



Published in final edited form as:

Pharm Res. ; 36(8): 113. doi:10.1007/s11095-019-2645-0.

Interlaboratory Variability in Human Hepatocyte Intrinsic Clearance Values and Trends with Physicochemical Properties

Christine M. Bowman¹, Leslie Z. Benet¹

¹Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, University of California San Francisco, San Francisco, California 94143-0912, USA

Abstract

Purpose—To examine the interlaboratory variability in CL_{int} values generated with human hepatocytes and determine trends in variability and clearance prediction accuracy using physicochemical and pharmacokinetic parameters.

Methods—Data for 50 compounds from 14 papers were compiled with physicochemical and pharmacokinetic parameter values taken from various sources.

Results—Coefficients of variation were as high as 99.8% for individual compounds and variation was not dependent on the number of prediction values included in the analysis. When examining median values, it appeared that compounds with a lower number of rotatable bonds had more variability. When examining prediction uniformity, those compounds with uniform *in vivo* underpredictions had higher CL_{int} , *in vivo* values, while those with non-uniform predictions typically had lower CL_{int} , *in vivo* values. Of the compounds with uniform predictions, only a small number were uniformly predicted accurately. Based on this limited dataset, less lipophilic, lower intrinsic clearance, and lower protein binding compounds yield more accurate clearance predictions.

Conclusions—Caution should be taken when compiling *in vitro* CL_{int} values from different laboratories as variations in experimental procedures (such as extent of shaking during incubation) may yield different predictions for the same compound. The majority of compounds with uniform *in vitro* values had predictions that were inaccurate, emphasizing the need for a better mechanistic understanding of IVIVE. The non-uniform predictions, often with low turnover compounds, reaffirmed the experimental challenges for drugs in this clearance range. Separating new chemical entities by lipophilicity, intrinsic clearance, and protein binding may help instill more confidence in IVIVE predictions.

Keywords

hepatocytes; intrinsic clearance; *in vitro-in vivo* extrapolation; variability

Leslie Z. Benet, Leslie.Benet@ucsf.edu.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11095-019-2645-0>) contains supplementary material, which is available to authorized users.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

INTRODUCTION

Clearance is one of the most fundamental pharmacokinetic parameters, and its accurate *in vivo* prediction is necessary for compound prioritization and first-in-human estimates. However, the surprising inaccuracy in predictions from *in vitro* to *in vivo* extrapolation (IVIVE) has recently been reviewed (1,2).

The typical IVIVE process involves measuring an intrinsic clearance (CL_{int}) in microsomes or hepatocytes and applying biological scaling factors and a model of hepatic disposition to estimate an *in vivo* hepatic clearance (CL_H). In an attempt to eliminate the systematic error with IVIVE, groups have begun applying regression or empirical based scaling factors (3).

When examining the widespread IVIVE error, significant interlaboratory *in vitro* variability has been noted (1,4,5). While variability may result from interdonor differences, pooled lots are now commonly used to reduce lot-to-lot variation, or may result from differences in the biological scaling factors applied, efforts have been directed toward reaching a consensus (6,7). There could also be variation due to the use of fresh *vs.* cryopreserved hepatocytes, however previous studies have not found significant differences (8,9).

When collating *in vivo* hepatic clearance values from intravenous studies, Stringer *et al.* (5) found low variability; however, upon examining *in vitro* hepatocyte CL_{int} values, the authors found large coefficients of variation (CVs), which increased with increasing CL_{int} . Nagilla *et al.* (4) noted the paucity and variability of *in vitro* literature data, explaining that CL_{int} values should be taken from a consistent assay rather than arbitrarily chosen from different literature sources. Now that more data have been generated, we reexamine the interlaboratory variability, and search for trends with variability and physicochemical and pharmacokinetic parameters. We also examine trends in prediction accuracy for compounds with uniform *in vitro* values.

