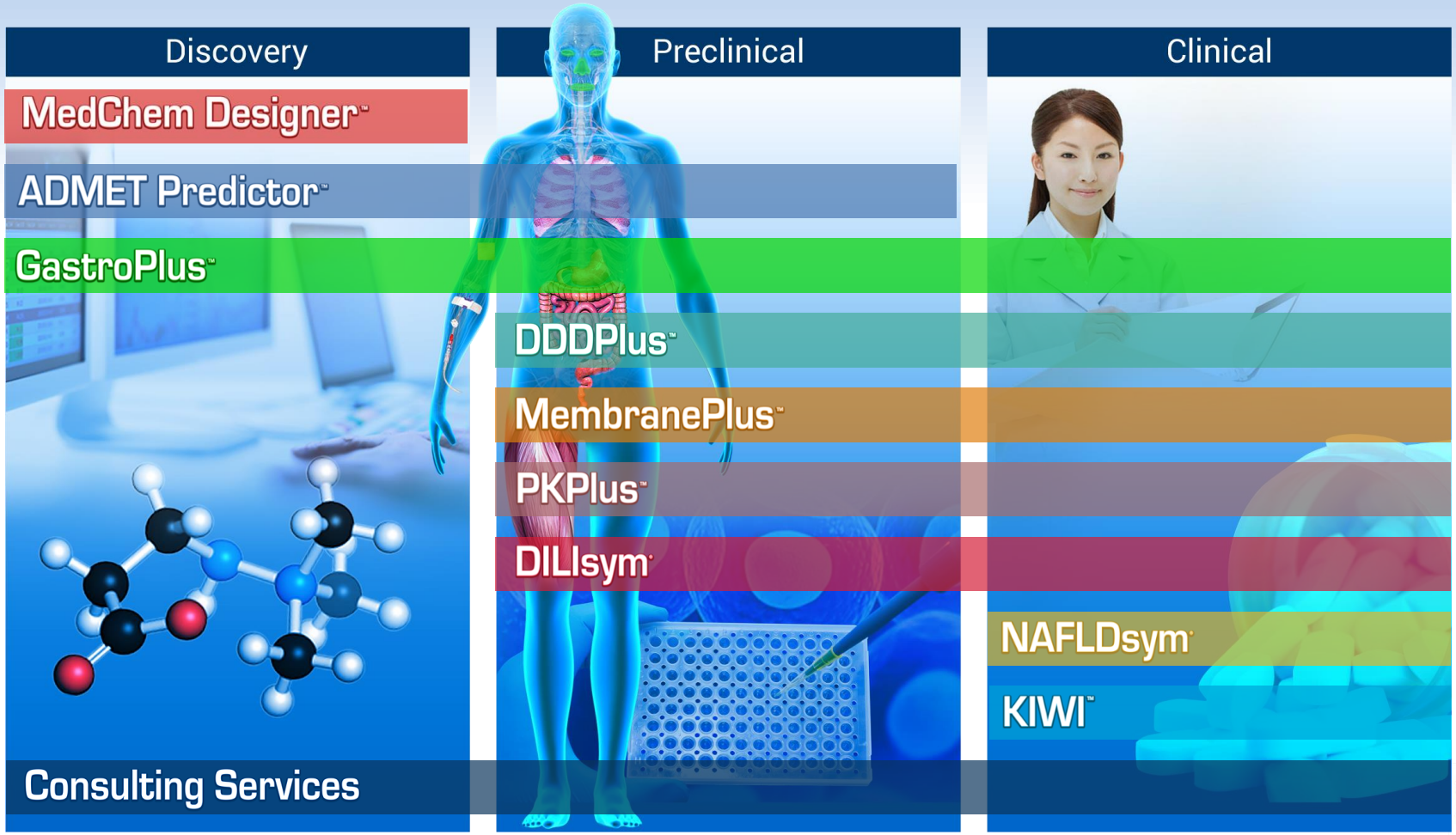

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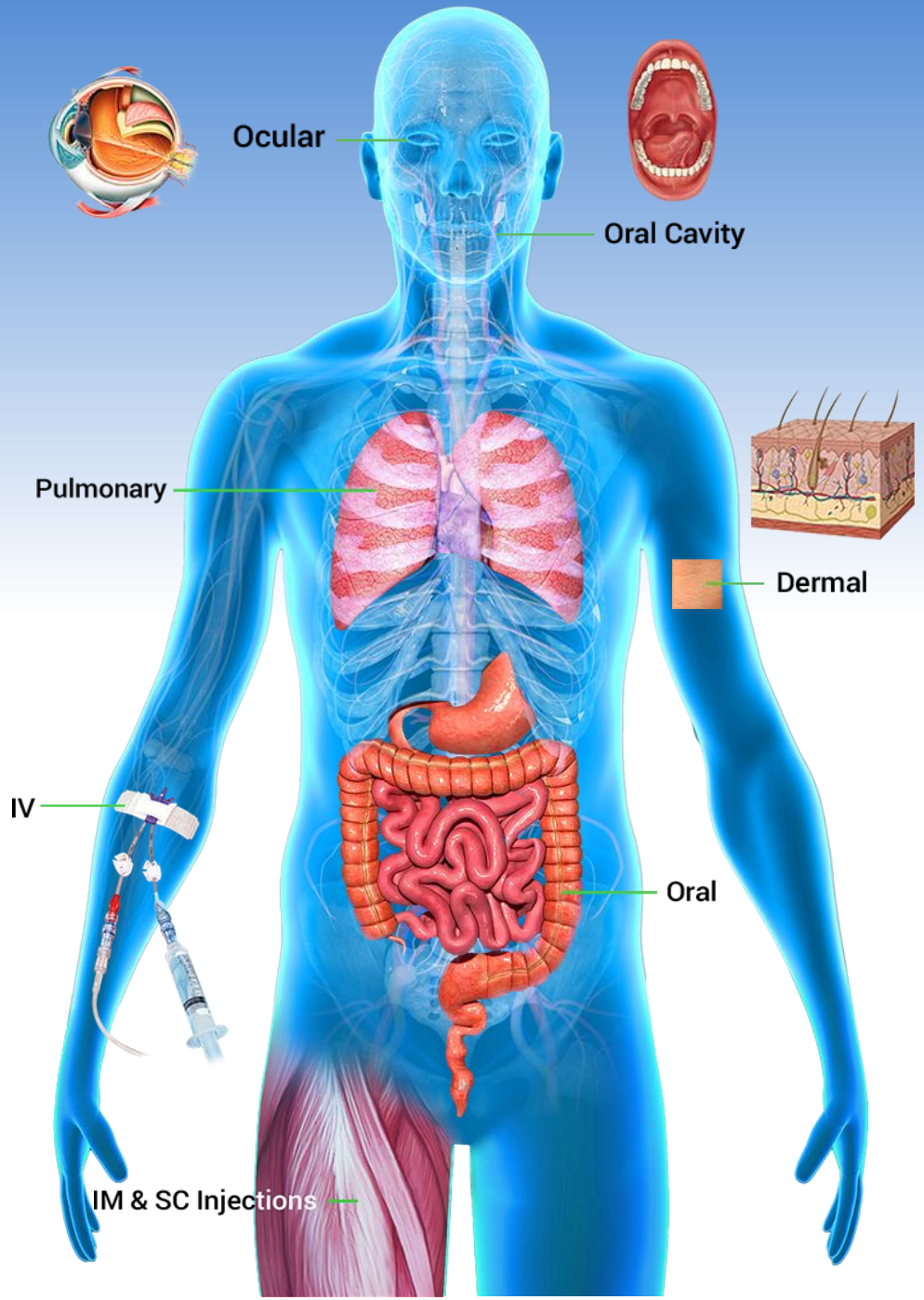
- GastroPlus includes mechanistic absorption models for variety of administration routes
- This presentation focuses on oral administration but similar principles could be applied in development of formulations for non-oral administration routes



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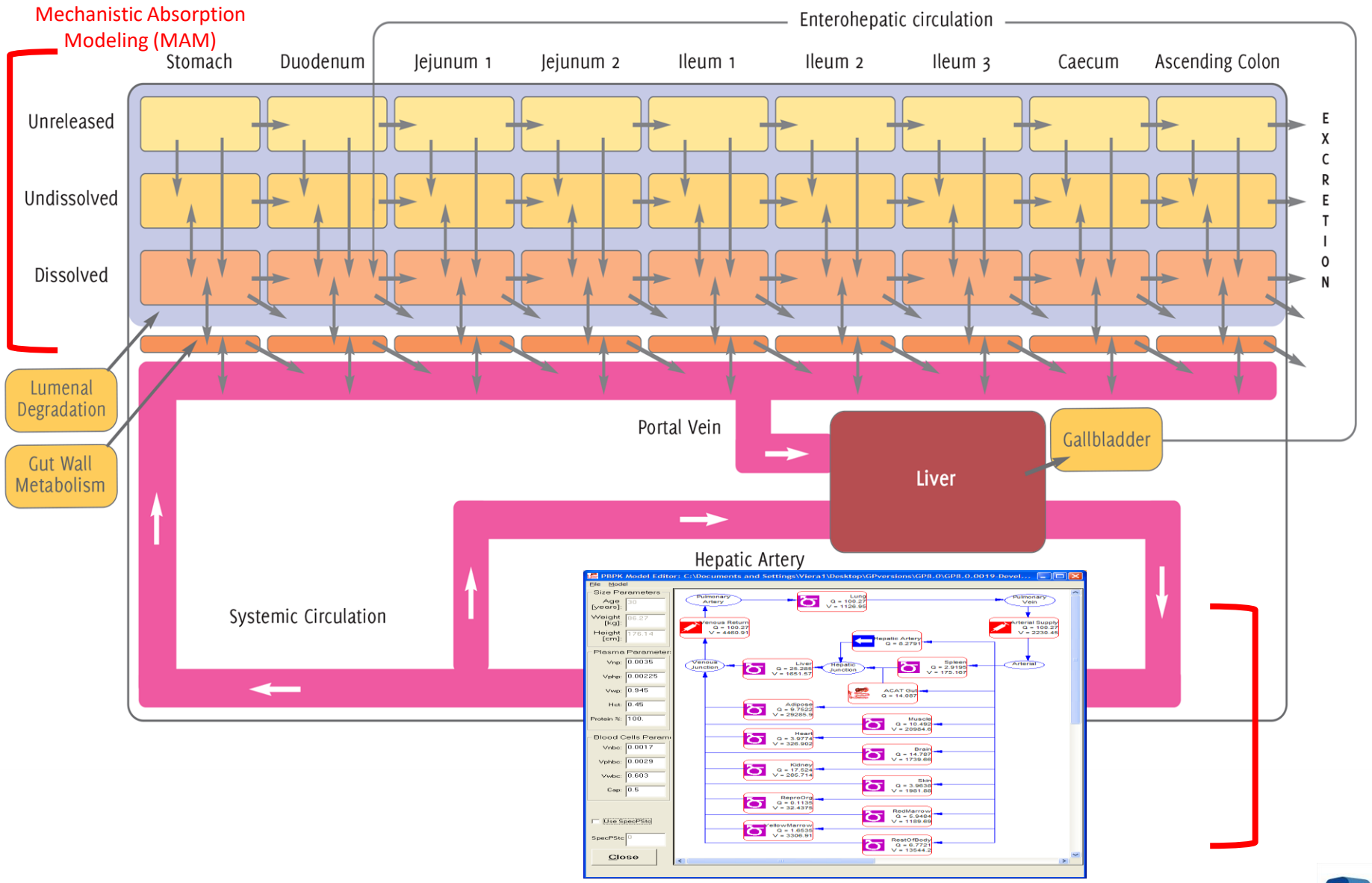
Advanced Compartmental Absorption and Transit Model (ACAT™)



