Teaching of Drug Disposition using Physiologically Based Pharmacokinetic Modeling Software: GastroPlus as an Educational Tool

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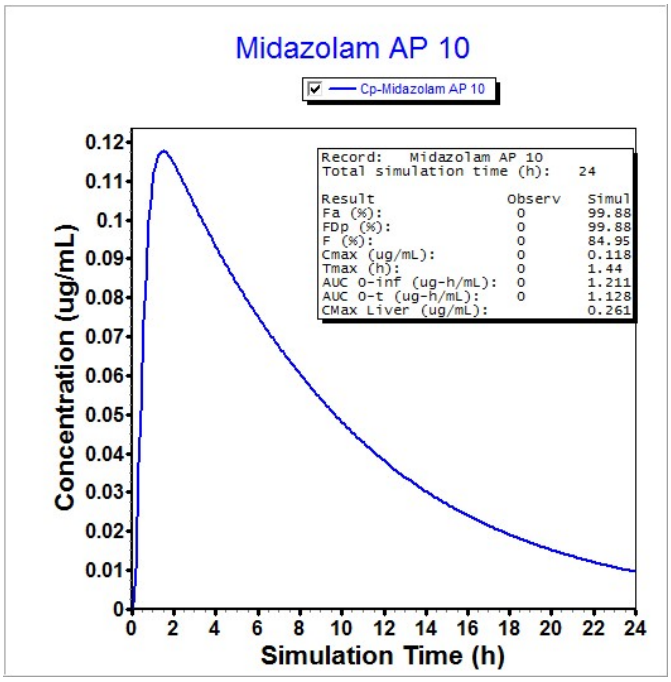
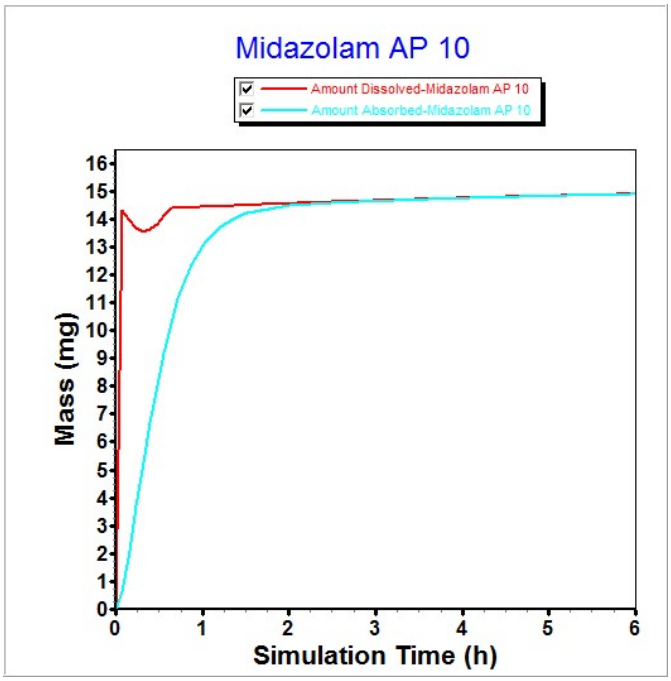
Supporting Information

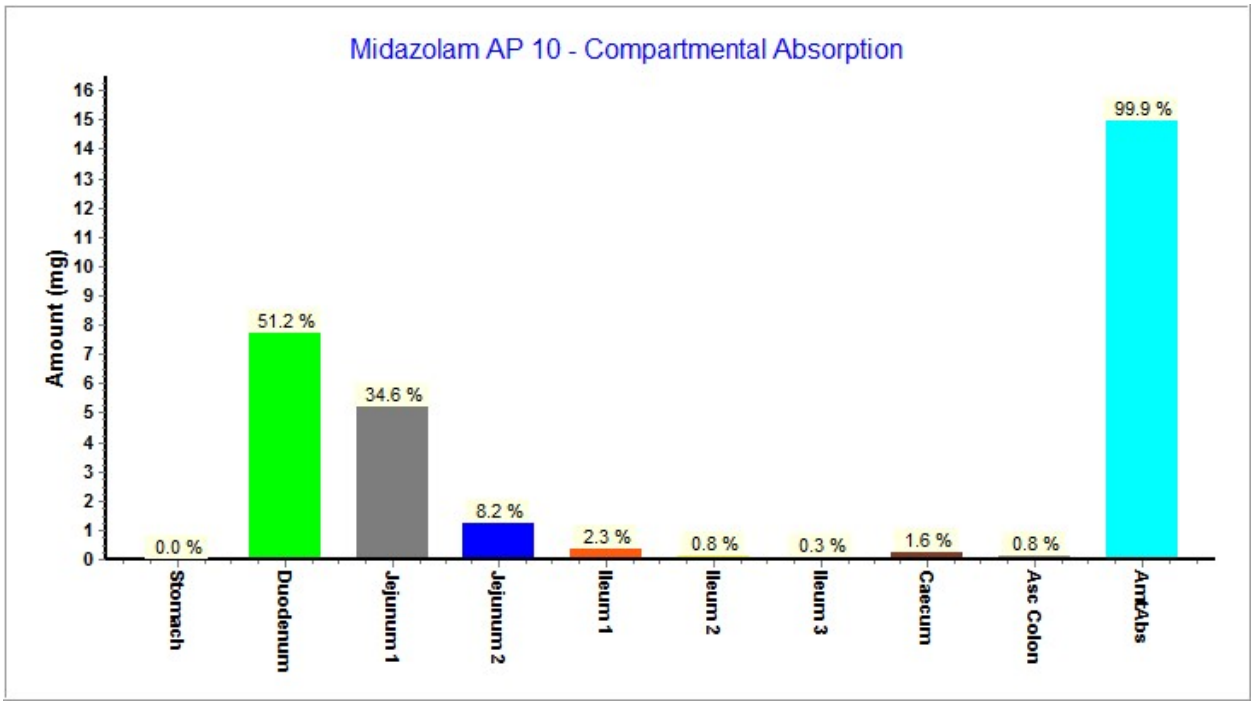
Supplementary Figure 1: GastroPlus screenshots of example project with midazolam with scientific explanations. This is a 24-part figure labeled A-X.



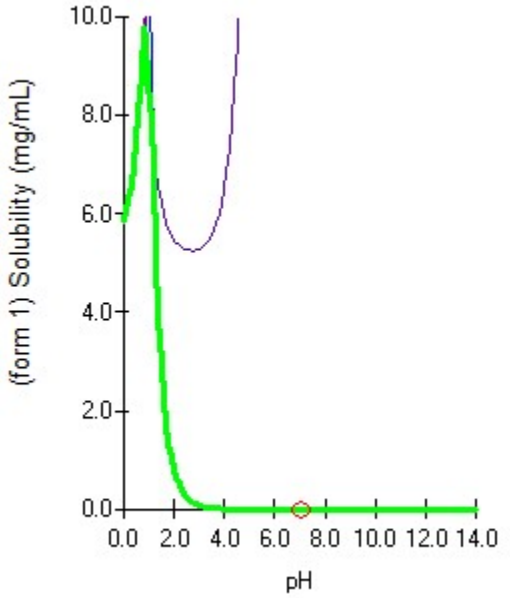
A. Cp-time profile and summary of results from the midazolam model based on properties predicted in silico by ADMET Predictor® (AP) v10.0. This simulation used a dose of a single 15 mg oral tablet.



