

Drug Metabolism and Disposition

Application of a physiologically-based pharmacokinetic model to assess propofol hepatic and renal glucuronidation in isolation; utility of in vitro and in vivo data – Supplementary Material

Katherine L Gill, Michael Gertz, J Brian Houston and Aleksandra Galetin

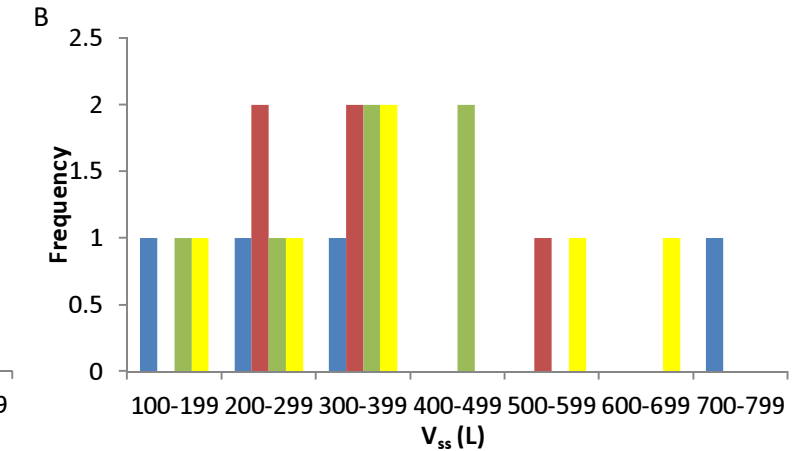
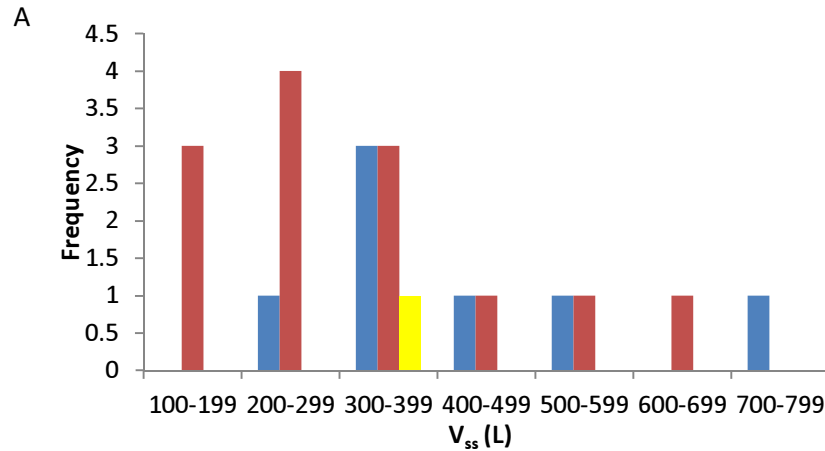
Supplemental Table 1: Organ weight, blood flow and tissue to plasma partition coefficients used in a whole body PBPK model for prediction of propofol in vivo clearance and blood concentration-time profiles from in vitro metabolism data

Tissue	Mass ^a (% total body weight)	Blood Flow ^a (% total cardiac output)	Tissue to Plasma Partition Coefficient ^b
Adipose	20	5.0	9.29
Brain	2.0	12	6.47
Enterocyte	0.13	4.6	5.18
Gut	0.76	5.4	5.18
Heart	0.46	4.0	1.66
Kidney	0.43	19	2.51
Liver	2.5	26	3.12
Lung	0.69	100	0.68
Muscle	40	17	2.63
Pancreas	0.19	1.0	4.12
Bone	15	5.0	7.10
Skin	4.6	5.0	3.17

Spleen	0.21	3.0	2.53
Stomach	0.21	1.0	5.18
Large Intestine	0.51	4.0	5.18
Arterial Blood	2.6	-	-
Venous Blood	5.2	-	-

