Published in final edited form as:

J Pharm Sci. 2019 July ; 108(7): 2500–2504. doi:10.1016/j.xphs.2019.02.009.

***In vitro-in vivo* extrapolation and hepatic clearance dependent underprediction**

Christine M. Bowman, Leslie Z. Benet

Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco (CMB, LZB)

Abstract

Accurately predicting the hepatic clearance of compounds using *in vitro* to *in vivo* extrapolation (IVIVE) is crucial within the pharmaceutical industry. However several groups have recently highlighted the large error in the process. While empirical or regression-based scaling factors may be used to mitigate the common underprediction, they provide unsatisfying solutions since the reasoning behind the underlying error has yet to be determined. One previously noted trend was intrinsic clearance-dependent underprediction, highlighting the limitations of current *in vitro* systems. When applying these generated *in vitro* intrinsic clearance values during drug development and making first-in-human dose predictions for new chemical entities though, hepatic clearance is the parameter that must be estimated using a model of hepatic disposition such as the well-stirred model. Here we examine error across hepatic clearance ranges and find a similar hepatic clearance-dependent trend, with high clearance compounds not predicted to be so, demonstrating another gap in the field.

Keywords

Clearance; Hepatic clearance; In Vitro/In Vivo (IVIVC) Correlation(s)

Introduction

Given that many drugs are primarily eliminated by metabolism, the accurate prediction of hepatic clearance (CL_H) is crucial for both evaluating and optimizing new chemical entities as well as estimating first-in-human doses. Successful predictions could help reduce the high attrition¹ associated with the current drug discovery and development process. While allometric scaling may be attempted for prediction, it is more accurate for renally cleared compounds^{2,3}. Alternatively, *in vitro* to *in vivo* extrapolation (IVIVE) is commonly used to predict hepatic clearance.

When implementing IVIVE, microsomes or hepatocytes can be used to determine an *in vitro* intrinsic clearance (CL_{int}). The *in vitro* value is then scaled to an *in vivo* CL_{int} using physiologically based parameters such as microsomal protein content/hepatocellularity and

Corresponding author: Leslie Z. Benet, Department of Bioengineering and Therapeutic Sciences, University of California, 533 Parnassus Ave., Room U-68, San Francisco, California 94143-0912, 415-476-3853 (telephone), 415-476-8887 (fax), leslie.benet@ucsf.edu.

liver weight. Ultimately the scaled value is input into a model of hepatic disposition such as the well-stirred model to estimate hepatic clearance.

Several publications have examined the accuracy of IVIVE predictions with rat^{4–6} and human^{7–11} data and further comparisons have been made with data generated in microsomes vs. hepatocytes^{12–14}. One review found that on average, human microsomes underpredict clearance by 9 fold, while human hepatocytes underpredict by 3–6 fold¹⁵. This would be expected given that hepatocytes contain transporters, both phase I and II enzymes, and the natural localization of organelles and cofactors, unlike microsomes. However, examining a larger quantity of data, groups have recently reported the error between the two systems to be more comparable^{16,17}.

Several hypotheses have been proposed to account for the systematic underprediction observed. Concerns with hepatocyte cryopreservation have been expressed, however studies have shown no significant differences between cryopreserved and fresh cells^{4,8,13,18}. Similarly, the impact of donor variability is frequently discussed¹⁸, however both over- and underprediction would be expected¹⁵ and many groups now use pooled microsomes and hepatocytes. Other proposed reasons for the inaccuracy have included differences in liver sample viability and preparation¹⁹, differences in the use of binding terms^{7,20}, inaccuracies in the measurement of fraction unbound^{21,22}, the presence of inhibitory long-chain unsaturated fatty acids in microsomal incubations^{23,24}, ignoring extra-hepatic metabolism^{15,25}, and simplifying the complex interplay between uptake, metabolism, biliary secretion, and efflux²⁶.