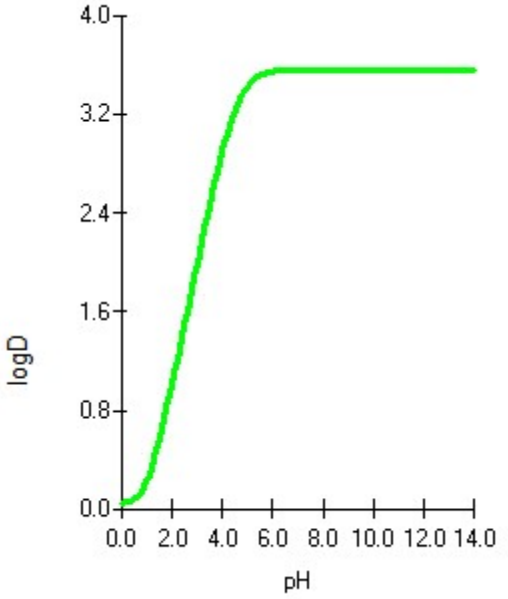
B. Dissolution and absorption curves from the midazolam model based on properties predicted in silico by ADMET Predictor (AP) v10.0. Note that while some precipitation is occurring, absorption is limited by permeation.



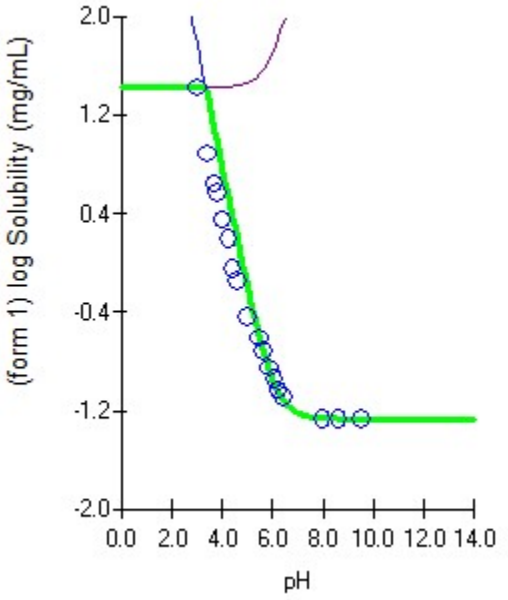
C. Regional absorption plot for midazolam based on properties predicted in silico by ADMET Predictor (AP) v10.0.



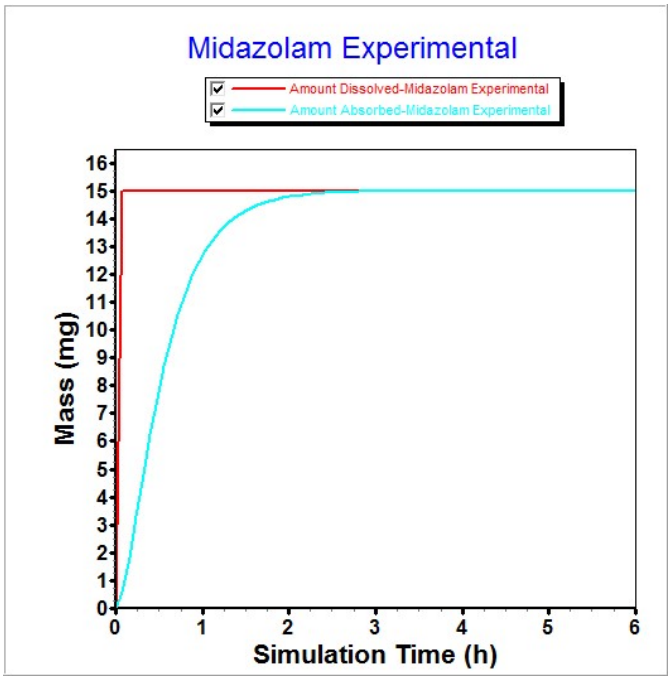
D. Predicted pH-solubility profile of midazolam. The AP-predicted pKa values are: 4.57 (base) and 0.84 (base), with a predicted solubility factor of 2488.5. Note that midazolam is ionized and increasingly soluble at low pH, such as that in the fasted stomach (~1.2). As the pH approaches that of the small intestine (~6), solubility decreases substantially and precipitation may occur, as seen in B.



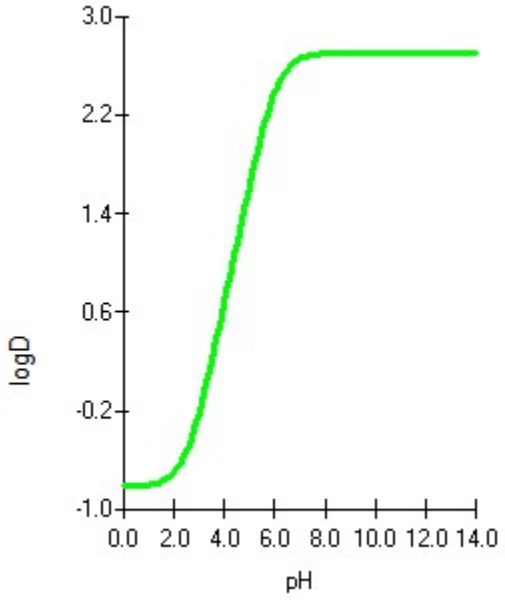
E. pH-LogD profile of midazolam based on predicted pKa (D) and logP (3.56) values. Note that logD has increased to a maximum value by pH 6, implying that most molecules are in the neutral lipophilic form that can be absorbed via passive diffusion. This explains why most of the compound absorbs in the duodenum (C), and almost all remaining molecules (i.e. those that transit through the duodenum before being absorbed) are absorbed quickly in the jejunum. This also explains the relatively early Tmax of ~1.5 h (A).



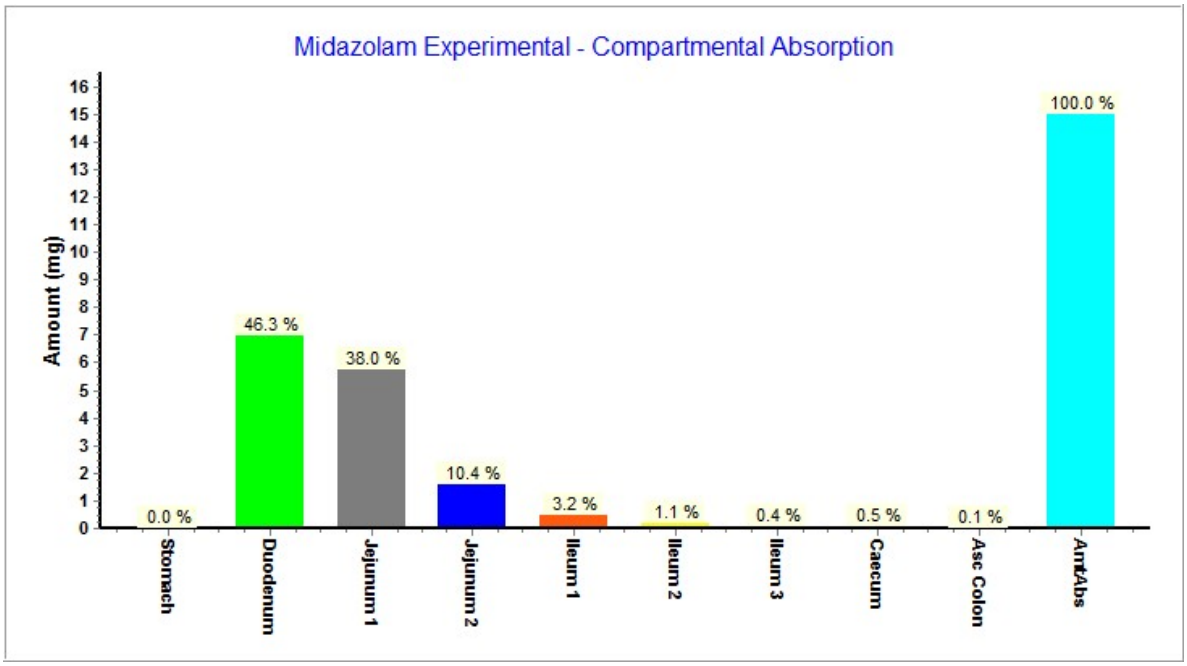
F. pH-solubility profile of midazolam fit to *in vitro* solubility data (pKa = 6.04, Solubility Factor = 487). Observed data are shown as blue circles.