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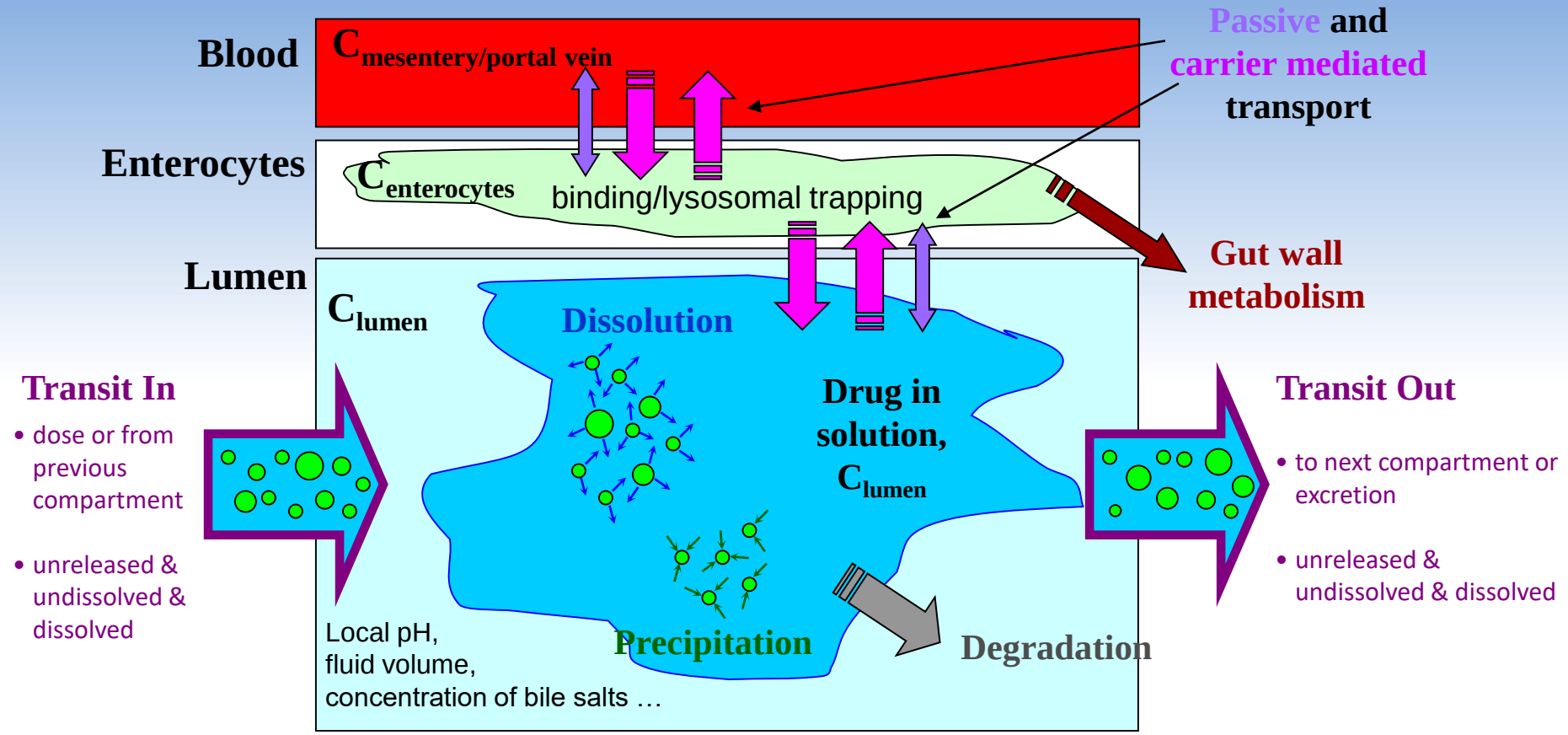
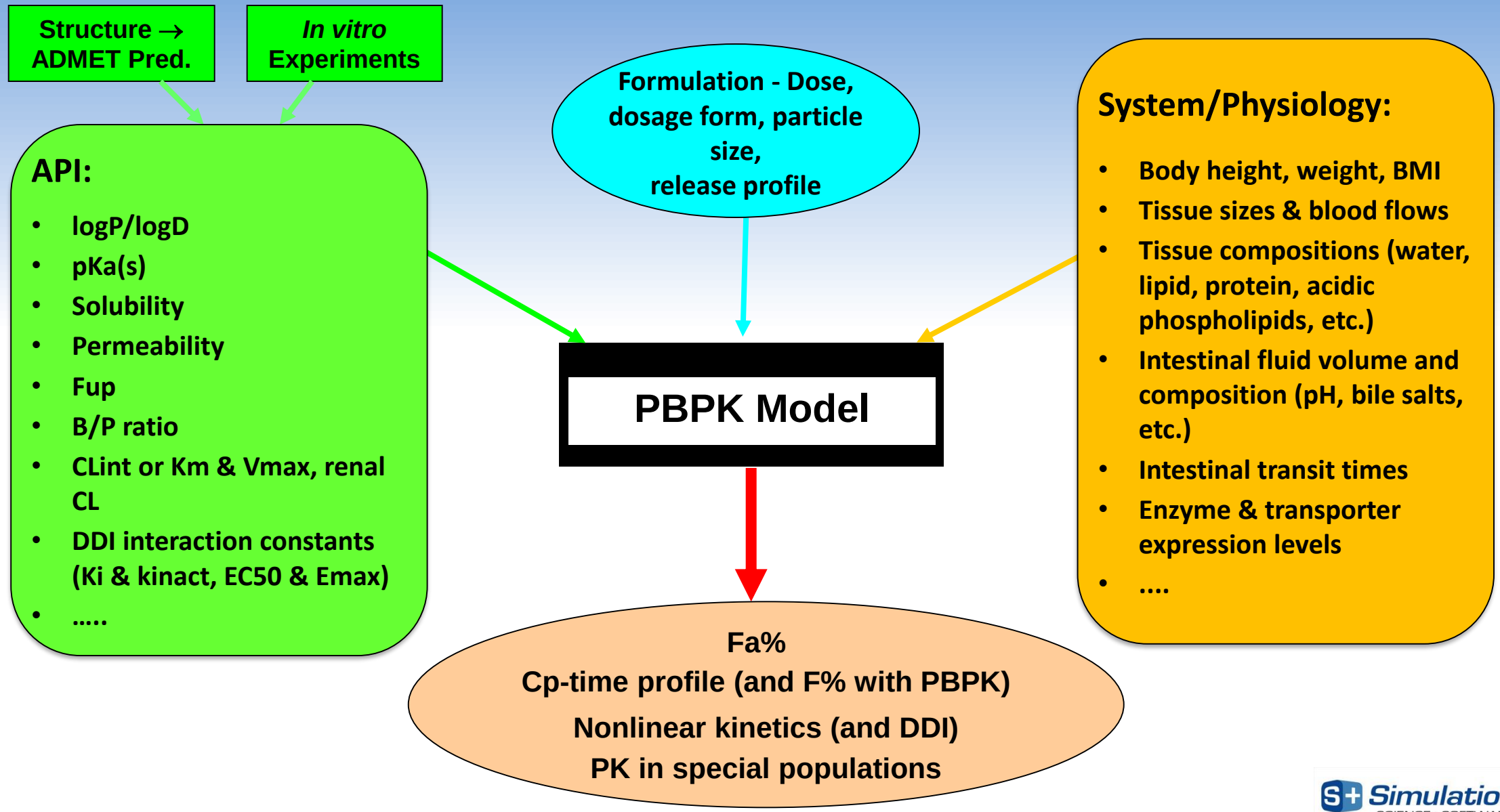
Processes Involved in Oral Absorption



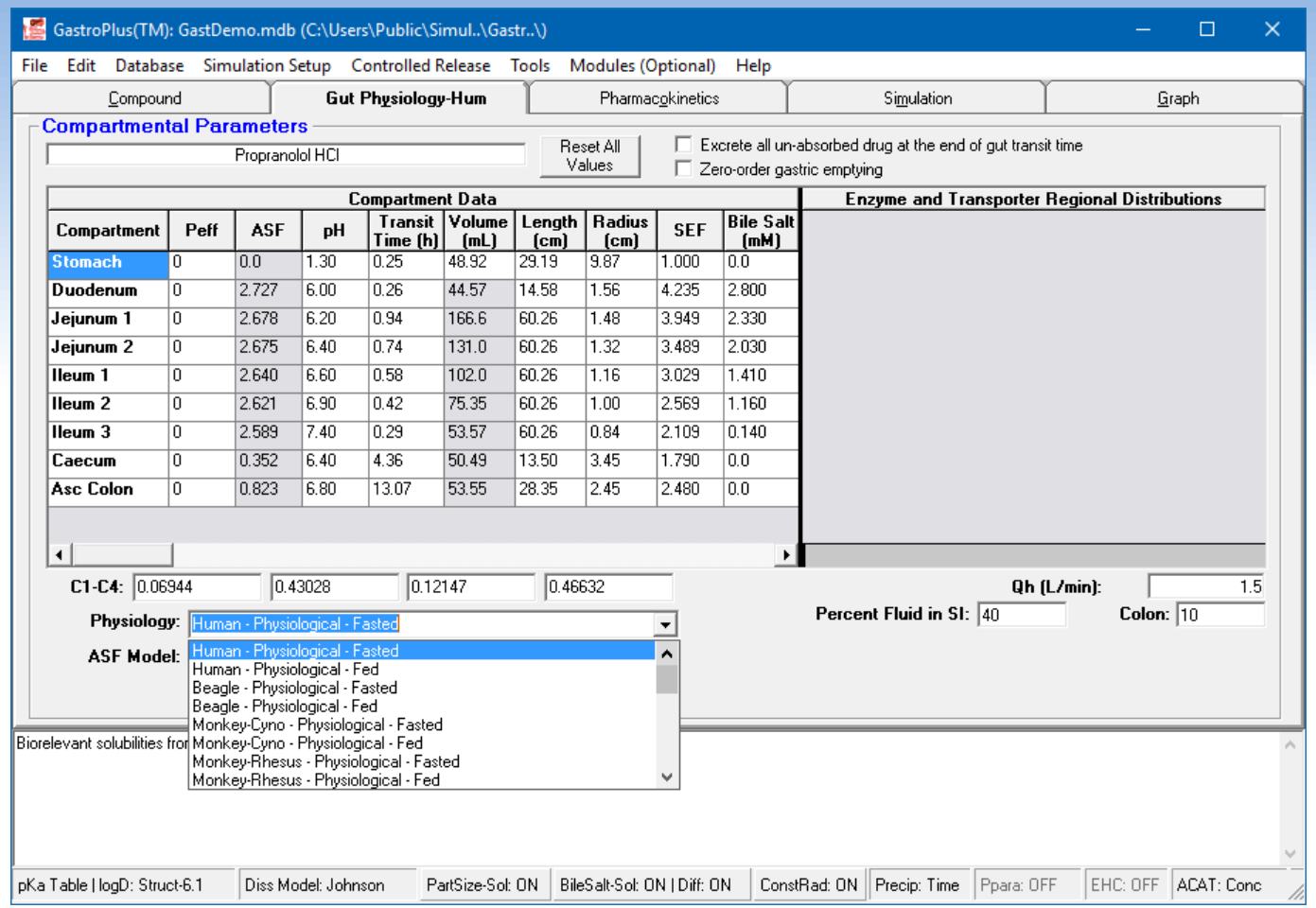
These phenomena:

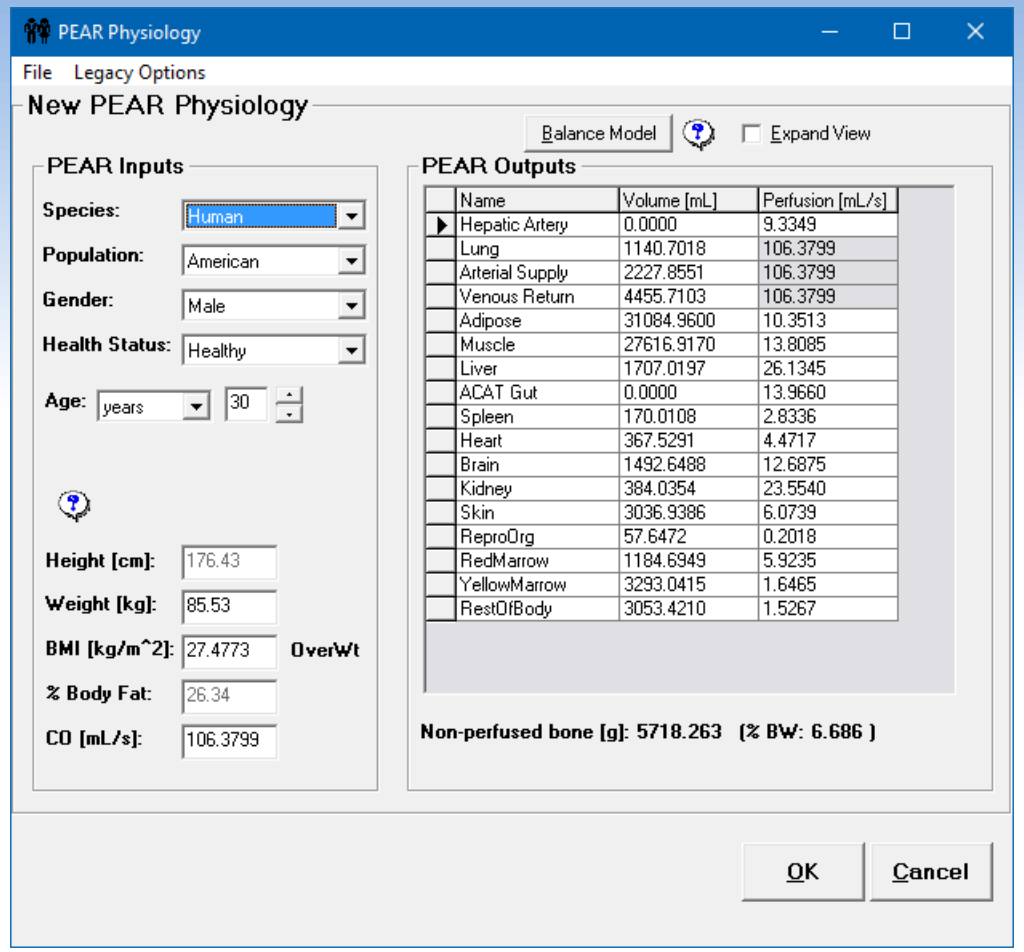
- **are happening simultaneously**
- **are repeated in each of the compartments of the gastrointestinal tract**

The Big Picture



System/Physiology Parameters: Built in the Program, Editable by User





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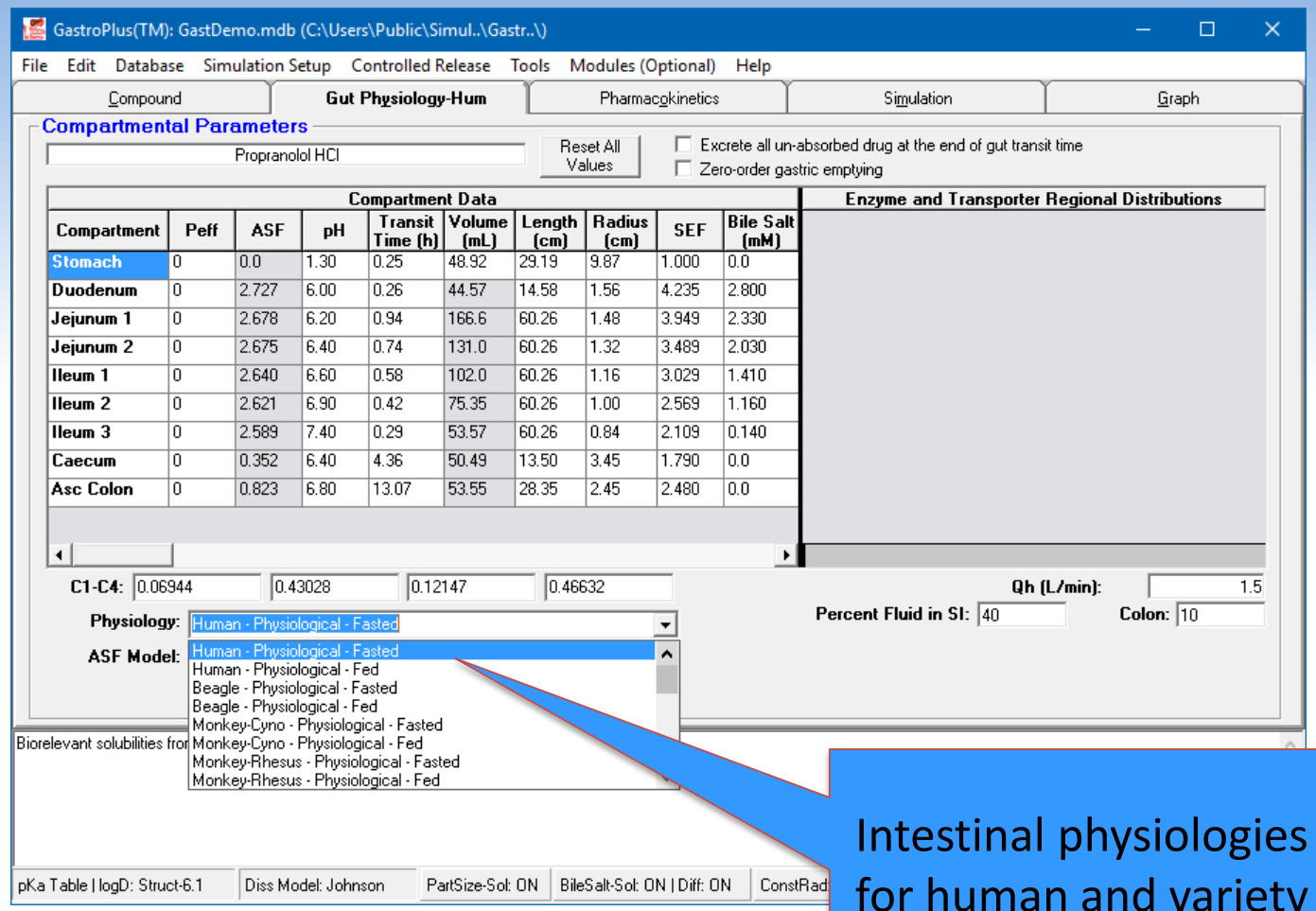

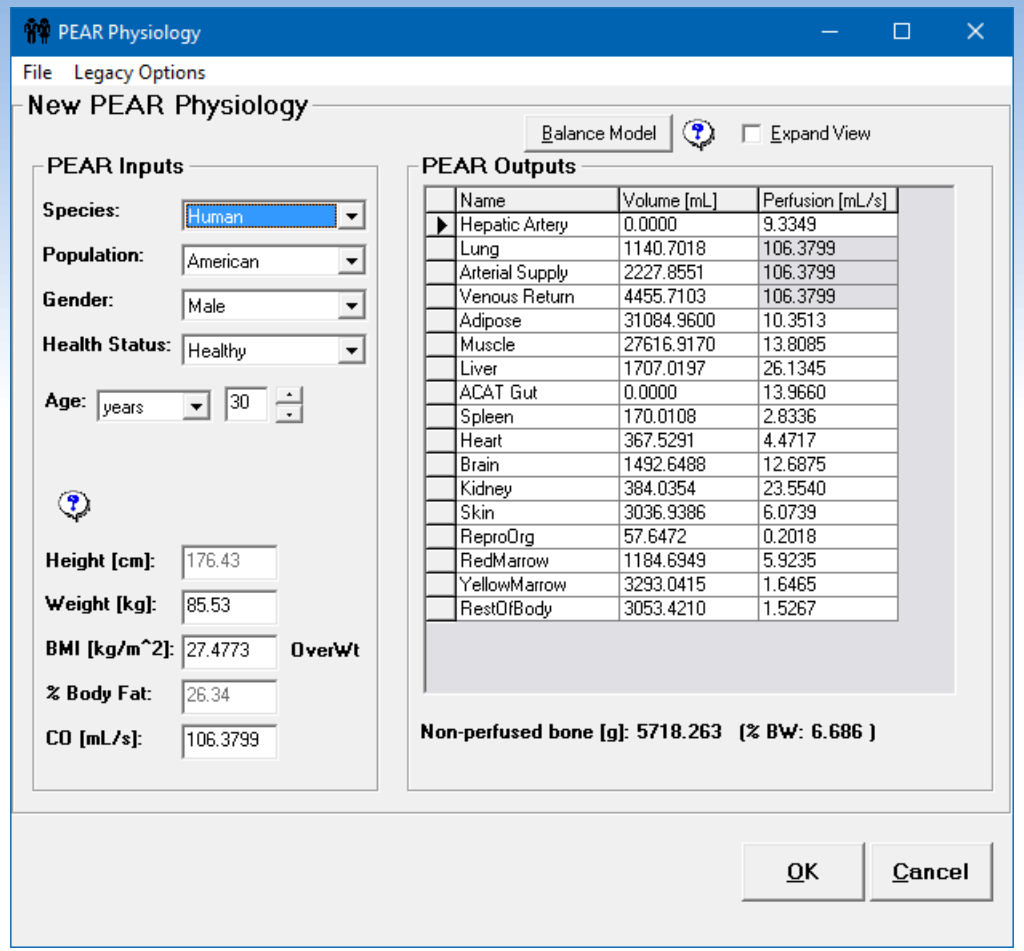

Intestinal physiologies for human and variety of animal species

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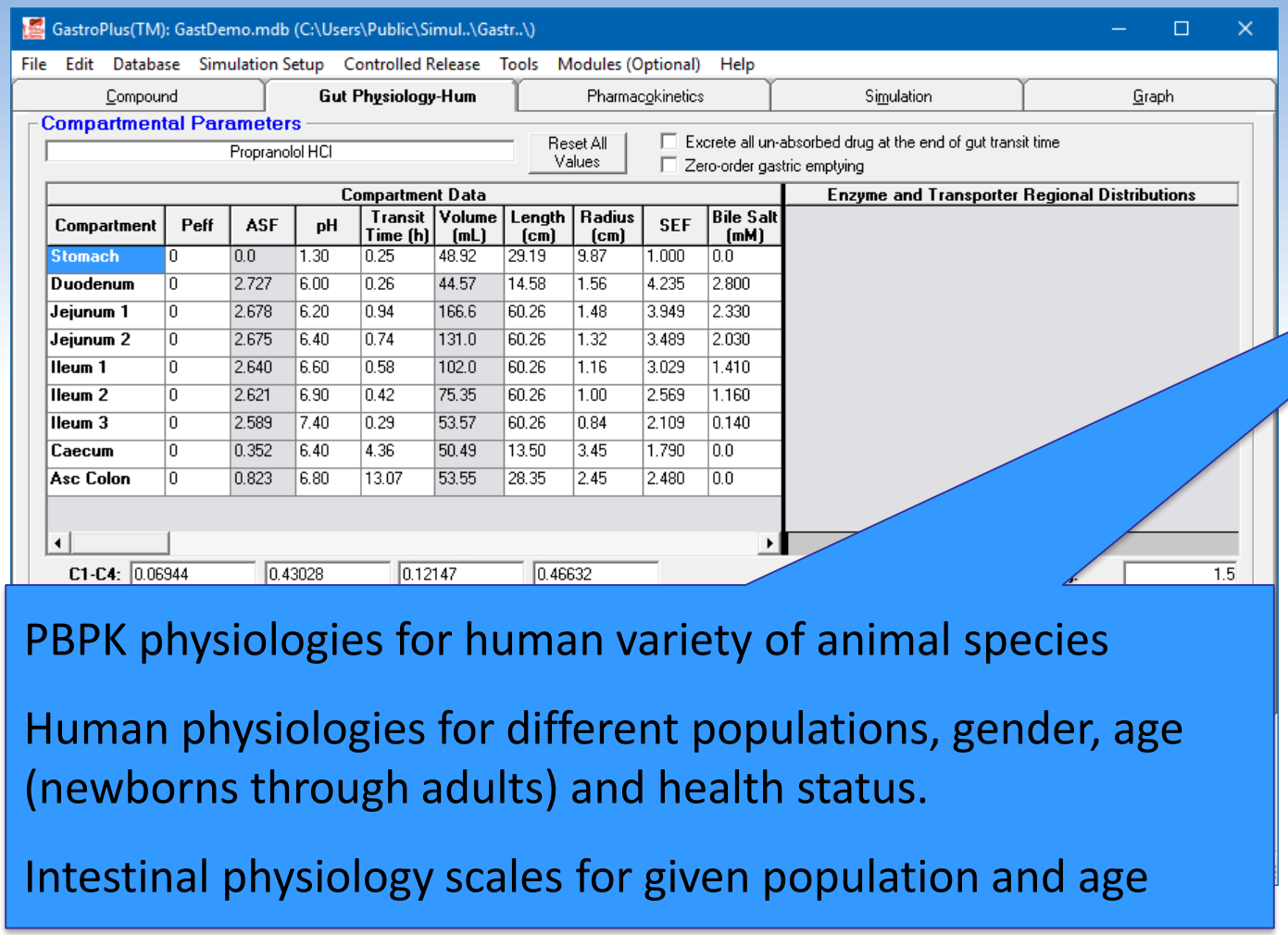