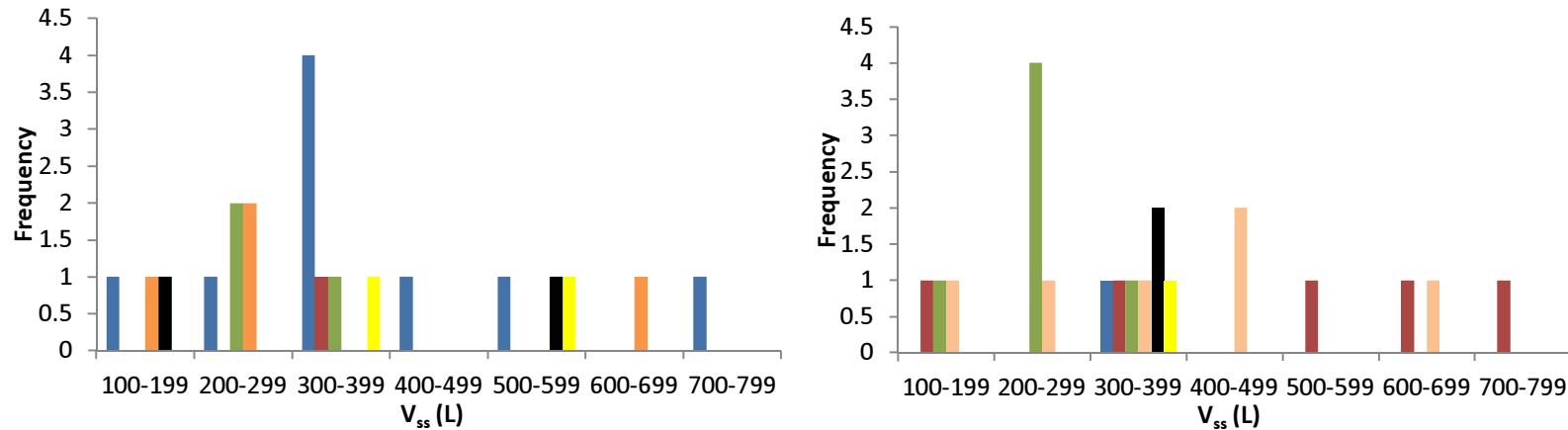
^a Remaining body mass and blood flow were accounted for in a 'rest of body' compartment. K_p for the 'rest of body' compartment was assumed to be the same as muscle.

^b predicted using the Rodgers and Rowland method and normalised for observed V_{ss} by a uniform scalar (Scaling factor for volume = $\frac{V_{ss,obs} - \text{blood volume}}{V_{ss,pred} - \text{blood volume}}$)