When exploring reasons for error, groups have also considered clearance-dependent trends. While reducing the clearance of compounds is often a goal to facilitate lower dosage requirements and longer half-lives, measuring low clearance *in vitro* is experimentally challenging. Stringer et al.²⁷ found that of compounds with an *in vivo* CL_{int} of 1–10 ml/min/kg, only 8% had a measurable value in microsomes and 13% in hepatocytes. Given that enzyme activity begins declining in microsomes after 1 hour of incubation, and cell viability begins decreasing in hepatocytes at 4–6 hours, a low turnover compound can have large uncertainty in its clearance and first dose estimations²⁸. A study examining predictions in hepatocyte preparations from four species found poorer accuracy with low clearance compounds⁴. However newer methods such as the hepatocyte relay method^{29,30}, and hepatocyte culture systems containing flow and/or cell coculture^{31,32}, have been developed to try to address the error.

At the other extreme, studies have seen an increase in error with increasing *in vivo* CL_{int} in hepatocytes^{17,33,34} and microsomes¹⁷ in both human and rat preparations¹⁷. Suggested reasons for this trend include endogenous cofactor depletion, loss of enzymatic activity, permeability limitation, and rate limiting diffusion through the unstirred water layer^{13,33,34,35}.

While recognizing CL_{int} trends are important for determining the limitations of the cell systems currently utilized, ultimately, an accurate scaled CL_H is needed for new chemical entities and first-in-human dose predictions. Hepatic clearance is directly related to other

pharmacokinetic parameters including half-life, bioavailability, and exposure, which drive the dosing regimen and efficacy/toxicity profiles of potential compounds. Here we explore the accuracy of hepatic clearance predictions across extraction ratio ranges to determine where the most improvement is needed.

Materials and Methods

The large database, including human (n=101, hepatocytes; n=83, microsomes) and rat (n=128 hepatocytes; n=71 microsomes) values, which was recently compiled by Wood et al.¹⁷, was utilized for this analysis. Hepatic clearance was calculated using the well-stirred model

$$CL_H = \frac{Q_H \cdot \left(\frac{f_{u,B}}{f_{u,inc}} \right) \cdot CL_{int}}{Q_H + \left(\frac{f_{u,B}}{f_{u,inc}} \right) \cdot CL_{int}} \quad (1)$$

where Q_H is liver blood flow and $f_{u,B}$ and $f_{u,inc}$ are fraction unbound in the blood and incubation, respectively. Physiologically based scaling factors, not empirical or regression-based factors were used. Details on the specific values and scaling factors can be found in the original source¹⁷.

The coefficient of determination, R^2 , was used to examine the potential of clearance-dependent error. The overall bias in predictions was measured by calculating the average fold error (AFE) and precision was measured with the root mean squared error (RMSE) as follows:

$$AFE = 10^{\frac{1}{N} \sum \log \left(\frac{observed}{predicted} \right)} \quad (2)$$

$$RMSE = \sqrt{\frac{1}{N} \sum (predicted - observed)^2} \quad (3)$$

Additionally, the accuracy of predictions was determined based on whether the predictions fell within 2-fold of the true *in vivo* values, as has been a standard cutoff in previous studies^{8,12,36}. As was done by Wood et al.¹⁷, an empirical scaling factor (ESF) was calculated to determine the error associated with each prediction

$$ESF = \frac{observed CL_H}{predicted CL_H} \quad (4)$$

The data were divided into difference clearance ranges: low extraction ratio (ER) (<30% of liver blood flow (LBF)), intermediate (30–70%), and high (>70%) where LBF was assumed to be 20.7 and 100 ml/min/kg for human and rat, respectively¹⁷.