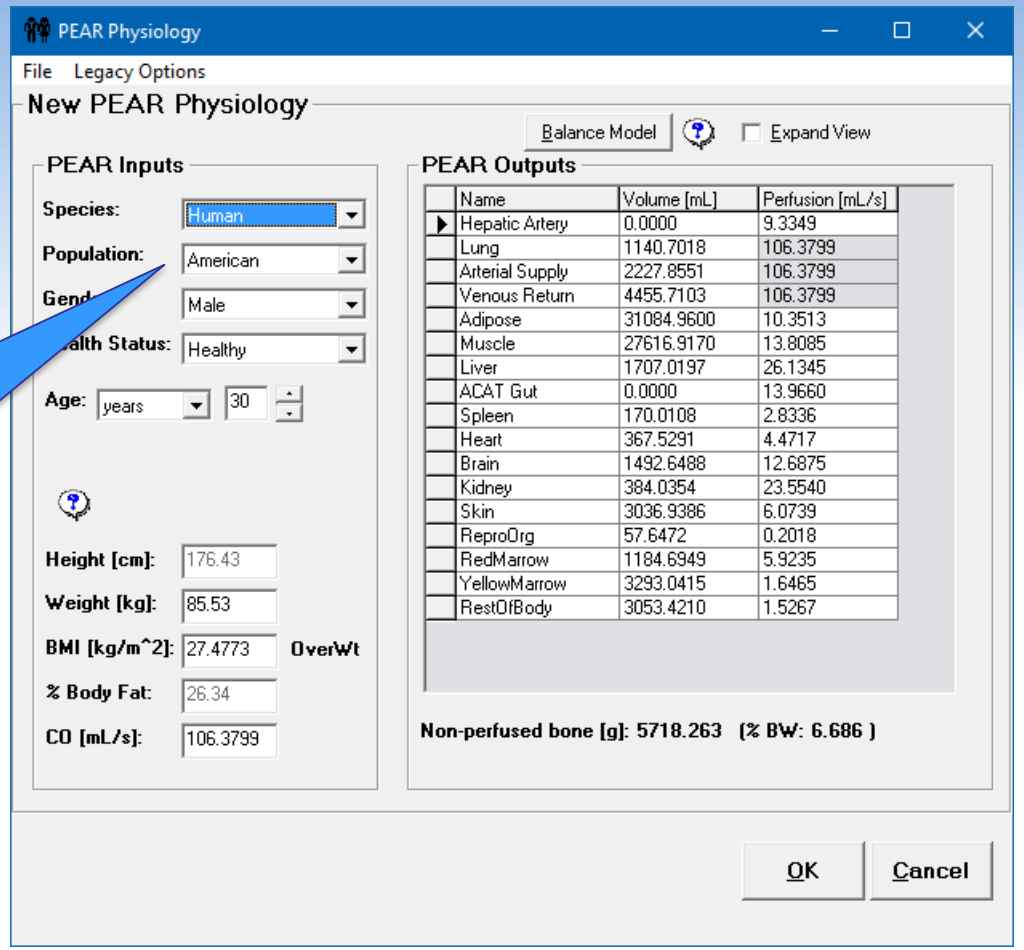

PBPK physiologies for human variety of animal species

Human physiologies for different populations, gender, age (newborns through adults) and health status.

Intestinal physiology scales for given population and age

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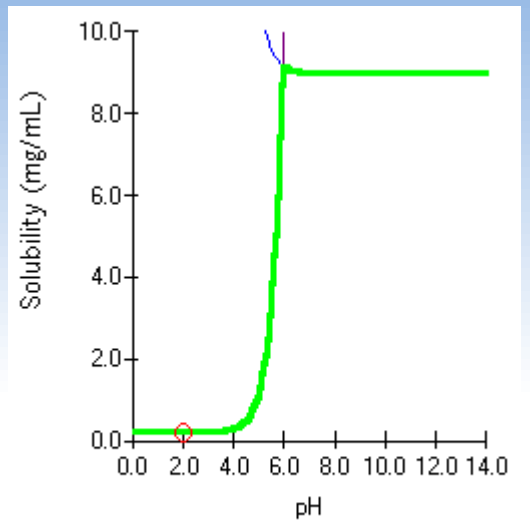
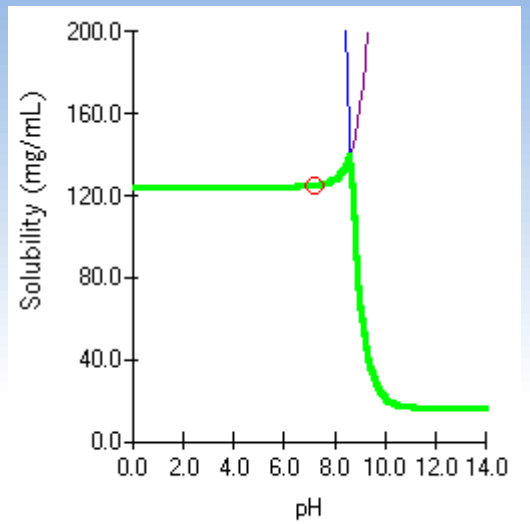
Species differences: Solubility/Dissolution

Changes in ionization result in chanes in solubility in different regions of the intestine





pH and bile salt concentrations

fasted:

human:

Compartment Data		
Compartment	pH	Bile Salt (mM)
Stomach	1.30	0.0
Duodenum	6.00	2.800
Jejunum 1	6.20	2.330
Jejunum 2	6.40	2.030
Ileum 1	6.60	1.410
Ileum 2	6.90	1.160
Ileum 3	7.40	0.140
Caecum	6.40	0.0
Asc Colon	6.80	0.0

rat:

Compartment Data		
Compartment	pH	Bile Salt (mM)
Stomach	3.90	0.0
Duodenum	5.89	20.00
Jejunum 1	6.13	17.29
Jejunum 2	6.13	6.980
Ileum 1	5.93	2.820
Ileum 2	5.93	1.300
Ileum 3	5.93	1.240
Caecum	6.58	0.0
Asc Colon	6.23	0.0

dog:

Compartment Data		
Compartment	pH	Bile Salt (mM)
Stomach	3.00	0.0
Duodenum	6.20	5.000
Jejunum 1	6.20	4.050
Jejunum 2	6.20	1.820
Ileum 1	6.40	0.610
Ileum 2	6.60	0.440
Ileum 3	6.68	0.310
Caecum	6.75	0.0
Asc Colon	6.45	0.0

fed:

Compartment Data		
Compartment	pH	Bile Salt (mM)
Stomach	4.90	0.0
Duodenum	5.40	14.44
Jejunum 1	5.40	12.02
Jejunum 2	6.00	10.46
Ileum 1	6.60	7.280
Ileum 2	6.90	5.990
Ileum 3	7.40	0.730
Caecum	6.40	0.0
Asc Colon	6.80	0.0

Compartment Data		
Compartment	pH	Bile Salt (mM)
Stomach	3.20	0.0
Duodenum	5.00	20.00
Jejunum 1	5.10	17.29
Jejunum 2	5.10	6.980
Ileum 1	5.94	2.820
Ileum 2	5.94	1.300
Ileum 3	5.94	1.240
Caecum	5.90	0.0
Asc Colon	5.51	0.0

Compartment Data		
Compartment	pH	Bile Salt (mM)
Stomach	5.00	0.0
Duodenum	6.20	15.40
Jejunum 1	6.20	12.50
Jejunum 2	6.20	5.600
Ileum 1	6.40	1.900
Ileum 2	6.60	1.340
Ileum 3	7.05	0.950
Caecum	7.50	0.0
Asc Colon	6.45	0.0

Changes in bile salt concentrations in different regions of the intestine may result in changes in solubility (especially for more lipophilic compounds)

$$Sol_{bile,pH} = Sol_{aq,pH}\left(1+\frac{MWt_{H_2O}}{\rho_{H_2O}}\times SR\times C_{bile}\right)$$

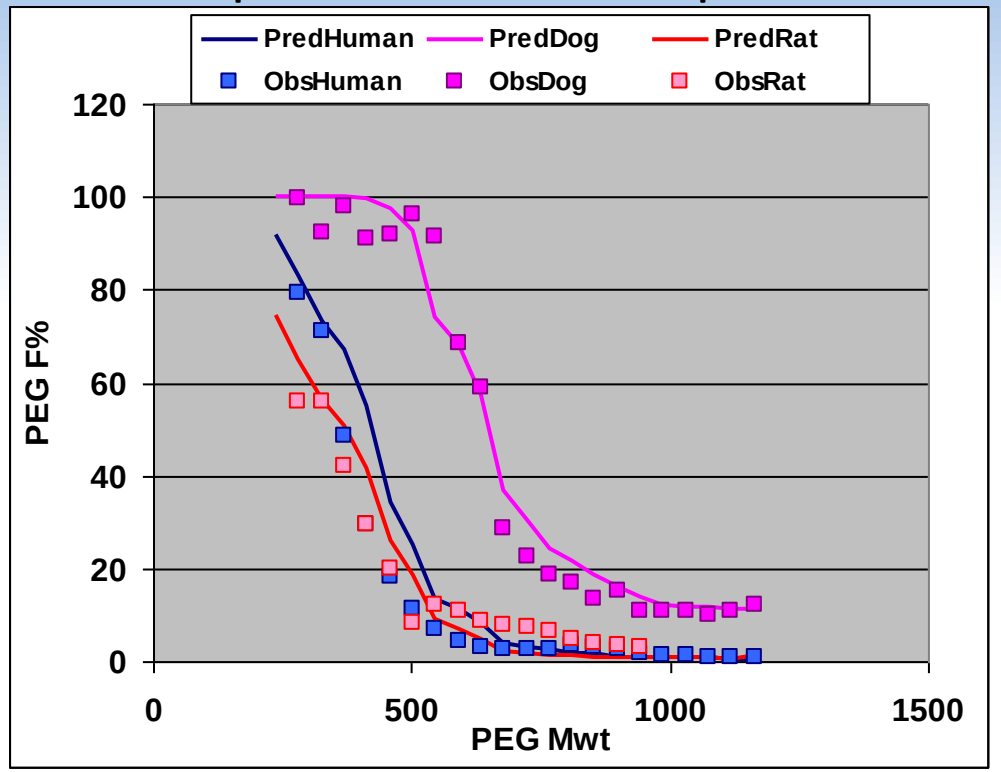
Mithani, Pharm Res 1996, 13:163-167

Species differences: Absorption

The model accounts for:

- Difference in pH
- Difference in absorptive surface area
- Difference in pore sizes (tight junctions) and porosities
- Difference in distribution of transporter and enzyme expression levels (where known)

Example of interspecies differences in paracellular absorption



Observed data from He-JPharmSci 1998, 87: 626-633

Species differences: Absorption

The model accounts for:

- Difference in pH
- Difference in absorptive surface area
- Difference in pore sizes (tight junctions) and porosities
- Difference in distribution of transporter and enzyme expression levels (where known)

Example of interspecies differences in transporter distributions (mRNA)

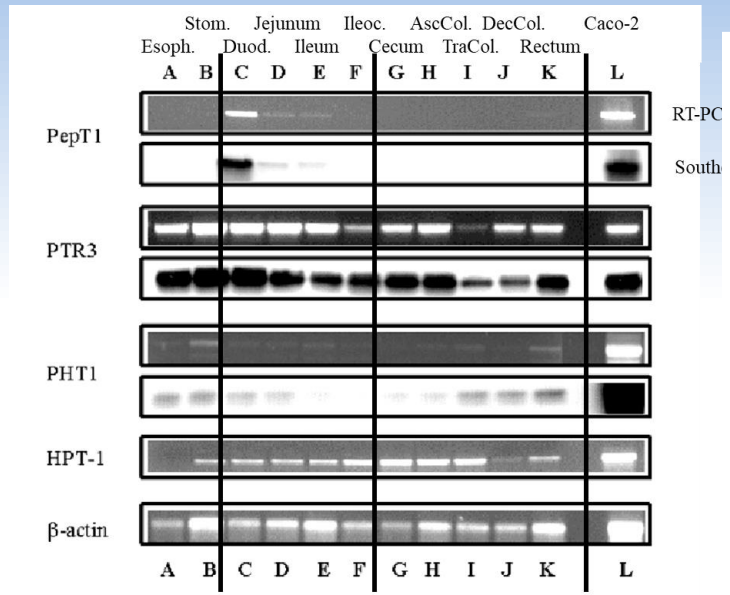


Figure 1. RT-PCR analysis of human PepT1, PTR3, PHT1, and HPT-1 mRNAs in the human esophagus (A), stomach (B), duodenum (C), jejunum (D), ileum (E), ileocecum (F), cecum (G), ascending colon (H), transverse colon (I), descending colon (J), rectum (K), and in Caco-2 cells (L). RT-PCR was performed with specific primers for each mRNA and amplified products of PepT1, PTR3, PHT1, and HPT-1 were 588, 470, 443, and 1004 bp, respectively. Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Commercially available human β-actin primers were used to generate a mRNA expression positive control, amplifying a product of 303 bp.