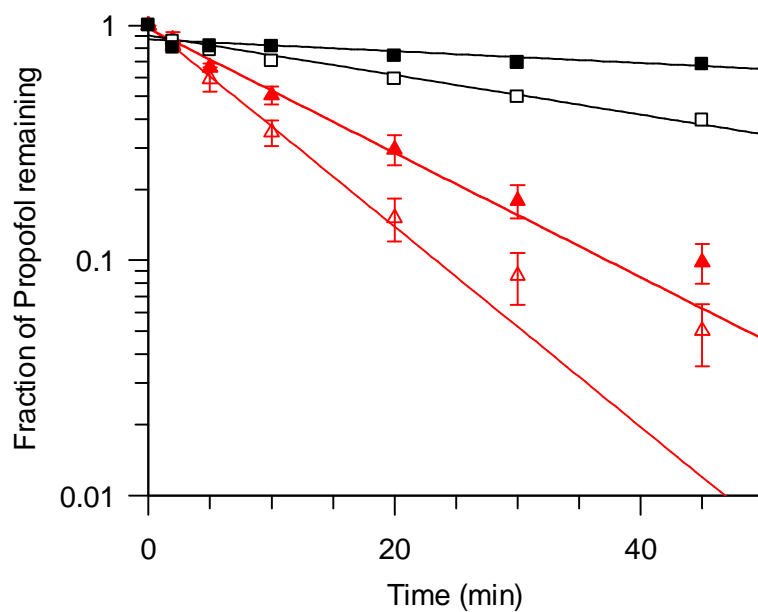


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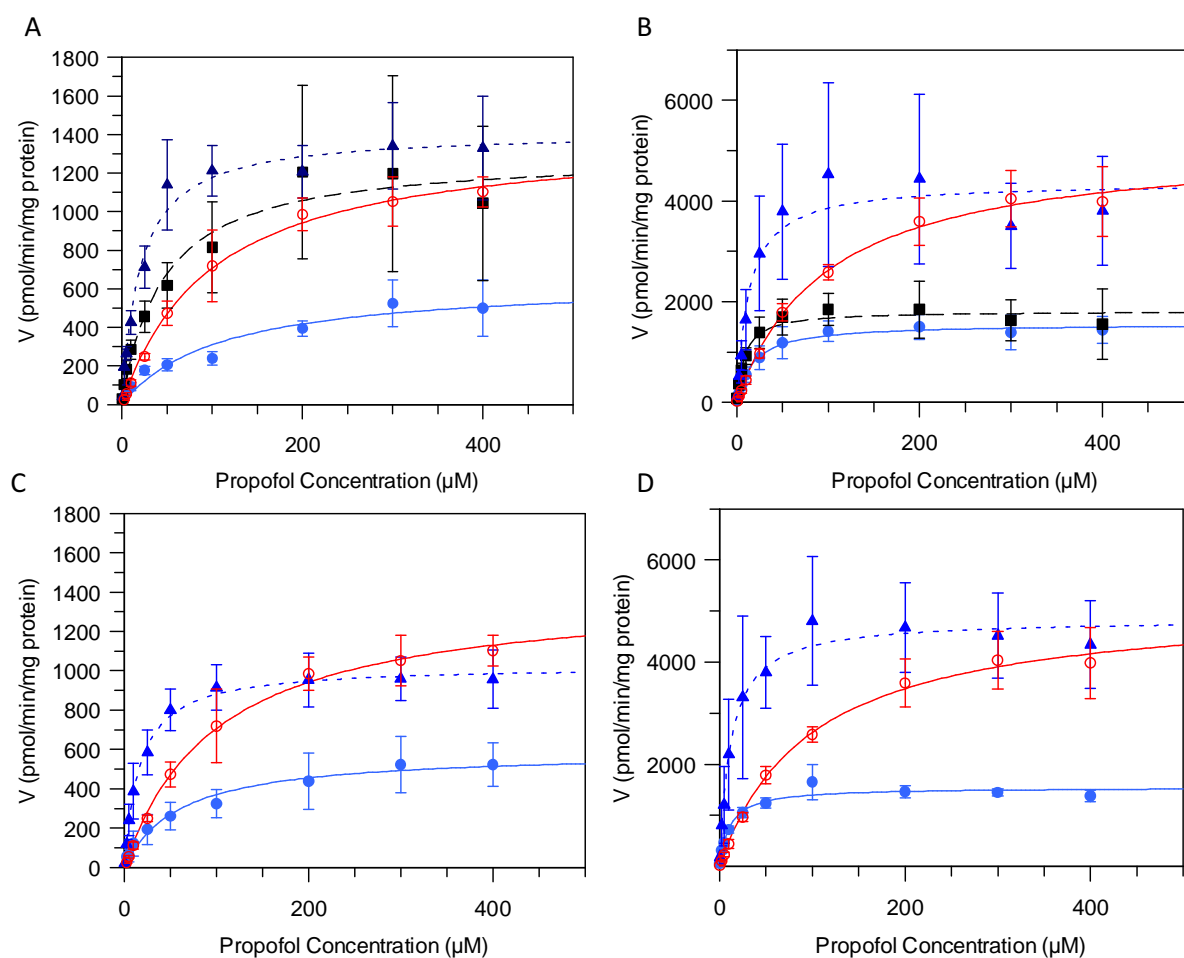
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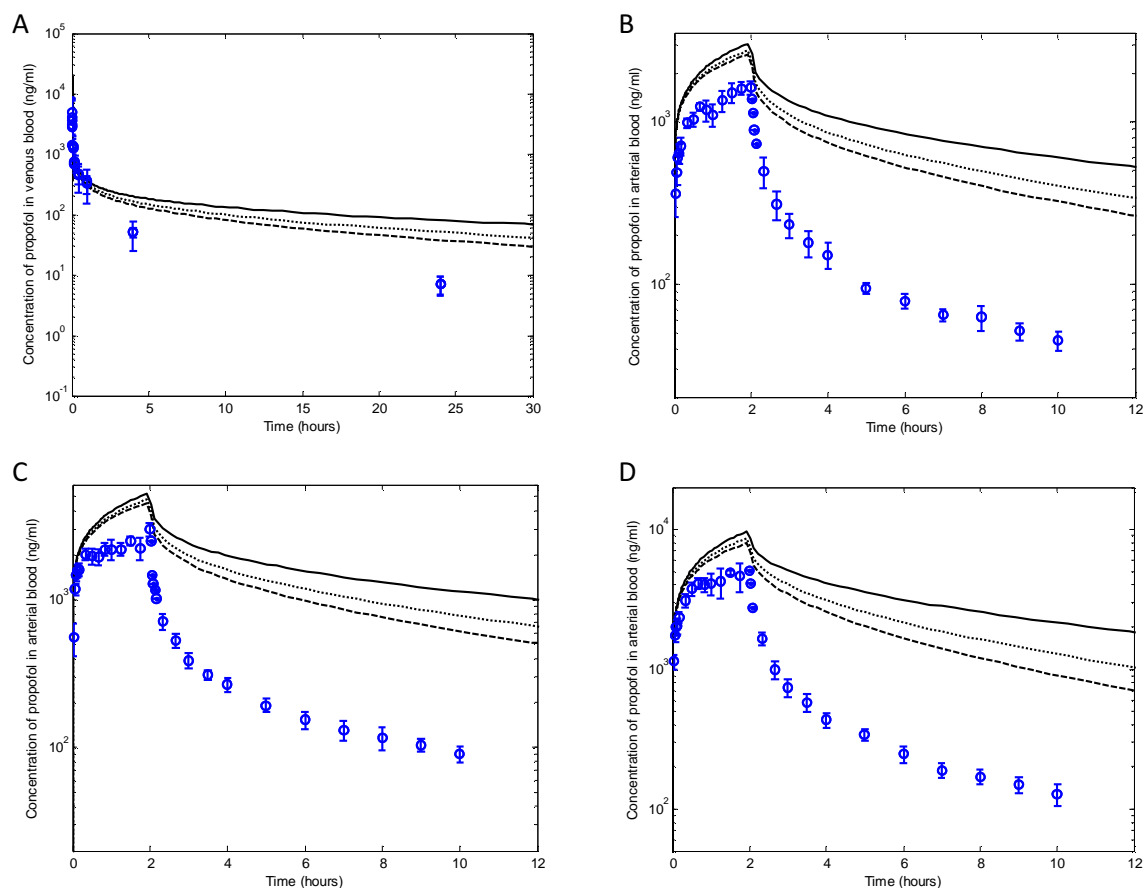
Supplemental Figure 1: Frequency of mean reported propofol V_{ss} values within the literature. A: comparisons based on dose type; ■, ■ and ■ represent bolus, infusion or unknown dosing, respectively. B: comparisons based on gender; ■, ■, ■ and ■ represent males, females, mixed or unknown gender, respectively. C: comparisons based on dose level; ■, ■, ■, ■ and ■ represent doses of 0-5, 5-10, 10-15, 15-20, 20-22 mg/kg or unknown dose, respectively. D: comparisons based on age; ■, ■, ■, ■ and ■ represent mean population age of 20-30, 30-40, 40-50, 50-60, 60-70 years or unknown age, respectively. Data collected from the following studies (Gepts et al., 1987; Servin et al., 1988a; Servin et al., 1988b; Simons et al., 1988; Gill et al., 1990; Servin et al., 1990; Gin et al., 1991; Servin et al., 1993; Wessén et al., 1994; Frenkel et al., 1995; Doenicke et al., 1997; Knibbe et al., 2000; Servin et al., 2003; Obach et al., 2008).