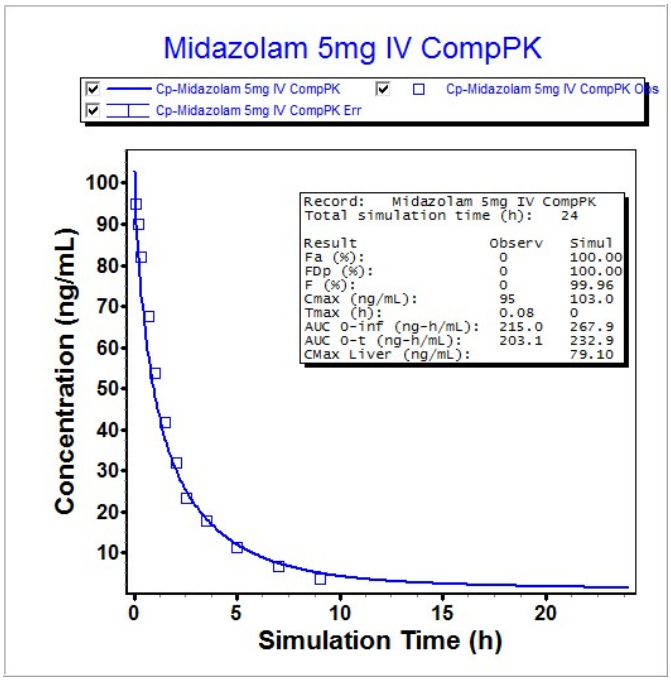
G. Dissolution and absorption plot for midazolam using the new pH-solubility and pKa inputs (F). Note that dissolution is slightly slower now that reference solubility and solubility factor are reduced, but no precipitation occurs as the drug enters the higher pH of the small intestine, as the higher pKa of ~6 (F) results in sufficient drug being ionized at the duodenal pH of ~6.



H. pH-LogD profile of midazolam using the aforementioned pKa and experimentally measured LogP of 2.7. The profile is shifted slightly to right, and has a lower maximum LogD, than the predicted profile in C.



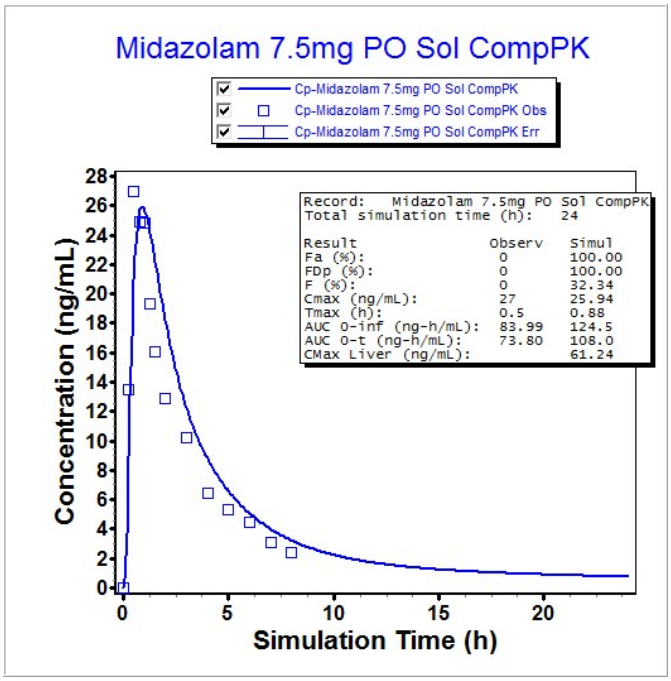
I. Regional absorption plot for midazolam model based on the new pH-LogD profile (H). The drug is still predicted to be completely absorbed, but absorption is occurring slightly later in the small intestine (as pH increases further and more time passes), reflective of both the lower LogP value used and the shift of the pH-LogD profile (H) to the right.



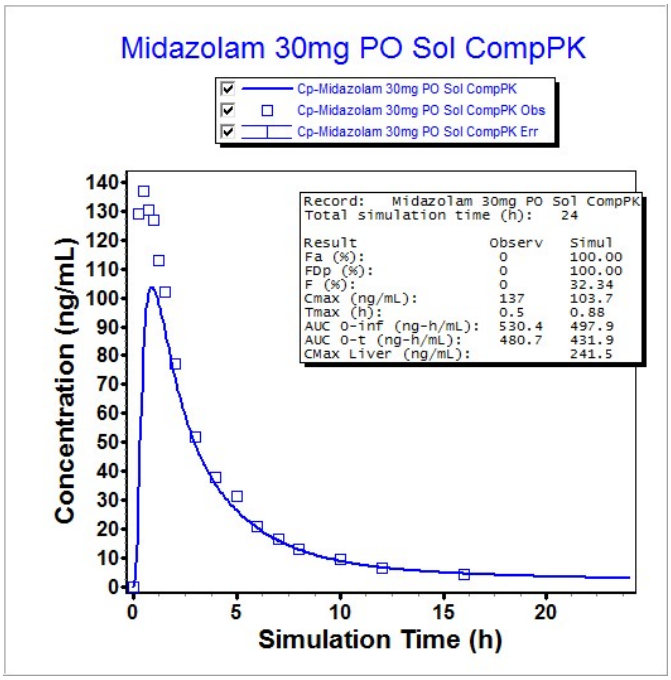
J. Cp-time profile and summary results for midazolam 5 mg IV, using a compartmental pharmacokinetic model fit to the IV data. As may be expected, the model that is fit to these data describes it well. Blue squares represent observed data.

		Renal Clearance CLr (L/h/kg):	0
CL (L/h):	19.113	or (L/h/kg):	0.26454
		Vc (L/kg):	0.6936
		T 1/2 (h):	16.59
K12 (1/h):	0.32843	K13 (1/h):	0.1583
K21 (1/h):	0.8857	K31 (1/h):	0.06224
V2 (L/kg):	0.2572	V3 (L/kg):	1.7641

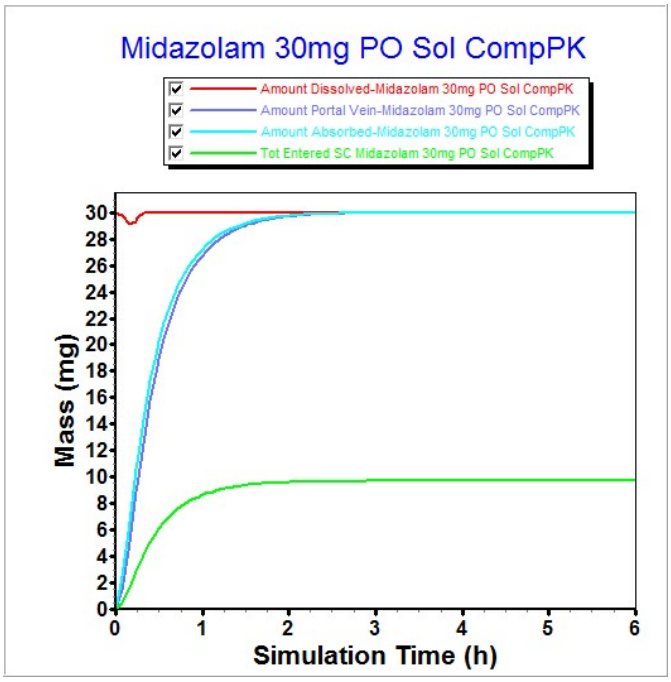
K. Compartmental PK parameters for model in J. Values were fit using the PKPlus™ module in GastroPlus.



L. Cp-time profile and summary results for midazolam 7.5 mg PO solution, using the compartmental model from J-K and the oral absorption model that uses experimental data (F-I). For this dose, the compartmental model and current oral absorption model describe the observed data relatively well. Note that a fixed first pass extraction (FPE) of 67% (fit in PKPlus from oral and IV data) is used in this model.