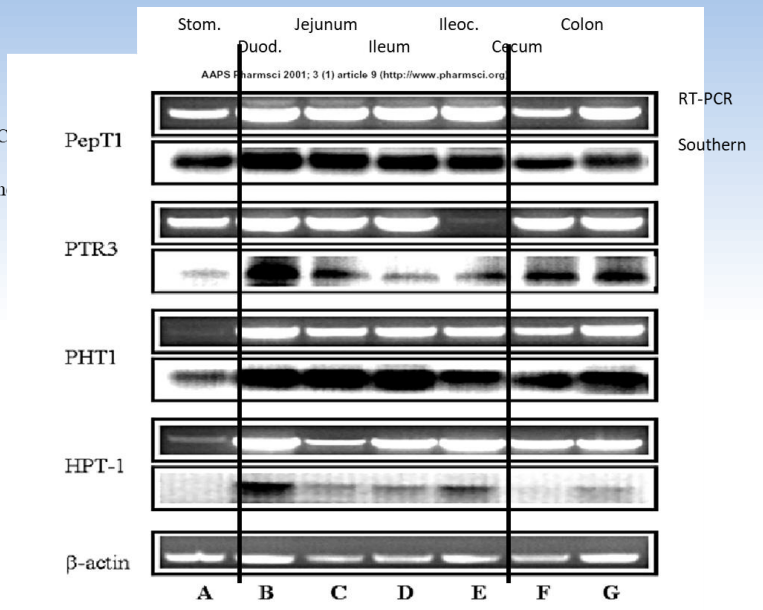


Figure 2. RT-PCR analysis of rat PepT1, PTR3, PHT1, and RPT1 mRNAs in the rat stomach (A), duodenum (B), jejunum (C), ileum (D), ileocecal junction (E), cecum (F), and colon (G). RT-PCR was performed using specific primers for rat PepT1, PHT1, and RPT-1 mRNAs amplifying products of 523, 437, and 860 bp, respectively. Analysis of PTR3 mRNA expression in the rat tissues was performed using primers designed from the human PTR3 mRNA sequence (Table 1). Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Rat-specific β-actin primers were designed to generate a mRNA expression-positive control, amplifying a product of 375 bp.

Herrera-Ruiz AAPS PharmSciTech 2001, 3(1) article 9

Examples

Salt Selection I

- Mechanistic absorption model was used for sensitivity analysis to determine solubility requirements for the select salt form
- Animal PK study (rat) was conducted to verify the predictions

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DOI: 10.1208/s12248-013-9519-x

Research Article

Incorporation of Physiologically Based Pharmacokinetic Modeling in the Evaluation of Solubility Requirements for the Salt Selection Process: A Case Study Using Phenytoin

Po-Chang Chiang[1,3] and Harvey Wong[2,3]

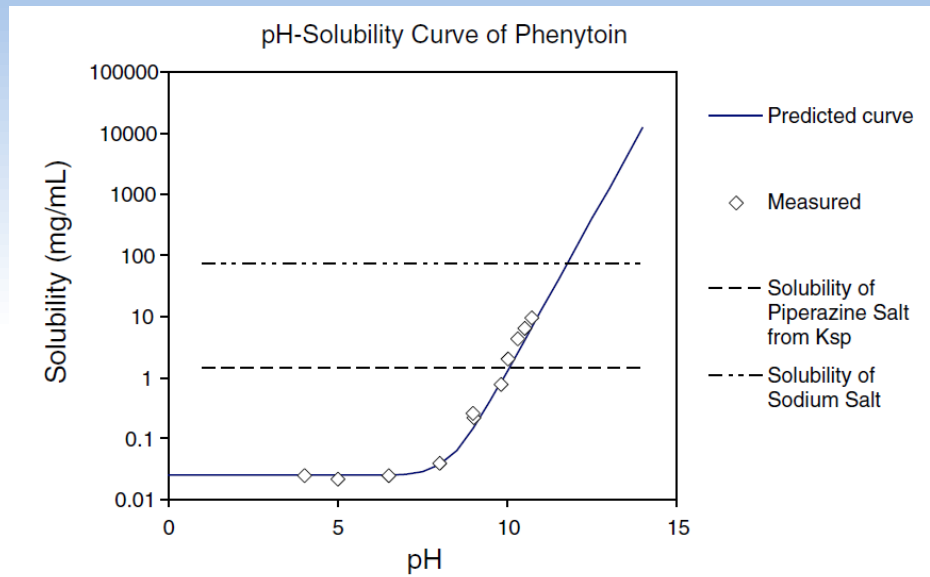

Fig. 1. A pH solubility curve of phenytoin and calculated solubility of the piperazine salt (K_{sp}) and sodium salt (assuming pH_{max} reached)

Table I. Phenytoin Salt Solubility Evaluation

Salt	Solubility from water (mg/mL)	Main component of salt sample recovered from solubility testing determined by PXRD and FT-Raman	Supersaturation ratio (S_x)
Ethylenediamine	9.5	Free acid	475
Ethanolamine	10.3	Free acid	515
Sodium	73.4	Salt (major component)+Free acid	3670
Piperazine	1.2	Salt	60
Piperidine	8.5	Free acid	425

Salt Selection II

PBPK model was developed and verified by predicting the exposure after administration of phenytoin free acid administration

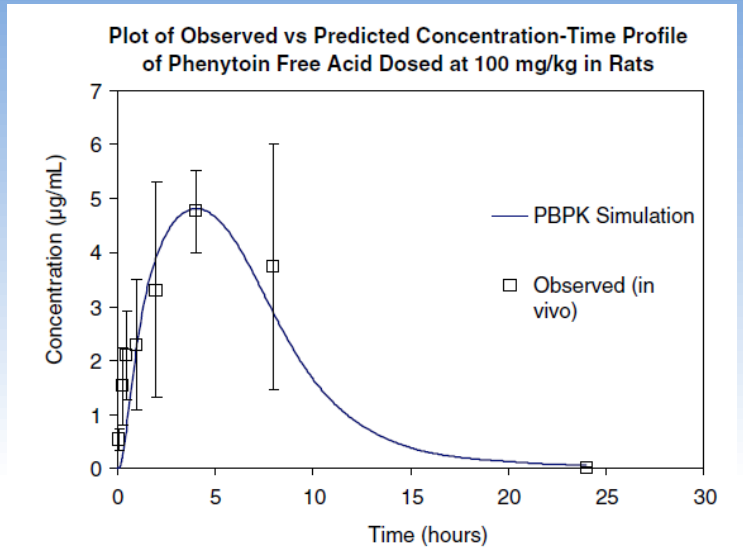

Fig. 2. Plot of observed *vs.* predicted concentration–time profile of phenytoin free acid dosed at 100 mg/kg in rats (n=3). Simulations were performed using an oral rat PBPK model (See supplemental Fig. 3 for more information on simulation)

Sensitivity analysis showed significantly increased absorption for even the lowest salt solubility

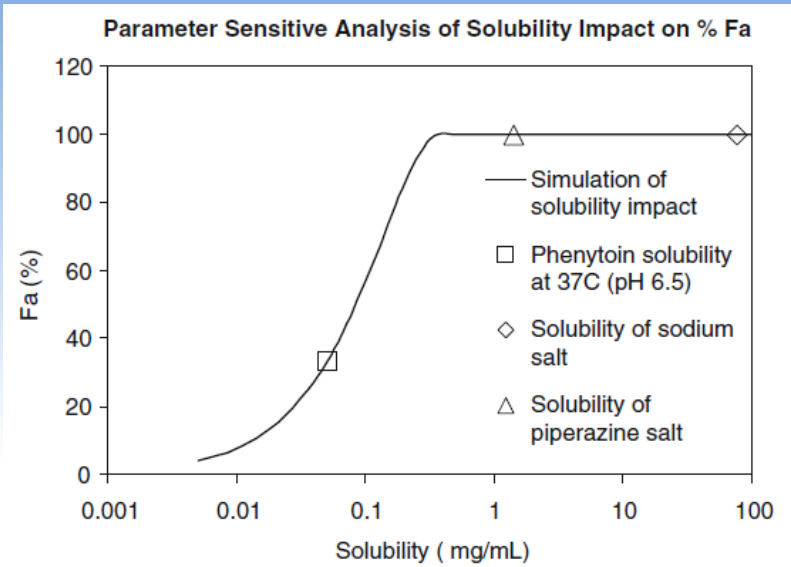

Fig. 3. A parameter sensitive analysis showing the relationship between solubility and the percent of a 100 mg/kg phenytoin dose that is absorbed

in vivo study in rat confirmed the results of sensitivity analysis

Table II. *In Vivo* Pharmacokinetics of Phenytoin Following Administration of a 100 mg/kg Oral Dose to Rats in the Form of the Free Acid or Salts (n=3 per Dose Group)

Group	Form dosed	Precipitation inhibitor	AUC (µM×h)	C_{max} (µM)	%F
1	Free acid	No	155±61	18±1	34±8
2	Na salt	No	444±96*	36±6*	97±22*
4	Piperazine salt	No	493±159*	36±12*	107±40*
3	Na salt	Yes	405±168*	35±15*	88±36*
5	Piperazine salt	Yes	405±74*	30±4*	88±19*

AUC area under the concentration–time profile, C_{max} maximum observed concentration
*p<0.05; significantly different than group 1 using ANOVA followed by the least significant difference (LSD) post-hoc test

Select Formulation to Mitigate Food Effect I

AAPS PharmSciTech, Vol. 14, No. 3, September 2013 (© 2013)
DOI: 10.1208/s12249-013-0018-2

Research Article

Theme: Leveraging BCS Classification and in-silico Modeling for Product Development
Guest Editors: Divyakant Desai, John Crison, and Peter Timmins

Utility of Physiologically Based Modeling and Preclinical *In Vitro*/*In Vivo* Data to Mitigate Positive Food Effect in a BCS Class 2 Compound

Binfeng Xia,[1] Tycho Heimbach,[1,4] Tsu-han Lin,[1] Shoufeng Li,[2] Hefei Zhang,[3] Jennifer Sheng,[3] and Handan He[1]

NVS123

- weak base with pH-dependent and limited solubility
- when administered as dry filled capsules displayed positive food effect
- *in vitro, in vivo* preclinical (F1-F4) and/or clinical (F1-F3) studies and PBPK modeling was used to evaluate formulation strategies to mitigate the food effect.