Supplemental Figure 2: Mean fraction of propofol remaining \pm SD over time in human hepatic microsomes in the presence and absence of cytochrome P450 cofactors and 2% bovine serum albumin. Open and closed symbols represent depletion in the absence and presence of 2% BSA, respectively. Red triangles represent depletion in human hepatic microsomes in the presence of cytochrome P450 cofactors. Black squares represent non-enzymatic loss of propofol in human hepatic microsomes in the absence of cytochrome P450 cofactors.

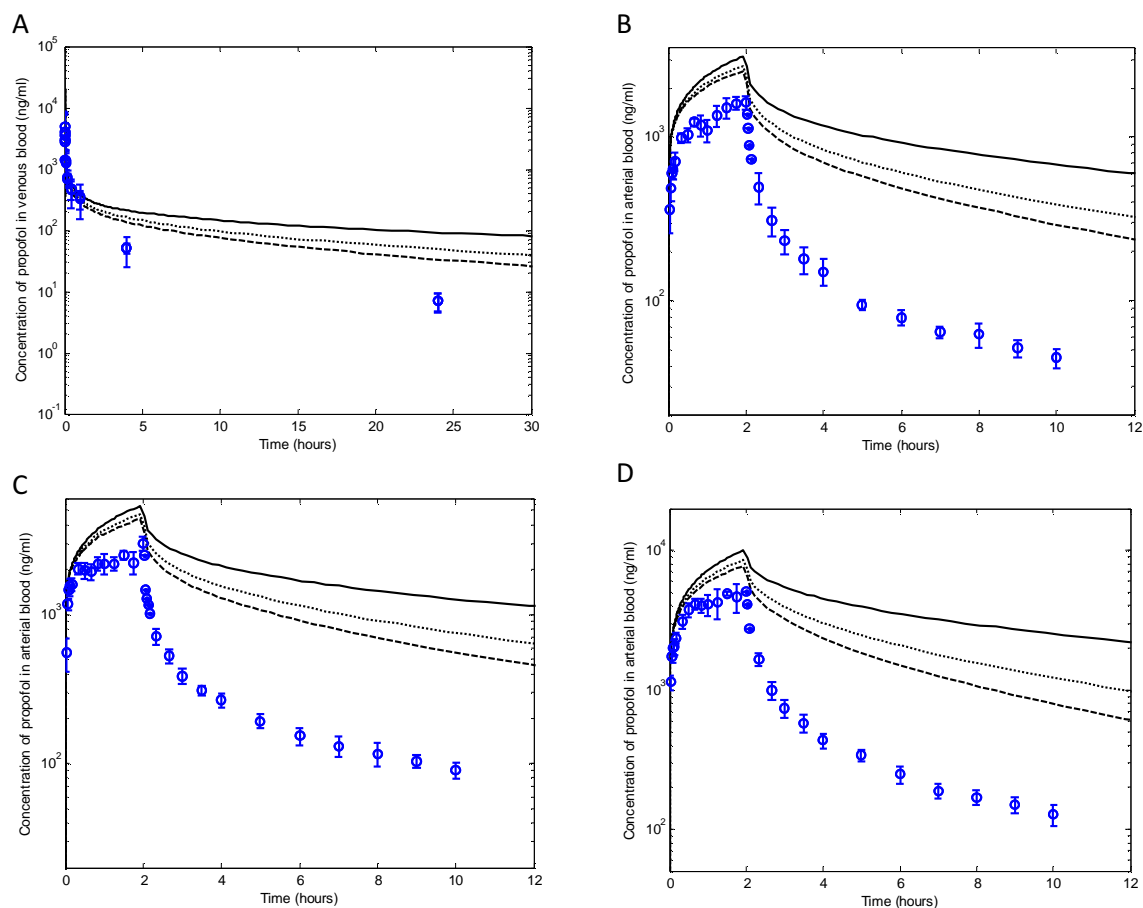


Supplemental Figure 3: Effect of 1 or 2% BSA on propofol glucuronide formation in human hepatic and renal microsomes at different microsomal protein concentrations. A and B: Effect of 2% BSA on propofol glucuronide formation in human hepatic and renal microsomes, respectively; C and D: Effect of 1% BSA on propofol glucuronide formation in human hepatic and renal microsomes, respectively. ○ represents propofol glucuronide formation in the absence of BSA; ●, ■ and ▲ represent propofol glucuronide formation in the presence of BSA at microsomal protein concentrations of 0.05, 0.075 and 0.1 mg/ml, respectively. Data represent the mean of three experiments, each performed in duplicate. Error bars represent the standard deviation.



Supplemental Figure 4: Propofol blood concentration-time profiles predicted using in vitro CL_{int} from the substrate depletion method in microsomes with/without hepatocyte CL_{int} data.

A: Data from Doenicke et al (1987); B: Data from 6 mg/kg dose level from Gepts et al (1987); C: Data from 12 mg/kg dose level from Gepts et al (1987); D: Data from 18 mg/kg dose level from Gepts et al (1987). \circ represent mean observed data \pm SD; solid line represents predicted concentrations using in vitro CL_{int} data from microsomal assays in the absence of BSA; dashed line represents predicted concentrations using in vitro CL_{int} data from microsomal assays in the presence of BSA; dotted line represents predicted concentrations using in vitro CL_{int} data from microsomal assays in the presence of BSA for kidney and intestine and hepatocyte CL_{int} data for liver.