M. Cp-time profile and summary results for midazolam 30 mg PO solution, using the compartmental model from J-K and the oral absorption model that uses experimental data (F-I). For this dose, the compartmental model (including FPE from L) underpredicts the observed data. This is likely due to the fact that the compartmental model used here cannot capture saturable processes, such as enzymatic FPE in the gut wall and/or liver.

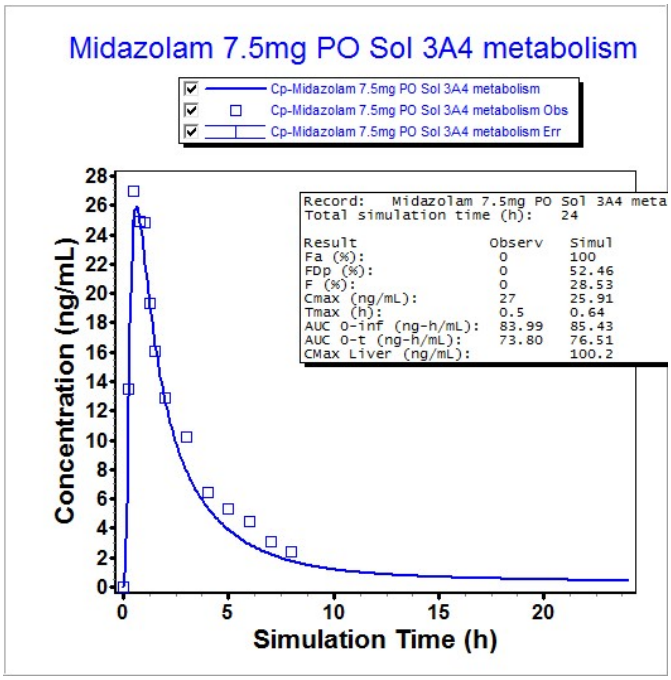


N. Dissolution, absorption, and amount reaching the portal vein and systemic circulation profiles for midazolam 30 mg PO solution (corresponds to Cp-time profile in M). We note that minor precipitation occurs with this dose, but absorption is still permeability limited. Amount entering the portal vein closely follows amount absorbed, reflective of having no FPE or trapping in the gut wall in this model. The amount entering systemic circulation is markedly lower than that in the portal vein, due to the fixed FPE of 67% assigned to the liver (L). As seen in M, the Tmax of this model aligns closely with that of the observed data, indicating that permeation, as the rate limiting step, is likely adequate in this model and does not need to be further adjusted at this time. Similarly, given that the terminal phase in M matches the

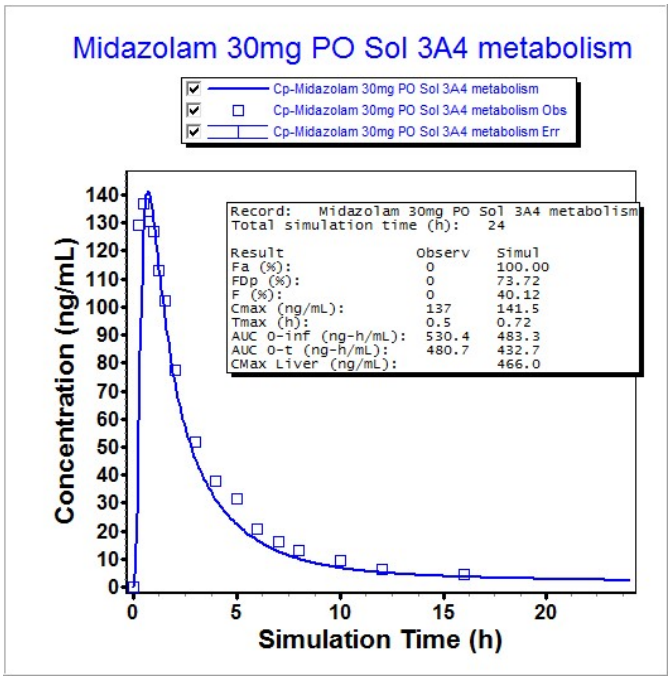
observed data, volume of distribution and systemic clearance are also adequate. This leaves FPE as the most likely explanation for the differences in the simulated and observed data for this dose. A review of the literature indicates that CYP3A4 is the enzyme responsible for midazolam metabolism, and its presence in both the gut wall and the liver can explain the saturable FPE process.

	Generic	Enzyme	Location	Data Source	Vmax (mg/s) or (mg/s/mg-enz)	Km (mg/L)	M
▶	Midazolam 7.5mg PO Sol 3A4	3A4	PBPK	Microsomes	0.000884	0.977	N
	Midazolam 7.5mg PO Sol 3A4 me	3A4	Liver	Microsomes	0.32	0.977	N
	Midazolam 7.5mg PO Sol 3A4 me	3A4	Gut	Microsomes	0.32	0.977	N
*							

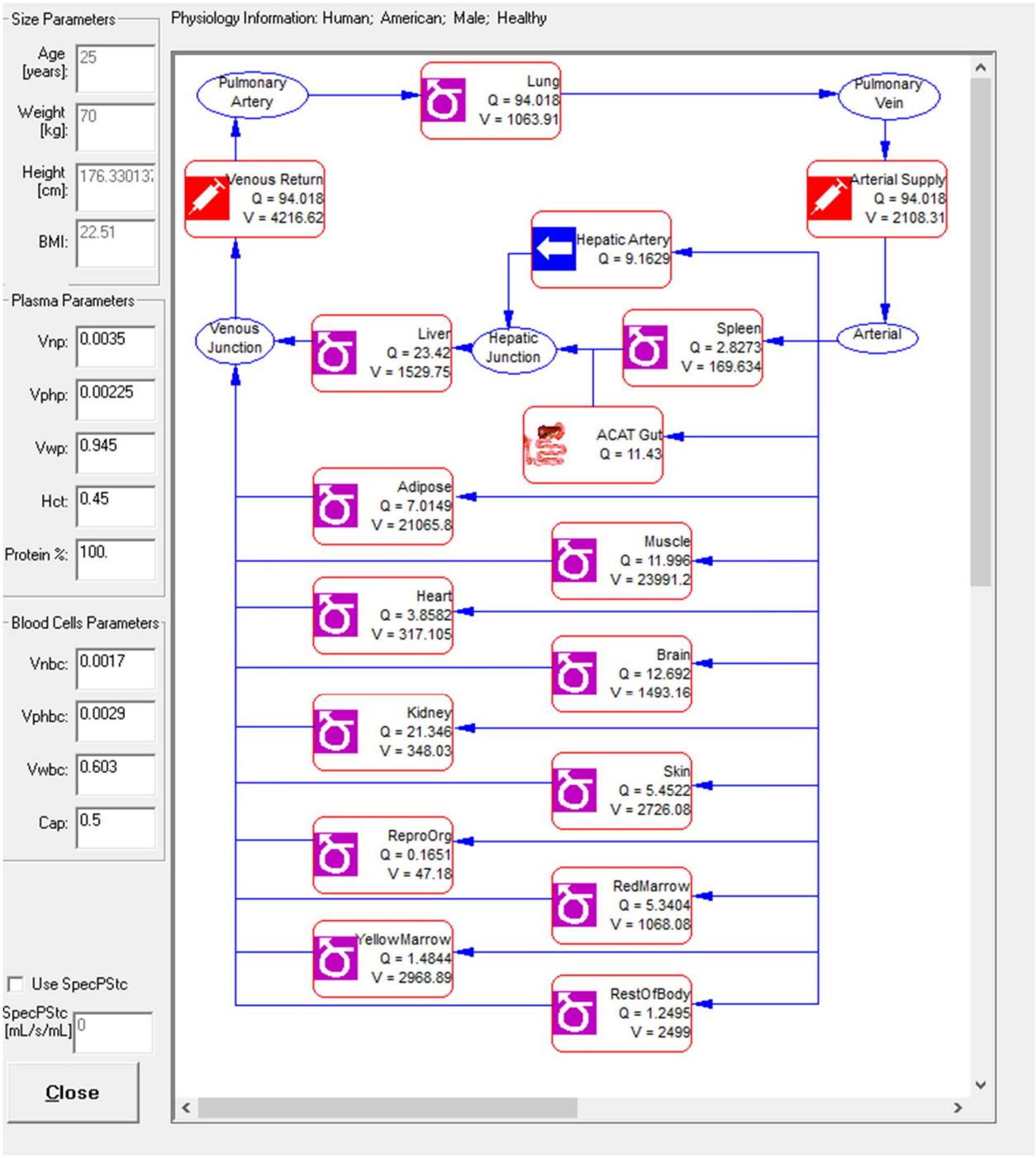
O. *in vivo* kinetic parameters for CYP3A4 metabolism of midazolam added to the model, replacing systemic CL and fixed FPE. *in vitro* metabolic data were taken from the literature, and converted to *in vivo* inputs using the Metabolism and Transporter module in GastroPlus. For compartmental models with nonlinear processes, the "Gut" and "Liver" locations will be used (GastroPlus handles this by attaching a fourth 'liver' compartment capable of calculating nonlinear metabolism to the existing 3-compartment model), while a whole-body PBPK model will use the "Gut" and "PBPK" locations (the PBPK model includes its own Liver, separate from the 'compartmental' liver seen in the second row). Enzyme kinetic data shown were used for all doses.