Select Formulation to Mitigate Food Effect II

- *In vivo data* from animal (dog) study was used to analyze formulation behavior and determine the most likely cause of observed food effect
- Differences in precipitation rates explained differences between fasted and fed state for the four formulations; these differences were supported also by *in vitro* experiments where precipitation was fasted in FaSSIF than in FeSSIF media for F1-F3.

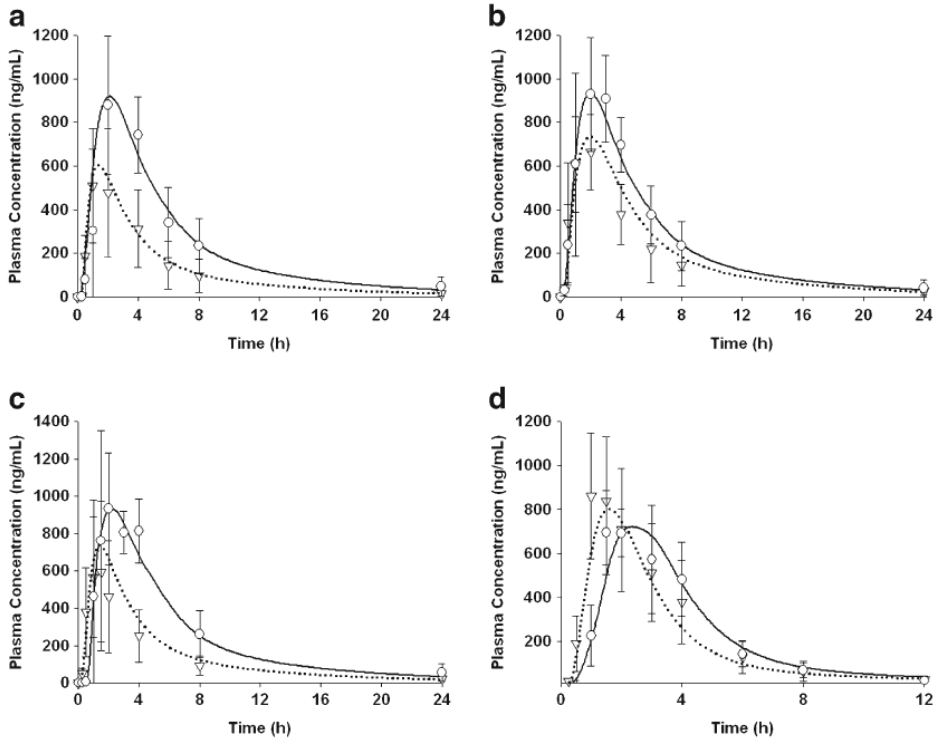

Fig. 2. Observed and simulated mean plasma concentrations after a single administration of 50 mg of NVS123 given as F1 (**a**), F2 (**b**), F3 (**c**), or F4 (**d**) formulation in dogs under fasted and fed state. Symbol annotation: *open triangles* observed fasted concentration with standard deviation; *open circles* observed fed concentration with standard deviation; *dotted curve* simulated mean fasted concentration; and *solid curve* simulated fed concentration

Table II. Comparison of Simulated (SIM) *vs.* Observed (OBS) Mean Plasma PK Parameters of NVS123 in Dogs

	F1		F2		F3		F4	
Parameters	OBS	SIM	OBS	SIM	OBS	SIM	OBS	SIM
Dose (mg)								
Fasted	50	50	50	50	50	50	50	50
Fed								
C_{max} (ng/mL)								
Fasted	510	610	670	735	595	737	560	801
Fed	880	920	929	935	934	932	695	720
AUC_{0-inf} (μg×h/mL)								
Fasted	3.22	3.20	4.57	4.71	3.31	3.81	3.15	2.80
Fed	6.60	6.10	7.02	6.12	7.75	6.16	2.89	3.13
t_{max} (h)								
Fasted	1	1.4	2	1.7	1.5	1.4	1	1.6
Fed	2	2.1	2	2	2	2.2	1.5	2.3
Precipitation time (s)[a]								
Fasted	N/A	1,000	N/A	1,800	N/A	1,500	N/A	4,000
Fed	N/A	3,500	N/A	3,000	N/A	3,000	N/A	4,000
Correlation coefficient (R^2)[b]								
Fasted	N/A	0.98	N/A	0.94	N/A	0.9	N/A	0.91
Fed	N/A	0.9	N/A	0.99	N/A	0.98	N/A	0.92
RMSE[c]								
Fasted	N/A	32.1	N/A	77.7	N/A	99.5	N/A	95.0
Fed	N/A	104	N/A	37.3	N/A	47.3	N/A	77.0

[a] Precipitation time (T_p) was fitted against *in vivo* dog PK profiles in the model
[b] Correlation coefficient between the observed concentration and simulated values
[c] Root mean square prediction error (RMSE) of plasma concentration. $RMSE = \sqrt{\sum(SIM-OBS)^2/N}$ where N is the number of observed data points

Select Formulation to Mitigate Food Effect III

- *In vivo* data for the F1-F3 formulations in human was used to confirm that the fitted precipitation rates in dog translated to human and to evaluate relationship between *in vitro* and *in vivo* dissolution
- The methodology was applied to predict behavior F4 in fasted and fed condition in human

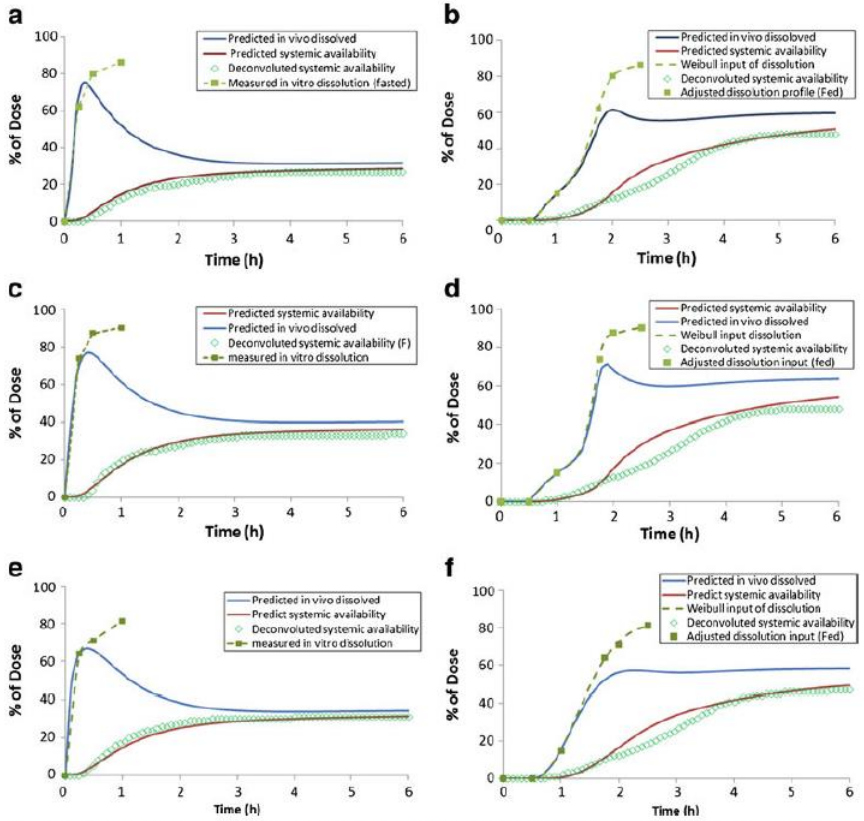

Fig. 3. Comparison of *in vitro* dissolution, adjusted Weibull input dissolution, and predicted *in vivo* dissolution as well as deconvoluted and predicted systemic availability for F1 (**a**, **b**), F2 (**c**, **d**), and F3 (**e**, **f**) formulation under fasted (**a**, **c**, **d**) and fed (**b**, **d**, **f**) state

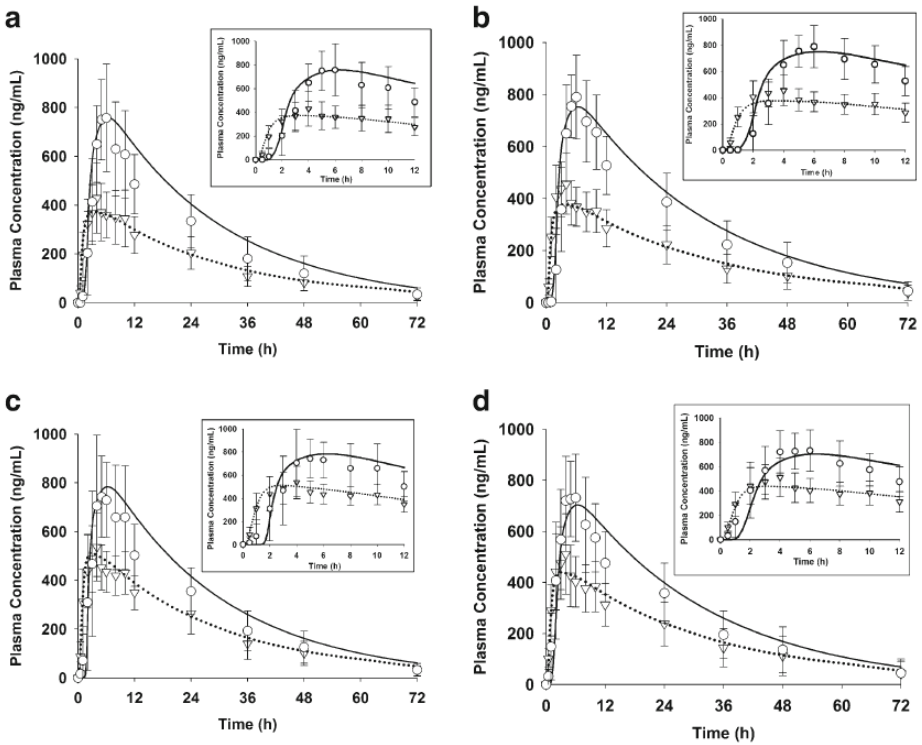

Fig. 4. Observed and simulated mean plasma concentrations after a single administration of 200 mg of NVS123 given as F1-arm 1 (**a**), F1-arm 2 (**b**), F2 (**c**), or F3 (**d**) formulation in humans under fasted and fed state. Symbol annotation: *open triangles* observed fasted concentration with standard deviation; *open circles* observed fed concentration with standard deviation; *dotted curve* simulated mean fasted concentration, and *solid curve* simulated fed concentration. *Insert panel*: observed and simulated mean plasma concentrations of each formulation from 0 to 12 h

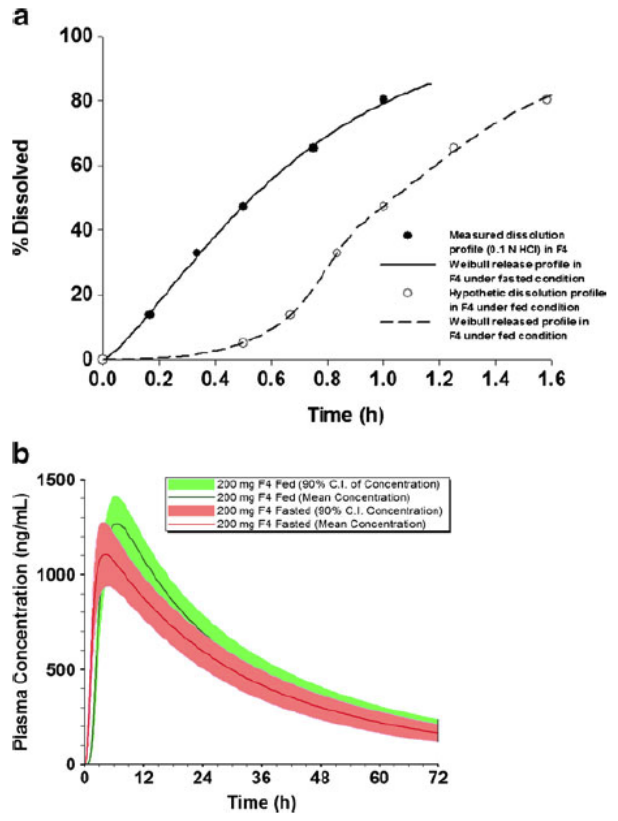

Fig. 6. **a** Comparison of *in vitro* dissolution and adjusted Weibull input dissolution for F4. **b** Simulated plasma concentration with 90% confidence interval (*CI*) after a single dose of F4 formulation (200 mg) under fasted and fed state