Supplemental Figure 5: Propofol blood concentration-time profiles predicted using in vitro $CL_{int,UGT}$ from the metabolite formation method in microsomes, $CL_{int,CYP}$ data from substrate depletion assays in microsomes with/without hepatocyte CL_{int} data. A: Data from Doenicke et al (1987); B: Data from 6 mg/kg dose level from Gepts et al (1987); C: Data from 12 mg/kg dose level from Gepts et al (1987); D: Data from 18 mg/kg dose level from Gepts et al (1987). \circ represent mean observed data \pm SD; solid line represents predicted concentrations using in vitro $CL_{int,UGT}$ data from microsomal assays in the absence of BSA; dashed line represents predicted concentrations using in vitro $CL_{int,UGT}$ data from microsomal assays in the presence of BSA; dotted line represents predicted concentrations using in vitro $CL_{int,UGT}$ data from microsomal assays in the presence of BSA for kidney and intestine and hepatocyte CL_{int} data for the liver.

Supplemental Table 2: Prediction accuracy for propofol in vivo clearance (CL) for individual dose groups using CL_{int} data derived from different in vitro systems

Clinical data source	In vivo CL (L/h)	In vitro method used to derive CL _{int,UGT}	Predicted CL (L/h)			Predicted CL / Observed CL (%)		
			In vitro system used			In vitro system used		
			Microsomes without BSA	Microsomes with BSA	Hepatocytes and HKM & HIM with BSA	Microsomes without BSA	Microsomes with BSA	Hepatocytes and HKM & HIM with BSA
Doenicke et al (1997) Group 1 / 2	98.0 / 100	Substrate depletion	18.7	37.2	29.4	19.1 / 18.7	37.9 / 37.9	30.0 / 29.4
		Metabolite formation	15.7	40.4	30.5	16.1 / 15.7	41.2 / 40.4	31.1 / 30.5 ^a
Gepts et al (1987) 6 mg/kg dose	116	Substrate depletion	17.4	33.0	26.9	15.1	28.6	23.3
		Metabolite formation	14.8	35.7	27.9	12.8	30.9	24.2 ^a
Gepts et al (1987) 12 mg/kg dose	114	Substrate depletion	16.0	31.3	30.0	14.1	27.6	26.4
		Metabolite formation	13.5	34.0	26.0	11.9	29.9	22.9 ^a
Gepts et al (1987) 18 mg/kg dose	91.9	Substrate depletion	15.6	31.3	29.8	17.0	34.0	32.4
		Metabolite formation	13.1	34.0	25.5	14.3	37.0	27.8 ^a