P. Cp-time profile and summary results for 7.5 mg midazolam PO solution, using the compartmental model with nonlinear CYP3A4 metabolism (O) included. This model is commensurate to the results seen in L.



Q. Cp-time profile and summary results for 30 mg midazolam PO solution, using the compartmental model with nonlinear CYP3A4 metabolism (O) included. This model is significantly improved compared to M.

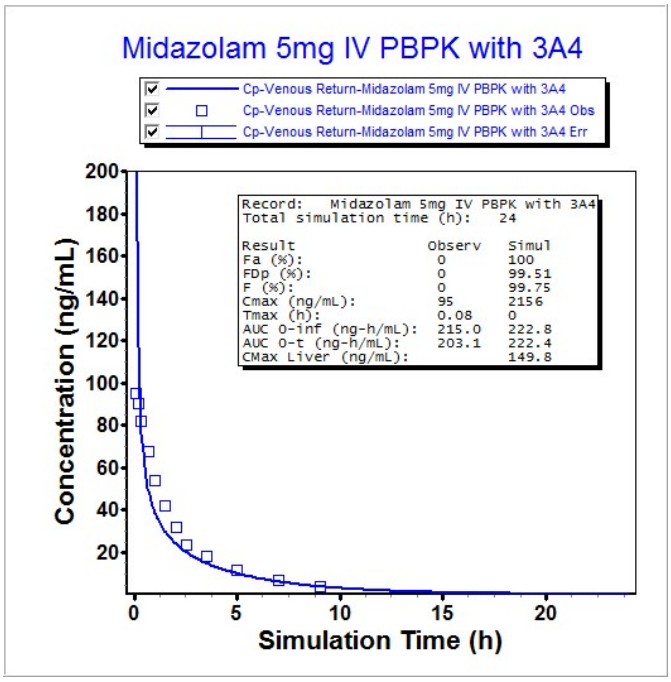


R. Physiology used in PBPK model for midazolam, based on the subject demographics from the clinical study. Note that the same oral absorption model as from previous iterations is used, delivering absorbed drug to portal circulation, which then feeds into the whole body model.

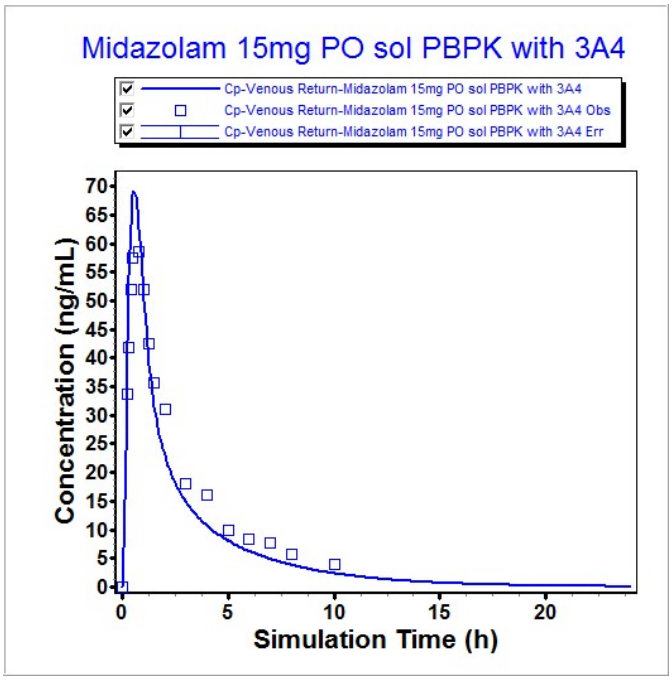
PBPK Summary

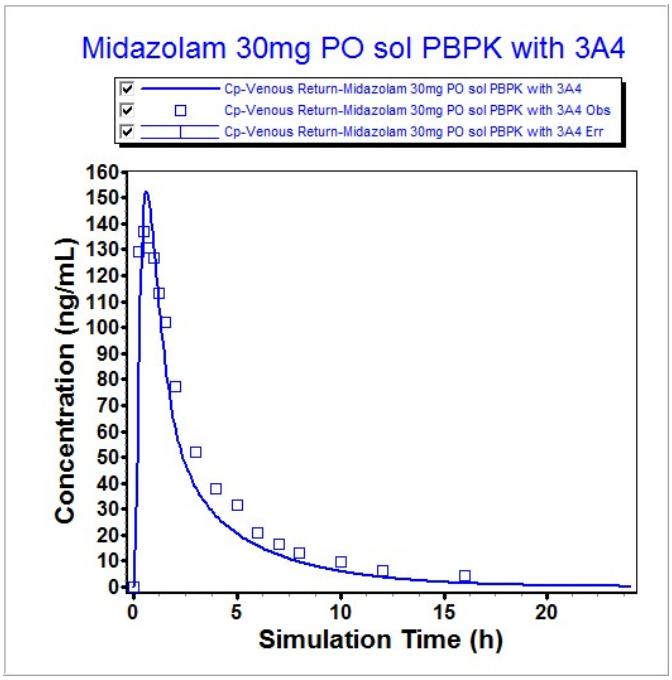
Tissue	Kp	CL	CLint	Fut/FuInt
Hepatic Artery	0.00	0.000	0.000	0.000
Lung	0.31	0.000	0.000	0.105
Arterial Supply	0.00	0.000	0.000	0.000
Venous Return	0.00	0.000	0.000	0.000
Adipose	1.40	0.000	0.000	0.023
Muscle	0.57	0.000	0.000	0.058
Liver	0.90	0.000	0.000	0.037
ACAT Gut	0.00	0.000	0.000	0.000
Spleen	0.60	0.000	0.000	0.055
Heart	0.47	0.000	0.000	0.070
Brain	1.35	0.000	0.000	0.024
Kidney	0.62	0.251	0.000	0.053
Skin	0.86	0.000	0.000	0.038
ReproOrg	0.63	0.000	0.000	0.052
RedMarrow	1.54	0.000	0.000	0.021
YellowMarrow	1.40	0.000	0.000	0.023
RestOfBody	0.62	0.000	0.000	0.053

S. Tissue/plasma partition coefficients (Kp), tissue clearance, and fraction unbound in tissue (Fut) parameters used in the midazolam PBPK model. Kps and Futs were calculated using the Lukacova equation, which uses the molecular properties of ionization, charge, lipophilicity, protein binding, and phospholipid binding, as well as the aqueous, neutral lipid, acidic phospholipid, and protein composition of individual tissues, to estimate partitioning. Renal clearance estimated from fraction unbound in plasma × glomerular filtration rate. This model also includes the enzymatic metabolism (not shown) from O.