Results and Discussion

When working with new chemical entities, CL_H is the parameter that would be used for predicting first-in-human doses and deciding whether to move a compound forward. Therefore, while a compound may have high CL_{int} , which could imply a likely error based on the CL_{int} trend^{17,33,34}, sizable error may not carry over for CL_H predictions. For instance, considering lorcinide and its human microsome data, its predicted CL_{int} is 449 vs. its observed value of 2559 ml/min/kg leads to a 5.7 fold difference¹⁷. However, when actually developing this compound, its predicted CL_H would have been 16.3, a value only 1.2 fold off from its 20.0 ml/min/kg observed CL_H . Table 1 highlights different *in vivo* CL_{int} ranges and the number of these compounds in each *in vivo* CL_H ER range. Given that not all low CL_{int} compounds have low *in vivo* CL_H for instance, it is crucial to examine potential CL_H dependent trends too.

When visually examining *in vivo* CL_H vs. ESF in Figure 1, a clearance-dependent trend does not strongly appear and the R^2 values are very low. However, this is expected as any clearance dependency would be suppressed due to the blood flow limitation at higher CL. Despite the potential suppression, the AFE moderately increased from low to high ER in all cases, with the largest AFEs for the human and rat hepatocyte data (Table 2). The lower number of high ER drugs particularly for rats should be noted though. The larger RMSE values for the rat data could be attributed to the higher CL range for the species, and the larger RMSE values noted in every case for the high ER drugs could be due to fewer compounds in this range.

The percentage of predictions falling within two-fold of observed data was generally consistent between ranges (Fig. 2) and surprisingly slightly increased across ER ranges in every system except human hepatocytes (Table 3). There were more underpredictions than overpredictions or accurate predictions in almost every case. While there appears to be consistent percentage accuracy between ER ranges, examining human microsome data for promethazine as an example, it has an accurate (within-two fold) *in vitro* prediction of 9.4 vs. the observed 16, but the prediction would be deemed an intermediate, not high ER compound. Correct determination of extraction ratio is crucial to understand if a compound will be sensitive to changes in protein binding, blood flow, and/or intrinsic clearance³⁷.

When examining the classification accuracy across ER ranges, similar trends were seen with both human and rat microsomes and hepatocytes (Table 4). The great majority of low ER drugs, >90% in all cases, were accurately predicted to be low ER drugs. However, the majority of intermediate and high ER drugs were also predicted to be low ER drugs. High ER drugs had the poorest accuracy, with 25% of high ER drugs predicted to have a high ER.

The predictions in Table 4 were made assuming the well-stirred model. Since it is generally believed that high ER drugs are better described by the dispersion and parallel tube models and it is known that for these latter models predicted ER values will always be greater than those predicted values from the well-stirred model¹⁵, we also did the calculations for the human hepatocyte data using the parallel tube model. In essence, there is no improvement

seen in Table 4 for human hepatocytes. One observed low ER drug is now predicted to be high ER; one observed intermediate drug is now predicted to be high ER; and two observed high ER drugs predicted to be low ER with the well-stirred model are now predicted to be intermediate ER.

Determining the mechanisms behind the likely multifactorial IVIVE error is crucial for moving the field forward and improving the efficiency of the drug discovery and development process. While several reasons have been proposed over the years and new technologies are being created to help combat extrinsic issues such cell viability and enzyme activity loss, systematic underprediction still remains. One phenomenon recently focused upon is CL_{int} -dependent underprediction, highlighting the limitations of current *in vitro* systems. When applying these generated *in vitro* values during drug development though, CL_H is the parameter that must be estimated. Here we show a similar trend of CL_H -dependent underprediction. This underprediction could be due to the CL_{int} error previously noted, errors in protein binding measurements or the understanding of protein binding if protein-facilitated uptake is occurring³⁸, or yet to be discovered mechanisms. The majority of high ER drugs are not predicted to have high or even intermediate ERs, highlighting a need for improved prediction methodologies especially in this range.

Acknowledgments

CMB was supported in part by the Pharmaceutical Research and Manufacturers of America Foundation Pre Doctoral Fellowship in Pharmaceutics and the National Science Foundation Graduate Research Fellowship Program [Grant 1144247]; LZB is a member of the UCSF Liver Center supported by NIH Grant [P30 DK026743].