^a Substrate depletion approach used to determine hepatocyte CL_{int,u} data.

Supplemental Table 3: Comparison of propofol glucuronide K_m , V_{max} and $CL_{int,UGT}$ in human liver, kidney and intestinal microsomes to those from previously published studies

Parameter	Source of data	HLM ^a	HKM ^b	HIM ^c	HLM ^d
		Without BSA			With BSA
K_m (μM)	Our data	107	91.0	458	5.22
	Published data	17.3-338	81-384	150-239	7.8-15.5
V_{max} (pmol/min/mg protein)	Our data	1460	5224	1280	1385
	Published data	580-3900	5560-7970	1370-1610	780-1048
$CL_{int,UGT}$ ($\mu L/min/mg$ protein)	Our data	14.9	57.5	2.82	266
	Published data	8-41	20-67	6.7-9.1	68-100

^a Kinetic data for HLM without BSA were collated from the following references: (Le Guellec et al., 1995; Raof et al., 1996; Soars et al., 2001; Soars et al., 2003; Al-Jahdari et al., 2006; Shimizu et al., 2007; Rowland et al., 2008; Liang et al., 2011; Walsky et al., 2012)

^b Kinetic data for HKM without BSA were collated from the following references: (Raof et al., 1996; Soars et al., 2001; Al-Jahdari et al., 2006)

^c Kinetic data for HIM without BSA were collated from the following references: (Raof et al., 1996; Shimizu et al., 2007)

^d Kinetic data for HLM with BSA were collated from the following references: (Rowland et al., 2008; Walsky et al., 2012)

References

- Al-Jahdari W, Yamamoto K, Hiraoka H, Nakamura K, Goto F, and Horiuchi R (2006) Prediction of total propofol clearance based on enzyme activities in microsomes from human kidney and liver. *Eur J Clin Pharmacol* **62**:527-533.
- Doenicke AW, Roizen MF, Rau J, O'Connor M, Kugler J, Klotz U, and Babl J (1997) Pharmacokinetics and pharmacodynamics of propofol in a new solvent. *Anesth Analg* **85**:1399-1403.
- Frenkel C, Schüttler J, Ihmsen H, Heye H, and Rommelsheim K (1995) Pharmacokinetics and pharmacodynamics of propofol/alfentanil infusions for sedation in ICU patients. *Intensive Care Med* **21**:981-988.
- Gepts EM, Camu FM, Cockshott IDP, and Douglas EJH (1987) Disposition of propofol administered as constant rate intravenous infusions in humans. *Anesth Analg* **66**:1256-1263.
- Gill SS, Wright EM, and Reilly CS (1990) Pharmacokinetic interaction of propofol and fentanyl: Single bolus injection study. *Br J Anaesth* **65**:760-765.
- Gin T, Yau G, Jong W, Tan P, Leung R KW, and Chan K (1991) Disposition of propofol at caesarean section and in the postpartum period. *Br J Anaesth* **67**:49-53.
- Knibbe CAJ, Aarts LPHJ, Kuks PFM, Voortman HJ, Lie-A-Huen L, Bras LJ, and Danhof M (2000) Pharmacokinetics and pharmacodynamics of propofol 6% SAZN versus propofol 1% SAZN and Diprivan-10 for short-term sedation following coronary artery bypass surgery. *Eur J Clin Pharmacol* **56**:89-95.
- Le Guellec C, Lacarelle B, Villard PH, Point H, Catalin J, and Durand A (1995) Glucuronidation of propofol in microsomal fractions from various tissues and species including humans: Effect of different drugs. *Anesth Analg* **81**:855-861.
- Liang S-C, Ge G-B, Liu H-X, Shang H-T, Wei H, Fang Z-Z, Zhu L-L, Mao Y-X, and Yang L (2011) Determination of propofol UDP-glucuronosyltransferase (UGT) activities in hepatic microsomes from different species by UFLC-ESI-MS. *J Pharm Biomed Anal* **54**:236-241.
- Obach RS, Lombardo F, and Waters NJ (2008) Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. *Drug Metab Dispos* **36**:1385-1405.
- Raouf AA, van Obbergh LJ, de Ville de Goyet J, and Verbeeck RK (1996) Extrahepatic glucuronidation of propofol in man: Possible contribution of gut wall and kidney. *Eur J Clin Pharmacol* **50**:91-96.