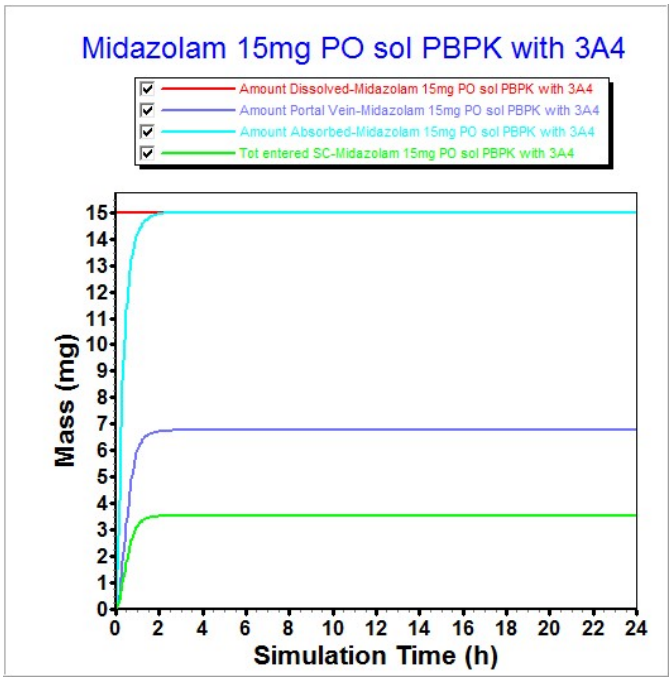


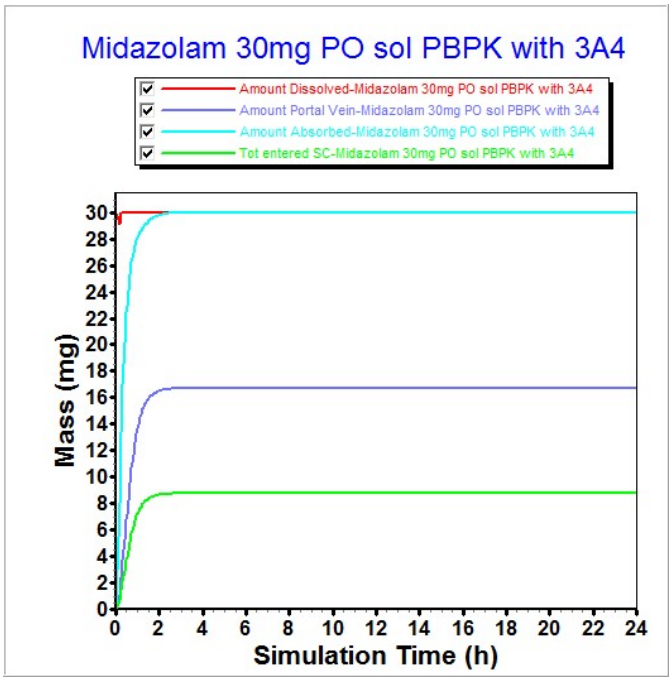
T. Performance of completed PBPK model for midazolam 5 mg IV.



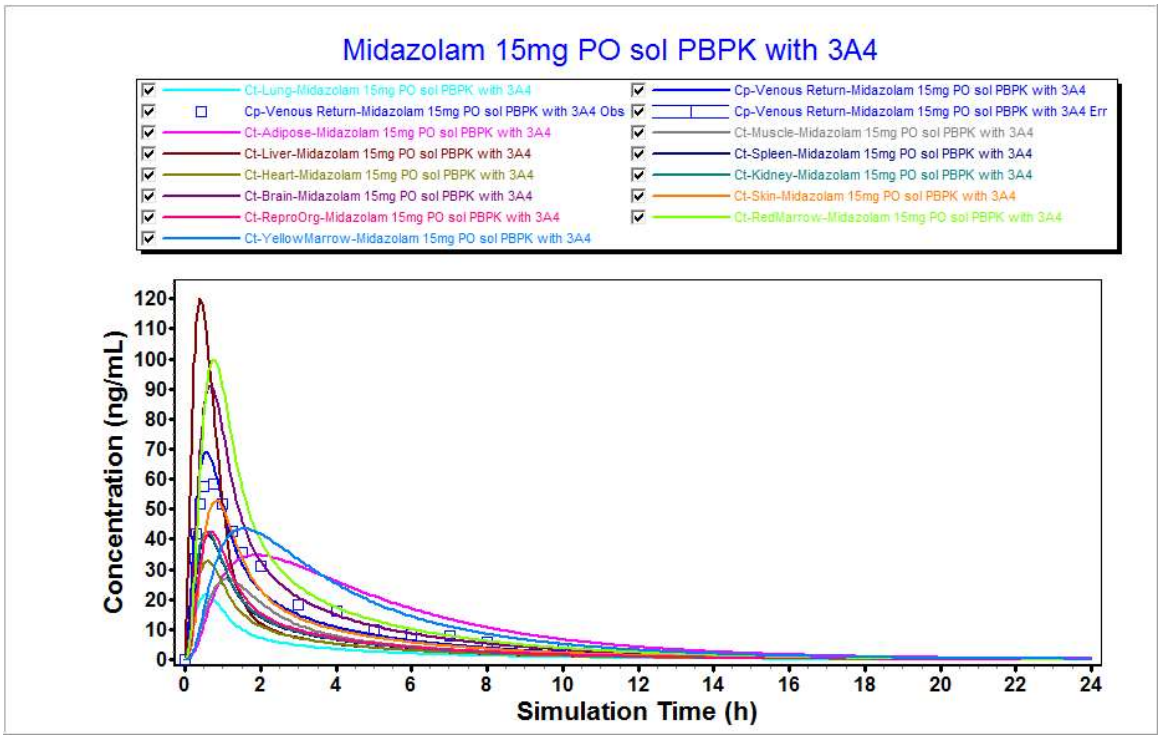


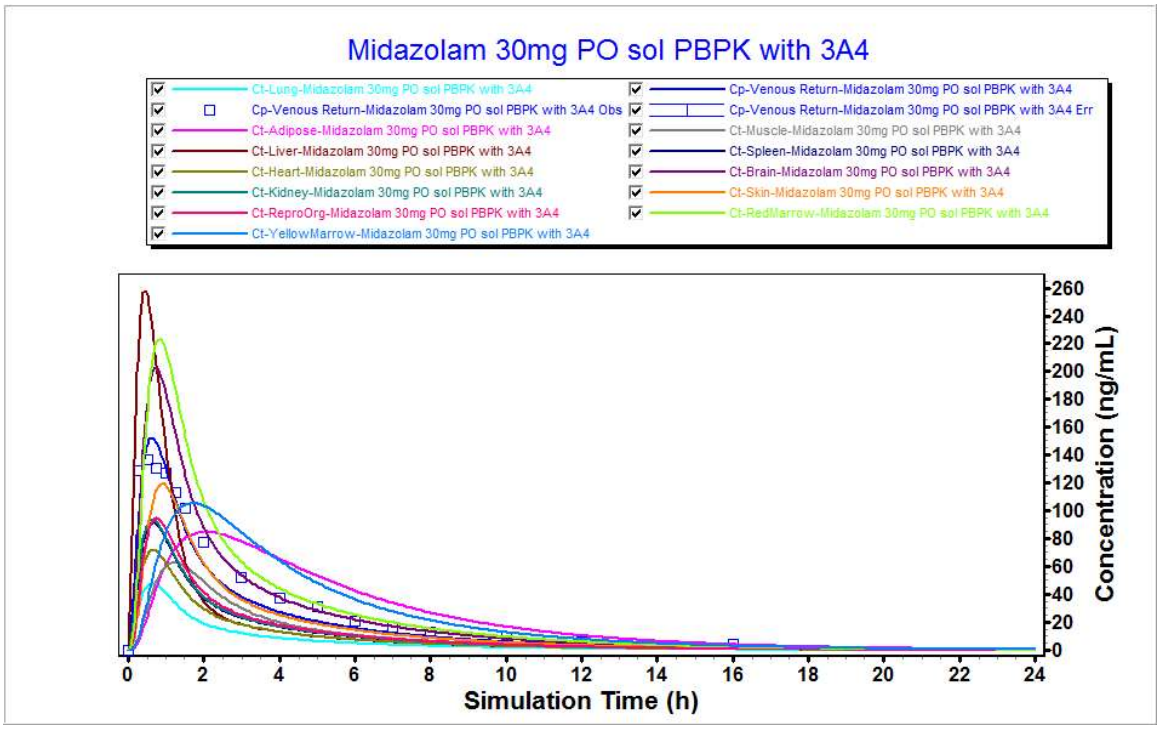
U. Performance of completed PBPK model for midazolam, for both 15 and 30 mg oral solutions. Note that the same model is used in both instances, only the dose and corresponding observed data are different.