Abbreviations:

AFE	average fold error
CL_{int}	intrinsic clearance
CL_H	hepatic clearance
ER	extraction ratio
ESF	empirical scaling factor
fu_b	fraction unbound in blood
IVIVE	<i>in vitro</i> to <i>in vivo</i> extrapolation
LBF	liver blood flow
RMSE	root mean squared error

References

1. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. *Nat Biotechnol.* 2014;32(1):40–51. [PubMed: 24406927]
2. Zuegge J, Schneider G, Coassolo P, Lavé T. Prediction of hepatic metabolic clearance: comparison and assessment of prediction models. *Clin Pharmacokinet.* 2001;40(7):553–563. [PubMed: 11510631]

3. Huh Y, Smith DE, Feng MR. Interspecies scaling and prediction of human clearance: comparison of small- and macro-molecule drugs. *Xenobiotica*. 2011;41(11):972–987. [PubMed: 21892879]
4. Lau YY, Sapidou E, Cui X, White RE, Cheng K-C. Development of a novel in vitro model to predict hepatic clearance using fresh, cryopreserved, and sandwich-cultured hepatocytes. *Drug Metab Dispos*. 2002;30(12):1446–1454. [PubMed: 12433818]
5. Naritomi Y, Terashita S, Kagayama A, Sugiyama Y. Utility of hepatocytes in predicting drug metabolism: comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab Dispos*. 2003;31(5):580–588. [PubMed: 12695346]
6. Ito K, Houston JB. Comparison of the use of liver models for predicting drug clearance using in vitro kinetic data from hepatic microsomes and isolated hepatocytes. *Pharm Res*. 2004;21(5):785–792. [PubMed: 15180335]
7. Obach RS. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos*. 1999;27(11):1350–1359. [PubMed: 10534321]
8. McGinnity DF, Soars MG, Urbanowicz RA, Riley RJ. Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for the prediction of metabolic clearance. *Drug Metab Dispos*. 2004;32(11):1247–1253. [PubMed: 15286053]
9. Brown HS, Griffin M, Houston JB. Evaluation of cryopreserved human hepatocytes as an alternative in vitro system to microsomes for the prediction of metabolic clearance. *Drug Metab Dispos*. 2007;35(2):293–301. [PubMed: 17132764]
10. Sohlenius-Sternbeck A-K, Afzelius L, Prusis AP, Neelissen J, Hoogstraate J, Johansson J, Floby E, Bengtsson A, Gissberg O, Sternbeck J, Petersson C. Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. *Xenobiotica*. 2010;40(9):637–649. [PubMed: 20624033]
11. Akabane T, Gerst N, Masters JN, Tamura K. A quantitative approach to hepatic clearance prediction of metabolism by aldehyde oxidase using custom pooled hepatocytes. *Xenobiotica*. 2012;42(9):863–871. [PubMed: 22448773]
12. Houston JB, Carlile DJ. Prediction of hepatic clearance from microsomes, hepatocytes, and liver slices. *Drug Metab Rev*. 1997;29(4):891–922. [PubMed: 9421679]
13. Lu C, Li P, Gallegos R, Uttamsingh V, Xia CQ, Miwa GT, Balani SK, Gan LS. Comparison of intrinsic clearance in liver microsomes and hepatocytes from rats and humans: evaluation of free fraction and uptake in hepatocytes. *Drug Metab Dispos*. 2006;34(9):1600–1605. [PubMed: 16790553]
14. Di L, Keefer C, Scott DO, Strelevitz TJ, Chang G, Bi Y-A, Lai Y, Duckworth J, Fenner K, Troutman MD, Obach RS. Mechanistic insights from comparing intrinsic clearance values between human liver microsomes and hepatocytes to guide drug design. *Eur J Med Chem*. 2012;57:441–448. [PubMed: 22840492]
15. Chiba M, Ishii Y, Sugiyama Y. Prediction of hepatic clearance from in vitro data for successful drug development. *AAPS J*. 2009;11(2):262–276. [PubMed: 19408130]
16. Bowman CM, Benet LZ. Hepatic clearance predictions from in vitro-in vivo extrapolation and the biopharmaceutics drug disposition classification system. *Drug Metab Dispos*. 2016;44(11):1731–1735. [PubMed: 27519549]
17. Wood FL, Houston JB, Hallifax D. Clearance prediction methodology needs fundamental improvement: trends common to rat and human hepatocytes/microsomes and implications for experimental methodology. *Drug Metab Dispos*. 2017;45(11):1178–1188. [PubMed: 28887366]
18. Floby E, Johansson J, Hoogstraate J, Hewitt NJ, Hill J, Sohlenius-Sternbeck A-K. Comparison of intrinsic metabolic clearance in fresh and cryopreserved human hepatocytes. *Xenobiotica*. 2009;39(9):656–662. [PubMed: 19622023]
19. Fisher RL, Gandolfi AJ, Brendel K. Human liver quality is a dominant factor in the outcome of in vitro studies. *Cell Biol Toxicol*. 2001;17(3):179–189. [PubMed: 11693579]
20. Riley RJ, McGinnity DF, Austin RP. A unified model for predicting human hepatic metabolic clearance from in vitro intrinsic clearance data in hepatocytes and microsomes. *Drug Metab Dispos*. 2005;33(9):1304–1311. [PubMed: 15932954]