Rowland A, Knights KM, Mackenzie PI, and Miners JO (2008) The "albumin effect" and drug glucuronidation: Bovine serum albumin and fatty acid-free human serum albumin enhance the glucuronidation of UDP-glucuronosyltransferase (UGT) 1A9 substrates but not UGT1A1 and UGT1A6 activities. *Drug Metab Dispos* **36**:1056-1062.

Servin F, Bougeois B, Gomeni R, Mentre F, Farinotti R, and Desmouts J-M (2003) Pharmacokinetics of propofol administered by target-controlled infusion to alcoholic patients. *Anesthesiology* **99**:576-585.

Servin F, Cockshott ID, Farinotti R, Haberer JP, Winckler C, and Desmouts JM (1990) Pharmacokinetics of propofol infusions in patients with cirrhosis. *Br J Anaesth* **65**:177-183.

Servin F, Desmouts JM, Farinotti R, Haberer JP, and Winckler C (1988a) Pharmacokinetics of propofol administered by continuous infusion in patients with cirrhosis. *Anaesthesia* **43**:23-24.

Servin F, Desmouts JM, Haberer JP, Cockshott ID, Plummer GF, and Farinotti R (1988b) Pharmacokinetics and protein binding of propofol in patients with cirrhosis. *Anesthesiology* **69**:887-891.

Servin F, Farinotti R, Haberer J-P, and Desmouts J-M (1993) Propofol infusion for maintenance of anesthesia in morbidly obese patients receiving nitrous oxide. A clinical and pharmacokinetic study. *Anesthesiology* **78**:657-665.

Shimizu M, Matsumoto Y, and Yamazaki H (2007) Effects of propofol analogs on glucuronidation of propofol, an anesthetic drug, by human liver microsomes. *Drug Metab Lett* **1**:77-79.

Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, and Rowland M (1988) Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of ¹⁴C-propofol. *Xenobiotica* **18**:429-440.

Soars MG, Riley RJ, Findlay KAB, Coffey MJ, and Burchell B (2001) Evidence for significant differences in microsomal drug glucuronidation by canine and human liver and kidney. *Drug Metab Dispos* **29**:121-126.

Soars MG, Ring BJ, and Wrighton SA (2003) The effect of incubation conditions on the enzyme kinetics of UDP-glucuronosyltransferases. *Drug Metab Dispos* **31**:762-767.

Walsky RL, Bauman JN, Bourcier K, Giddens G, Lapham K, Negahban A, Ryder TF, Obach RS, Hyland R, and Goosen TC (2012) Optimized assays for human UDP-glucuronosyltransferase (UGT) activities: Altered alamethicin concentration and utility to screen for UGT inhibitors. *Drug Metab Dispos* **40**:1051-1065.

Wessén A, Persson PM, Nilsson A, and Hartvig P (1994) Clinical pharmacokinetics of propofol given as a constant-rate infusion and in combination with epidural blockade. *J Clin Anesth* **6**:193-198.