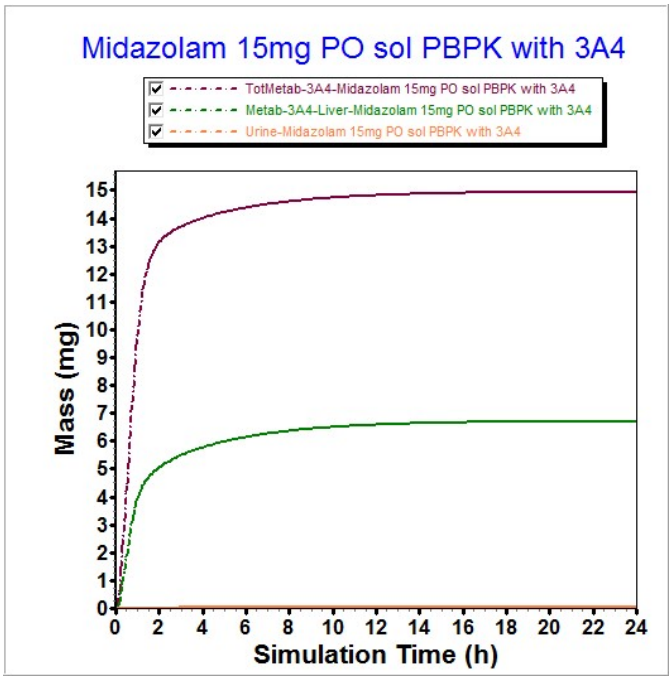


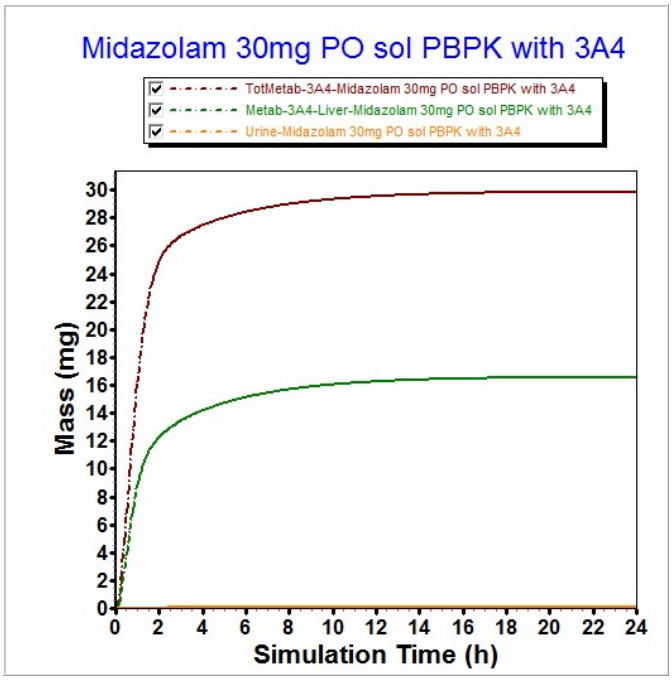
V. Dissolution, absorption, and mass entering portal and systemic circulation plots for PBPK model of midazolam at 15 and 30 mg PO solution doses.





W. Systemic distribution plots for PBPK model of midazolam at 15 and 30 mg PO solution doses.





X. Clearance plots for completed PBPK model of midazolam at 15 and 30 mg PO solution doses. Almost all clearance is through CYP3A4 metabolism, with approximately 55% and 45%, respectively, occurring in the gut wall and the remainder in the liver.

Supplementary Figure 2. Final presentation instructions

Presentation Outline

1. Overview of PBPK model (1-2 slides, 5 min, preferred 1 person)
    a. Review structure and basic physicochemical properties + known enzyme/transporter involvement and other clearance routes (brief recap)
    b. Model Setup
        i. PEAR Physiology
        ii. PBPK settings (include calculated Vss on slide)
        iii. Enzyme/transporter kinetics, dissolution settings, etc.
    c. Model fit
        i. Plot comparing predicted to observed for final PBPK model
        ii. Include oral and IV if applicable
2. Application of model (Per prompt: 1 person, 1-2 slides, 5-7 min)
    a. For each prompt, specify (2 min):
        i. The prompt
        ii. Your approach to modeling the scenario in question, what simulation inputs were shown
        iii. The Cp-time profile(s) and summary statistics of results (see example below)
    b. Discuss the results (3-5min)
        i. How do you interpret the results?
        ii. What specific processes do you identify?
        iii. Is the drug predicted to be safe and effective?
        iv. Include additional figures demonstrating how you reached these conclusions

Other Requirements

1. Always give Age, Weight, BMI, and Sex when showing results and/or observed data
    a. Helpful to include PK params as well (Vss, CL, enzyme/transporter kinetics)
2. Always give units
3. Create figures that are legible and consistent. This includes:
    a. Same Y-axis units and scale when comparing results
    b. Clear colors, markers, and line styles for plot series
    c. Chart title, axis labels, legend
    d. Legible font sizes and colors

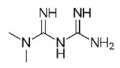
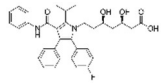
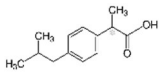
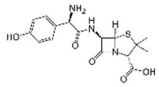
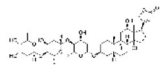
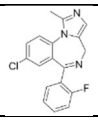
Supplementary Figure 3: Pre- and post-assessment question template

Pre- and Post-Course Assessment

Please answer the following questions using the data sheet provided. Each question should be answered in 1-2 sentences and include an explanation/ justification for the given answer.

1. What is the molecular basis for [drug] having a logP of [logP Value]?
2. Is [drug] ionizable? If so, is it an acid or a base? Justify your reasoning.
3. Where in the gastrointestinal tract does [drug] have the highest solubility and why?
4. What is the most likely mechanism of absorption for [drug], why do you reach this conclusion based on the molecular properties, and what is the charge of the species that crosses the gut wall?
5. What relationship do solubility and permeability have with the bioavailability of [drug]?
6. Based on the molecular properties of [drug], why is the F_{up} [F_{up} value]? What is the effect of [drug]'s F_{up} on volume of distribution and clearance?
7. What is the relationship between the pKa and LogP of [drug] and its distribution into [adipose/muscle] tissue, based on the nature of this tissue?
8. Will [drug] to be cleared primarily by the liver, the kidney, or both? Will [drug] be cleared mainly as the parent compound or as a metabolite?
9. What relationship do AUC and C_{max} have with dose, and why?
10. What are the differences in C_{max} and AUC profiles between a [dose/formulation 1] and a [dose/formulation 2], and what are possible reasons for any differences or lack thereof?
11. What is the effect of food on the pharmacokinetics of [drug] as compared to fasting conditions, and what is a likely explanation?
12. What is the reason for the effect of [interacting gene/interacting agent] on [drug] exposure, where in the body does this effect occur, and how does a comparison with other Cp-time data support your answer?