21. Kochansky CJ, McMasters DR, Lu P, Koeplinger KA, Kerr HH, Shou M, Korzekwa KR. Impact of pH on plasma protein binding in equilibrium dialysis. *Mol Pharm*. 2008;5(3):438–448. [PubMed: 18345638]
22. Zamek-Gliszczynski MJ, Ruterbories KJ, Ajamie RT, Wickremsinhe ER, Pothuri L, Rao MVS, Basavanakatti VN, Pinjari J, Ramanathan VK, Chaudhary AK. Validation of 96-well equilibrium dialysis with non-radiolabeled drug for definitive measurement of protein binding and application to clinical development of highly-bound drugs. *J Pharm Sci*. 2011;100(6):2498–2507. [PubMed: 21213309]
23. Rowland A, Gaganis P, Elliot DJ, Mackenzie PI, Knights KM, Miners JO. Binding of inhibitory fatty acids is responsible for the enhancement of UDP-glucuronosyltransferase 2B7 activity by albumin: implications for in vitro-in vivo extrapolation. *J Pharmacol Exp Ther*. 2007;321(1):137–147. [PubMed: 17237258]
24. Rowland A, Elliot DJ, Knights KM, Mackenzie PI, and Miners JO. The “albumin effect”: and in vitro-in vivo extrapolation: sequestration of long-chain unsaturated fatty acids enhances phenytoin hydroxylation by human liver microsomal and recombinant cytochrome P450 2C9. *Drug Metab Dispos*. 2008;36(5):870–877. [PubMed: 18256202]
25. De Kanter R, Monshouwer M, Draaisma AL, De Jager MH, de Graaf IA, Proost JH, Meijer DK, Groothuis GM. Prediction of whole-body metabolic clearance of drugs through the combined use of slices from rat liver, lung, kidney, small intestine and colon. *Xenobiotica*. 2004;34(3):229–241. [PubMed: 15204696]
26. Camenisch G, Umehara K. Predicting human hepatic clearance from in vitro drug metabolism and transport data: a scientific and pharmaceutical perspective for assessing drug-drug interactions. *Biopharm Drug Dispos*. 2012;33(4):179–194. [PubMed: 22407504]
27. Stringer R, Nicklin PL, Houston JB. Reliability of human cryopreserved hepatocytes and liver microsomes as in vitro systems to predict metabolic clearance. *Xenobiotica*. 2008;38(10):1313–1329. [PubMed: 18853387]
28. Di L, Obach RS. Addressing the challenges of low clearance in drug research. *AAPS J*. 2015;17(2):352–357. [PubMed: 25567366]
29. Di L, Trapa P, Obach RS, Atkinson K, Bi Y-A, Wolford AC, Tan B, McDonald TS, Lai Y, Tremaine LM. A novel relay method for determining low-clearance values. *Drug Metab Dispos*. 2012;40(9):1860–1865. [PubMed: 22645091]
30. Di L, Atkinson K, Orozco CC, Funk C, Zhang H, McDonald TS, Tan B, Lin J, Chang C, Obach RS. In vitro-in vivo correlation for low-clearance compounds using hepatocyte relay method. *Drug Metab Dispos*. 2013;41(12):2018–2023. [PubMed: 23857891]
31. Novik E, Maguire TJ, Chao P, Cheng KC, Yarmush ML. A microfluidic hepatic coculture platform for cell-based drug metabolism studies. *Biochem Pharmacol*. 2010;79(7):1036–1044. [PubMed: 19925779]
32. Chan TS, Yu H, Moore A, Khetani SR, Tweedie D. Meeting the challenge of predicting hepatic clearance of compounds slowly metabolized by cytochrome p450 using a novel hepatocyte model, Hepatopac. *Drug Metab Dispos*. 2013;41(12):2024–2032. [PubMed: 23959596]
33. Hallifax D, Foster JA, Houston JB. Prediction of human metabolic clearance from in vitro systems: retrospective analysis and prospective view. *Pharm Res*. 2010;27(10):2150–2161. [PubMed: 20661765]
34. Foster JA, Houston JB, Hallifax D. Comparison of intrinsic clearances in human liver microsomes and suspended hepatocytes from the same donor livers: clearance-dependent relationship and implications for prediction of in vivo clearance. *Xenobiotica*. 2011;41(2):124–136. [PubMed: 21058916]
35. Wood FL, Houston JB, Hallifax D. Importance of the unstirred water layer and hepatocyte membrane integrity in vitro for quantification of intrinsic metabolic clearance. *Drug Metab Dispos*. 2018;46(3):268–278. [PubMed: 29233818]
36. Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance, DJ, Wastall P. The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J Pharmacol Exp Ther*. 1997;283(1):46–58. [PubMed: 9336307]
37. Rowland M Protein binding and drug clearance. *Clin Pharmacokinet*. 1984;1:10–17.