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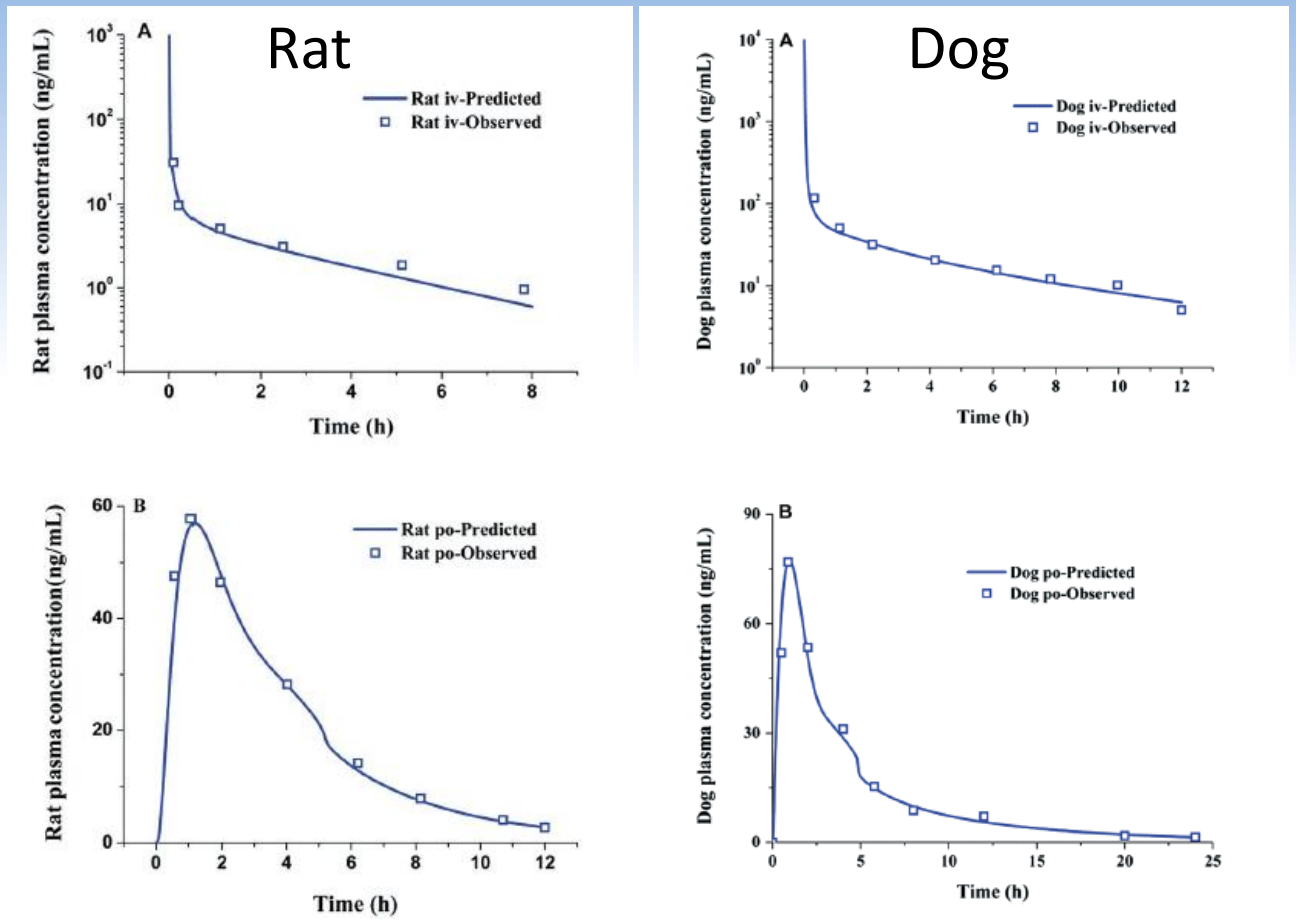
Select the Most Relevant *in vitro* Assay I

Lacidipine

- Rat and Dog data after IV and PO suspension administration were used to validate prediction of systemic distribution, elimination and intestinal absorption
- Dog PO data was used to select the most predictive *in vitro* dissolution experiment and validate methodology for *in vitro – in vivo* dissolution extrapolation



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Interspecies prediction of oral pharmacokinetics of different lacidipine formulations from dogs to human: physiologically based pharmacokinetic modelling combined with biorelevant dissolution

Chunnuan Wu,[a] Longfa Kou,[a] Panqin Ma,[b] Lifang Gao,[a] Bo Li,[a] Ran Li,[a] Cong Luo,[a] Jianzhong Shentu,[c] Zhonggui He[a] and Jin Sun*[ad]

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Select the Most Relevant *in vitro* Assay II

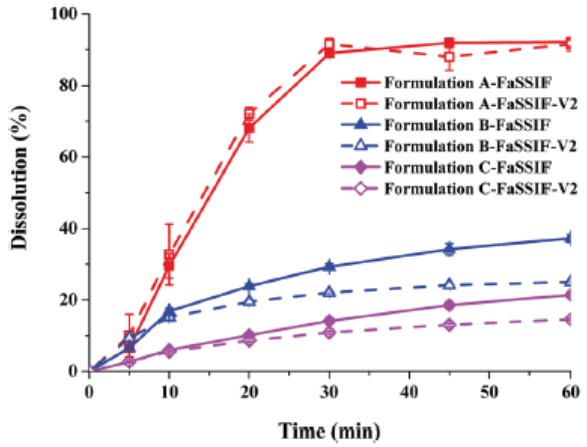


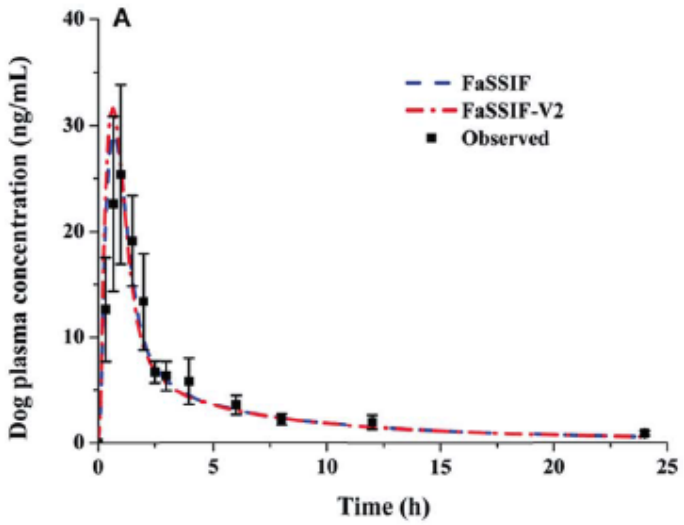
Fig. 1 Dissolution profiles of three lacidipine formulations in bio-relevant dissolution media (data are mean ± S.D., $n = 3$).

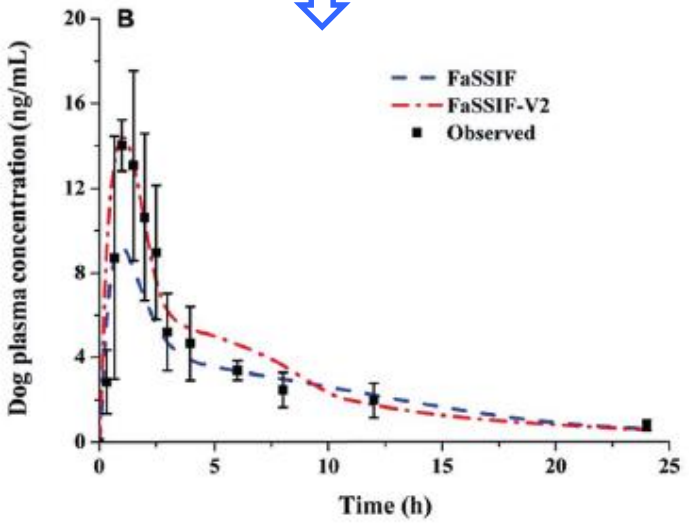
$$\frac{dM_D}{dt} = zM_{u,0}\left(\frac{M_{u,t}}{M_{u,0}}\right)^{2/3}(C_s - C_l)$$

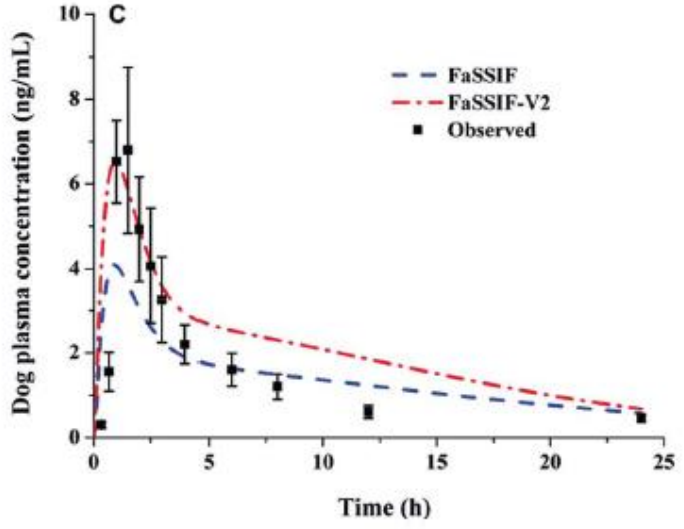
Table 3 The Z-factor values in different dissolution media for the three lacidipine formulations (unit: mL mg^{-1} s^{-1})

	Formulation A	Formulation B	Formulation C
FaSSIF	0.010	0.059	0.021
FaSSIF-V2	0.012	0.199	0.045

Dog data after PO administration of different formulations was used to select the most predictive *in vitro* dissolution experiment and test the methodology for prediction of *in vivo* dissolution







Select the Most Relevant *in vitro* Assay III

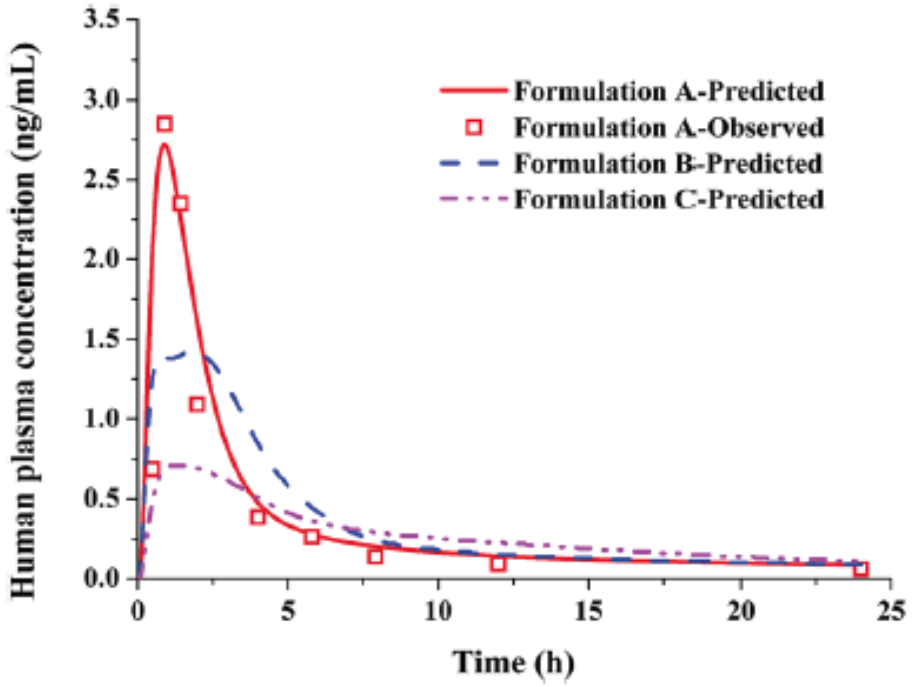


Fig. 8 The simulated and observed human *in vivo* PK profiles for the three lacidipine formulations using the *Z*-factor form FaSSIF-V2 dissolution media.