Supplementary Table 1: Drugs assigned in course project

Group	1	2	3	4	5	Instructors
Drug	Metformin	Atorvastatin	Ibuprofen	Amoxicillin	Digoxin	Midazolam
Structure						
Physicochemical properties	Highly ionized, soluble base	Highly lipophilic acid	Moderately lipophilic acid, low solubility	Hydrophilic zwitterion, formulated as salt	Nonionizable, high molecular weight, amphoteric	Lipophilic, weak base
Absorption	Paracellular	Passive, transporter mediated	Passive	Transporter mediated	Passive	Passive
Protein Binding	<1%	98-99%	99%	20%	25%	95%
Tissue Distribution	High, mainly liver	Liver and muscle	Limited	Soft tissues	Limited	Fatty tissues
Primary Clearance Mechanism	Renal	Hepatic	Hepatic	Renal	Renal	Hepatic
Metabolism		Gut, Liver (CYP3A4)	Liver (CYP)			Liver, Gut (CYP3A4)
Transport	Kidney (OCT2, MATE1), Liver (OCT1), Gut (OCT1)	Liver (OATP1B1), Gut (BCRP)		Gut, Kidney (PEPT1, PEPT2)	Gut, Liver, Kidney (Pgp)	
IV Cp-time Data	yes	no	yes	yes	yes	yes
Oral Cp-time Data	yes	yes	yes	yes	yes	yes

Supplementary Table 2: Experimental data used in midazolam model

A) Physicochemical properties and structure of Midazolam

Property	**Value**	**Reference**
logP	2.7	Roche FH-L: Midazolam (base) Safety Data Sheet. Basel, Switzerland, 2000
Human Ussing Chamber (cm/s)	3.80×10^{-5}	Sjöberg Å, Lutz M, Tannergren C, Wingolf C, Borde A, Ungell AL. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. *Eur J Pharm Sci*. 2013;48(1-2):166-180. doi:10.1016/j.ejps.2012.10.007
pKa (Base)	6.04	Andersin R. Solubility and acid-base behaviour of midazolam in media of different pH, studied by ultraviolet spectrophotometry with multicomponent software. *J Pharm Biomed Anal*. 1991;9(6):451-455. doi:10.1016/0731-7085(91)80246-6
Human fup (%)	4.4	de Vries JX, Rudi J, Walter-Sack I, Conradi R. The determination of total and unbound midazolam in human plasma. A comparison of high performance liquid chromatography, gas chromatography and gas chromatography/mass spectrometry. *Biomed Chromatogr*. 1990;4(1):28-33. doi:10.1002/bmc.1130040105
Human Blood/Plasma Concentration Ratio	0.55	Gertz M, Houston JB, Galetin A. Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. *Drug Metab Dispos*. 2011;39(9):1633-1642. doi:10.1124/dmd.111.039248

B) Mean 5 mg IV bolus Cp-time data (mean age = 25yr, mean weight = 70).

Digitized from: Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, Krähenbühl S. Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther*. 1995;58(1):20-28. doi:10.1016/0009-9236(95)90068-3

IV 5 mg Midazolam	
Time (h)	ng/mL
0.083	95.00
0.167	90.00
0.330	82.10
0.670	67.50
1.000	53.90
1.500	41.90
2.000	32.00
2.500	23.50
3.500	17.80
5.000	11.40
7.000	6.90
9.000	3.70

C) Mean 15 mg PO tablet Cp-time data (mean age = 25yr, mean weight = 70).

Digitized from: Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, Krähenbühl S. Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther*. 1995;58(1):20-28. doi:10.1016/0009-9236(95)90068-3

15 mg PO Tablet Midazolam	
Time (h)	ng/mL
0.00	0.00
0.25	8.28
0.45	45.20
0.70	48.60
1.00	40.30
1.50	36.10
2.00	31.90
2.50	24.70
3.50	15.20
5.00	6.81
7.00	3.65
9.00	2.23

D) Mean 7.5 mg PO solution Cp-time data (mean age = 25yr, mean weight = 70).

Digitized from: Bornemann LD, Min BH, Crews T, et al. Dose dependent pharmacokinetics of midazolam. *Eur J Clin Pharmacol*. 1985;29(1):91-95. doi:10.1007/BF00547375

Midazolam 7.5 mg PO Solution	
Time (h)	ng/mL
0.00	0.00
0.25	13.50
0.50	27.00
0.75	24.90
1.00	24.80
1.25	19.30
1.50	16.10
2.00	12.90
3.00	10.20
4.00	6.46
5.00	5.30
6.00	4.43
7.00	3.10
8.00	2.44

E) Mean 15 mg PO solution Cp-time data (mean age = 25yr, mean weight = 70).

Digitized from: Bornemann LD, Min BH, Crews T, et al. Dose dependent pharmacokinetics of midazolam. *Eur J Clin Pharmacol*. 1985;29(1):91-95. doi:10.1007/BF00547375

Midazolam 15 mg PO Solution	
Time (h)	ng/mL
0.00	0.00
0.25	33.60
0.30	41.80
0.40	51.90
0.50	57.40
0.75	58.50
1.00	51.90
1.25	42.60
1.50	35.60
2.00	31.00
3.00	18.10
4.00	16.10
5.00	10.00
6.00	8.36
7.00	7.73
8.00	5.74
10.00	3.86

F) Mean 30 mg PO solution Cp-time data (mean age = 25yr, mean weight = 70).

Digitized from: Bornemann LD, Min BH, Crews T, et al. Dose dependent pharmacokinetics of midazolam. *Eur J Clin Pharmacol*. 1985;29(1):91-95. doi:10.1007/BF00547375

Midazolam 30 mg PO Solution	
Time (h)	ng/mL
0.00	0.00
0.25	129.00
0.50	137.00
0.75	130.50
1.00	127.00
1.25	113.00
1.50	102.00
2.00	77.30
3.00	51.90
4.00	37.80
5.00	31.60
6.00	20.80
7.00	16.40
8.00	13.20
10.00	9.61
12.00	6.33
16.00	4.43

Supplementary Table 3: Prompts selected by groups for each project, with reference to Table 3

Group	Drug	Prompts
1	Metformin	1D. Is your drug expected to be safe and effective in a special population of renally impaired patients? 1E. Is your drug expected to be safe and effective in a special population of MATE1 and/or OCT2 variant patients? 2C. How would your drug's ADME be affected by changes in dosing schedule (qDay vs BID vs TID)?
2	Atorvastatin	1A. Is your drug expected to be safe and effective in a special population of elderly patients? 1E. Is your drug expected to be safe and effective in a special population of OATP1B1 variant patients? 2C. How would your drug's ADME be affected by changes in route of administration (IV vs PO)? 2D. How would your drug's ADME be affected by changes in food coadministration (fasted vs fed)?
3	Ibuprofen	1B. Is your drug expected to be safe and effective in a special population of pediatric patients? 1E. Is your drug expected to be safe and effective in a special population of CYP2C9 variant patients? 2A. How would your drug's ADME be affected by changes in formulation type (tablet vs solution)? 2D. How would your drug's ADME be affected by changes in food coadministration (fasted vs fed)?
4	Amoxicillin	1A. Is your drug expected to be safe and effective in a special population of elderly patients? 1B. Is your drug expected to be safe and effective in a special population of pediatric patients? 1E. Is your drug expected to be safe and effective in a special population of PepT1 variant patients? 2A. How would your drug's ADME be affected by changes in formulation type (tablet vs suspension)?
5	Digoxin	1A. Is your drug expected to be safe and effective in a special population of elderly patients? 1E. Is your drug expected to be safe and effective in a special population of P-glycoprotein variant patients? 2A. How would your drug's ADME be affected by changes in formulation type (tablet vs capsule vs suspension)? 2E. How would your drug's ADME be affected by coadministration of a P-glycoprotein inducer?