38. Bowman CM, Benet LZ. An examination of protein binding and protein-facilitated uptake relating to in vitro-in vivo extrapolation. *Eur J Pharm Sci.* 2018;123:502–514. [PubMed: 30098391]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1:

The relationship between ESF (ratio of observed to predicted hepatic clearance) and observed *in vivo* CL_H for hepatocytes (A and C) and microsomes (B and D) in human (A and B) and rat (C and D).

Figure 2:
The percentage of *in vitro* predictions falling within two-fold of observed *in vivo* values grouped by extraction ratio for hepatocytes (A) and microsomes (B).

Table 1:

Observed CL_{int} ranges and the number of compounds with observed low/intermediate/high ERs within those ranges.

CL _{int} (mL/min/kg)	<u>Human Hep.</u>			<u>Human Mic.</u>			<u>Rat Hep.</u>			<u>Rat Mic.</u>		
	<u>Low</u> <u>ER</u>	<u>Inter.</u> <u>ER</u>	<u>High</u> <u>ER</u>	<u>Low</u> <u>ER</u>	<u>Inter.</u> <u>ER</u>	<u>High</u> <u>ER</u>	<u>Low</u> <u>ER</u>	<u>Inter.</u> <u>ER</u>	<u>High</u> <u>ER</u>	<u>Low</u> <u>ER</u>	<u>Inter.</u> <u>ER</u>	<u>High</u> <u>ER</u>
<10–100	45	7	1	34	3	0	13	2	0	10	1	0
100–1000	10	19	11	7	16	11	43	23	1	25	8	1
1000–>10,000	0	2	6	0	4	8	13	25	8	5	14	7

Table 2:

The AFE and RMSE for human and rat hepatocytes and microsomes according to level of observed CL_H .