The data from most predictive *in vitro* dissolution experiment was used to predict human PK

Explore Interspecies Differences in Oral Absorption I

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Research Article

Theme: Revisiting IVIVC (In Vitro-In Vivo Correlation)
Guest Editors: Amin Rostami Hodjegan and Marilyn N. Martinez

Use of Modeling and Simulation Tools for Understanding the Impact of Formulation on the Absorption of a Low Solubility Compound: Ciprofloxacin

Marilyn Martinez,[1,2] Bipin Mistry,[1] Viera Lukacova,[1] Jim Polli,[1] Stephen Hoag,[1] Thomas Dowling,[1] Ravikanth Kona,[1] and Raafat Fahmy[1]

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DOI: 10.1208/s12248-017-0055-y

Research Article

Theme: Revisiting IVIVC (In Vitro-In Vivo Correlation)
Guest Editors: Amin Rostami Hodjegan and Marilyn N. Martinez

Exploring Canine-Human Differences in Product Performance. Part II: Use of Modeling and Simulation to Explore the Impact of Formulation on Ciprofloxacin *In Vivo* Absorption and Dissolution in Dogs

M. N. Martinez,[1,7] B. Mistry,[1] V. Lukacova,[2] K. A. Lentz,[3] J. E. Polli,[4] S. W. Hoag,[4] T. Dowling,[5] R. Kona,[6] and R. M. Fahmy[1]

Ciprofloxacin:

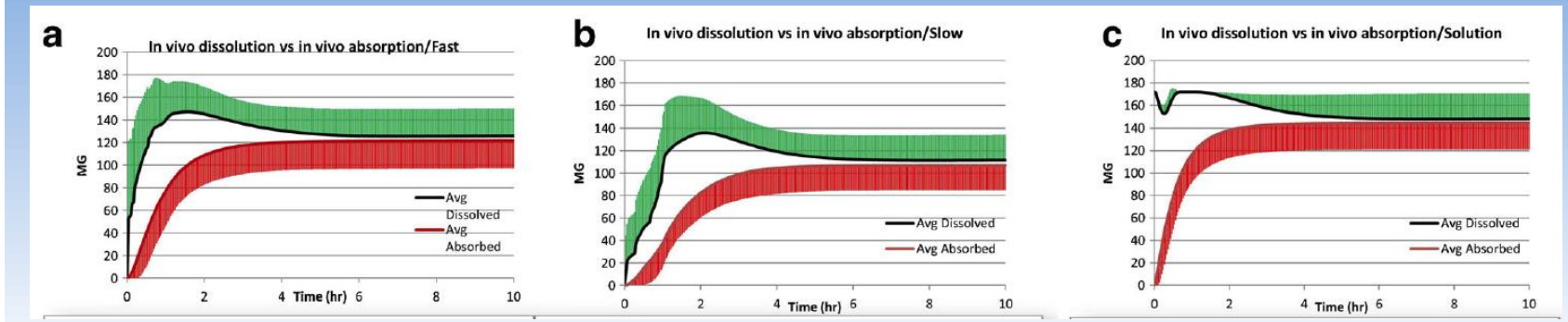
- Mechanistic absorption/pharmacokinetic models for ciprofloxacin were used to deconvolute dissolution and absorption behavior after oral administration (solution and two table formulations) in human and dog
- Deconvoluted dissolution and absorption profiles provided insights into causes of intersubject variability and interspecies differences in ciprofloxacin behavior *in vivo*

Explore Interspecies Differences in Oral Absorption II

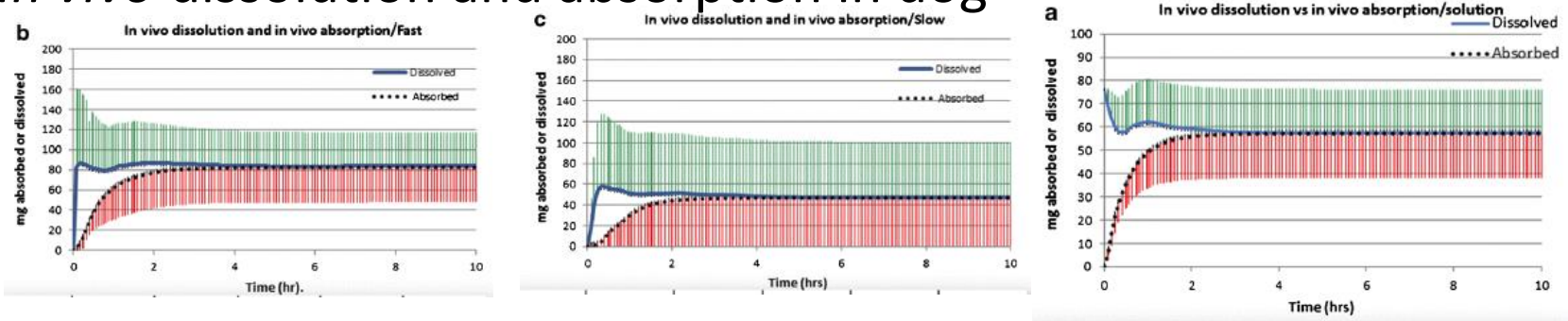
Table II. The ADMET predictor-generated ciprofloxacin physico-chemical characteristics

Molecular weight (g/mol)	331.35
pKa	
Acid	5.75
Base	8.9
Aqueous solubility	
pH 7.32	0.0266
Simulated gastric fluid	5.54
Fasted simulated small intestinal fluid	0.76
pH = 6.5	
Bile salt concentration = 3 mM	
Fed simulated small intestinal fluid	0.73
pH = 5.0	
Bile salt concentration = 15 mM	
Solubilization ratio	2.30E + 05
Log P	0.81
Log D	
pH = 1.2	−1.9
pH = 4.6	−1.67
pH = 6.8	−0.85
pH = 7.4	−0.83
Diffusion coefficient ($cm^2/s \times 10^5$)	0.76
Peff ($cm/s \times 10^4$)	0.56

in vivo dissolution and absorption in human



in vivo dissolution and absorption in dog



Explore Interspecies Differences in Oral Absorption III

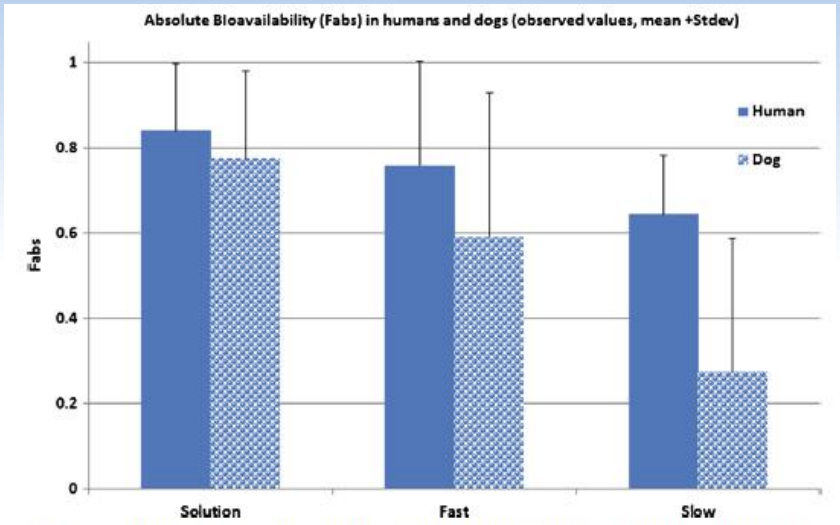


Fig. 2. Comparison of observed ciprofloxacin absolute bioavailability in the three formulations: dogs ($n = 5$) *versus* human ($n = 16$)

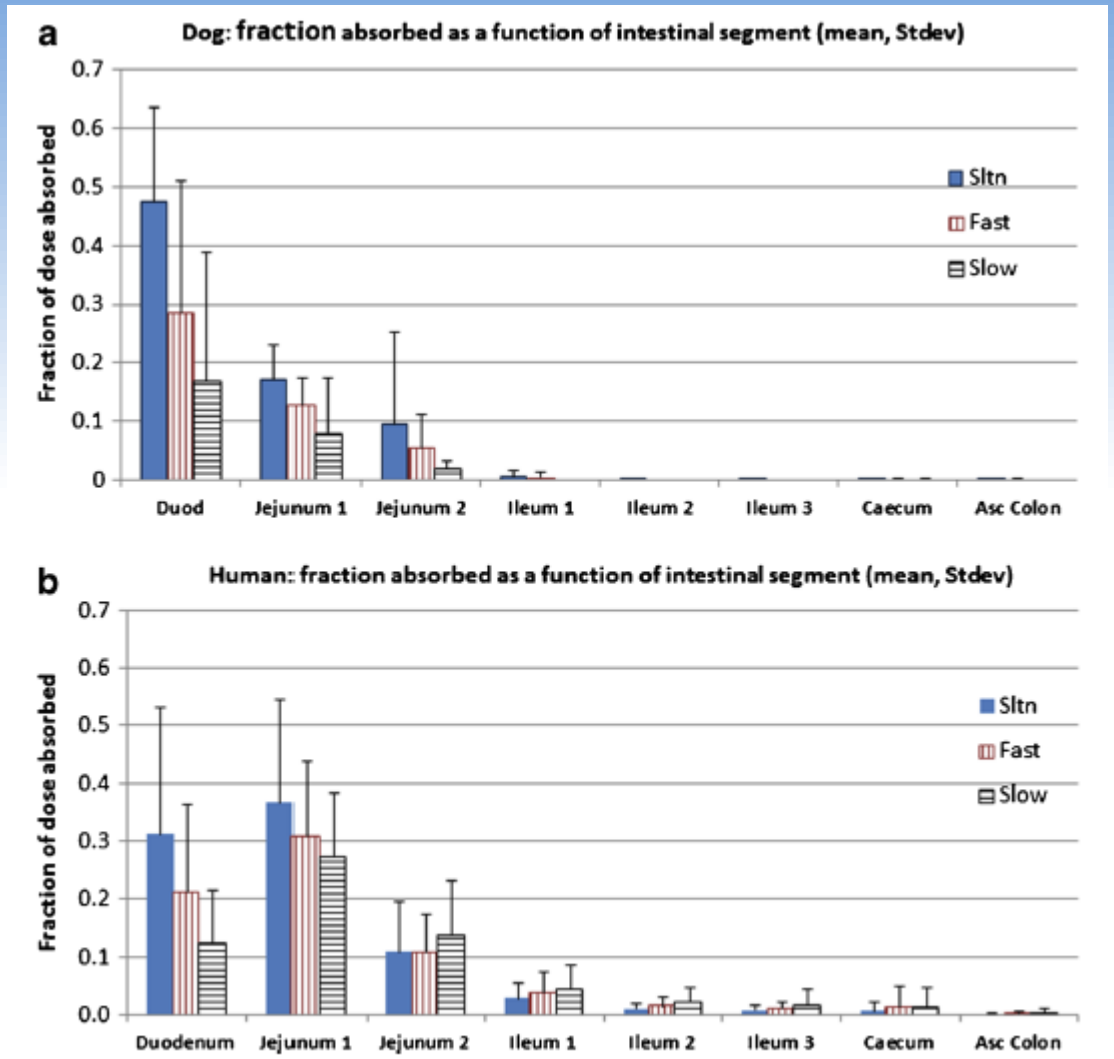


Fig. 5. Mean fraction (stdev) of administered dose absorbed as a function of formulation across the intestinal segments of dogs (**a**) and humans (**b**)

Summary

- PBPK models provide unique platform to combine information from *in vitro, in silico* and animal assays for accurate prediction of complex drug behavior *in vivo*
- These models are now routinely used to predict first-in-human exposure, and applications in the area of formulation design and development have also been increasing in last few years
- The models are useful not only for prediction of drug exposure before an *in vivo* study, but are invaluable tool in investigation of complex drug/formulation behaviors observed *in vivo*
- There are still gaps in characterization of physiologies (especially in animals), closing these gaps will further increase accuracy and utility of these models

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Consulting Studies

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Discovery Cheminformatics

Thank you for your kind attention!