CL_H (ml/min/kg)	Human Hepatocytes			Human Microsomes			Rat Hepatocytes			Rat Microsomes		
	AFE	n	RMSE	AFE	n	RMSE	AFE	n	RMSE	AFE	n	RMSE
All	2.7	101	6.6	2.0	83	6.4	3.8	128	28	2.2	71	29
Low ER	2.1	55	2.9	1.3	41	3.0	3.6	69	8.8	2.0	40	16
Intermediate ER	3.2	28	6.7	2.7	23	6.6	3.9	50	35	2.2	23	35
High ER	4.8	18	12	2.9	19	10	5.3	9	61	3.8	8	51

Table 3:

The percentage of predictions falling within two-fold, below, and above for the Wood et al. (2017) datasets grouped by CL_H range.

	CL _H (ml/min/kg)	All	Low ER	Intermediate ER	High ER
Human Hepatocytes	% within 2-fold (n)	30.7 (31)	34.6 (19)	35.7 (10)	11.1 (2)
	% below (n)	62.4 (63)	52.7 (29)	64.3 (18)	88.9 (16)
	% above (n)	6.90 (7)	12.7 (7)	0.00 (0)	0.00 (0)
Human Microsomes	% within 2-fold (n)	42.2 (35)	36.6 (15)	47.8 (11)	47.4 (9)
	% below (n)	48.2 (40)	43.9 (18)	52.2 (12)	52.6 (10)
	% above (n)	9.60 (8)	19.5 (8)	0.00 (0)	0.00 (0)
Rat Hepatocytes	% within 2-fold (n)	25.8 (33)	24.6 (17)	26.0 (13)	33.3 (3)
	% below (n)	69.5 (89)	72.5 (50)	66.0 (33)	66.7 (6)
	% above (n)	4.70 (6)	2.90 (2)	8.00 (4)	0.00 (0)
Rat Microsomes	% within 2-fold (n)	43.7 (31)	40.0 (16)	43.5 (10)	62.5 (5)
	% below (n)	47.9 (34)	52.5 (21)	43.5 (10)	37.5 (3)
	% above (n)	8.40 (6)	7.50 (3)	13.0 (3)	0.00 (0)

Table 4:

The number of compounds (%) in each extraction ratio range that have correct classifications.

Human Hepatocytes		Predicted to be Low ER	Predicted to be Intermediate ER	Predicted to be High ER
Well-stirred model	Observed Low ER	53 (96.4%)	2 (3.6%)	0 (0.0%)
	Observed Intermediate ER	18 (64.3%)	10 (35.7%)	0 (0.0%)
	Observed High ER	12 (66.7%)	5 (27.8%)	1 (5.5%)
Parallel tube model	Observed Low ER	53 (96.4%)	1 (1.8%)	1 (1.8%)
	Observed Intermediate ER	17 (60.7%)	10 (35.7%)	1 (3.6%)
	Observed High ER	10 (55.6%)	7 (38.9%)	1 (5.5%)
Human Microsomes				
Well-stirred model	Observed Low ER	37 (90.2%)	4 (9.8%)	0 (0.0%)
	Observed Intermediate ER	14 (60.9%)	9 (39.1%)	0 (0.0%)
	Observed High ER	8 (42.1%)	8 (42.1%)	3 (15.8%)
Rat Hepatocytes				
Well-stirred model	Observed Low ER	67 (97.1%)	2 (2.9%)	0 (0.0%)
	Observed Intermediate ER	37 (74.0%)	7 (14.0%)	6 (12.0%)
	Observed High ER	5 (55.6%)	2 (22.2%)	2 (22.2%)
Rat Microsomes				
Well-stirred model	Observed Low ER	38 (95.0%)	1 (2.5%)	1 (2.5%)
	Observed Intermediate ER	10 (43.5%)	8 (34.8%)	5 (21.7%)
	Observed High ER	3 (37.5%)	3 (37.5%)	2 (25.0%)