METHODS

A total of 14 papers were examined (Table I) and overlapping values were found for 50 compounds with data generated in human hepatocytes (Supplementary Table I). All *in vitro* CL_{int} values were scaled to a predicted $CL_{int, in vivo}$ (Eq. 1) using consistent scaling factors of 120×10^6 hepatocytes/g liver and 21.4 g liver/kg body weight, and the fraction unbound in the hepatocyte incubation ($f_{u_{hep}}$) values taken from the Wood *et al.* (2) database:

$$\text{Predicted } CL_{int, in vivo} = \frac{CL_{int, invitro}}{f_{u_{hep}}} \cdot 120 \cdot 21.4 \quad (1)$$

Coefficients of variation (CV) were determined as standard deviation divided by the average.

Values for hepatic clearance ($CL_{H, in vivo}$) (ml/min/kg), fraction unbound in the blood and plasma (f_{u_b} , f_{u_p}), and intrinsic clearance ($CL_{int, in vivo}$) (ml/min/kg) were taken from Wood *et al.* (2). $CL_{int, in vivo}$ values were calculated using the well-stirred model (since the difference in bias between the well-stirred and parallel tube model, the two extremes for models of hepatic disposition, was determined to be minimal) (2).

The ChEMBL database (<https://www.ebi.ac.uk/chembl>) (21) was used to obtain values for molecular weight (MW), logP, logD, polar surface area (PSA), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), number of rotatable bonds, and number of aromatic rings.

Values for the steady state volume of distribution (VD_{ss}) (l/kg) and mean residence time (MRT) (hr) were found for 45 compounds in Obach *et al.* (22).

Classification within the Biopharmaceuticals Drug Disposition Classification System (BDDCS) was determined using Benet *et al.* (23) and Hosey *et al.* (24).

Main metabolizing enzyme information was found for 33 compounds in El-Kattan *et al.* (25)

The relationship between variability and the properties was evaluated by examining the coefficient of correlation R^2 .

The accuracy of predictions was determined based on whether the predicted CL_{int} values fell within two fold of the observed CL_{int} values (Eq. 2).

$$0.5 \leq \frac{\text{observed } CL_{int}}{\text{predicted } CL_{int}} \leq 2 \quad (2)$$

RESULTS

Coefficients of Variation and Physicochemical Parameters

Data for 50 compounds were evaluated and each compound had values from 2 to 9 sources. Of the 50 compounds, 17 had $n = 2$, preventing a statistically relevant CV from being calculated. For the remaining 33 compounds, the CVs ranged from 8.53–99.8%. The potential for CV dependence on the number of values (n) was examined first. Pindolol with the second lowest CV of 19.0% had data from three sources, and triazolam with the second highest CV of 99.4% similarly had data from three sources. Imipramine, with $n = 5$ had the lowest CV of 8.53%. Therefore, a high value of n did not necessarily cause high CV values as shown in Fig. 1a. The fold difference between the highest and lowest predictions for each compound was also examined and there did not appear to be a dependence on n (Fig. 1b).

Sixteen physiochemical and pharmacokinetic properties were examined in relation to CV (Fig. 2) and there were no direct correlations here as the highest R^2 value was only 0.071. The 5 largest correlations are reported in Table II. The data were then divided into a lower CV group ($CV < 50\%$) and higher CV group ($CV \geq 50\%$) and median parameter values were examined (Table III). The largest relative difference was seen with fu_b and fu_p values, followed by the number of rotatable bonds. In the lower CV half, 29% of compounds had 7 rotatable bonds compared to 6.3% of compounds with higher CV.

BDDCS class, molecular species, and main metabolizing enzymes were also examined. BDDCS Class 1 drugs appeared to have a wider range of CV values than Class 2 drugs (Fig. 3a). When examining molecular species, neutral drugs had the highest CV values (Fig. 3b).

Looking at main metabolizing enzymes, compounds metabolized by CYP3A4 appeared to have the highest CV values (Fig. 3c). For CYP3A4 substrates, 38% had a CV > 90%, while no CYP2D6, CYP1A2, CYP2C, and UGT substrates had CVs > 90%.

Given the difference seen between BDDCS classes, the data were also split by class 1 and class 2 compounds ($n = 21$ and 11 respectively). Examining the same physicochemical properties with CV for both classes, there were no correlations for BDDCS class 1 compounds (every R^2 value was less than 0.10). For BDDCS class 2 though, there were potential trends (Fig. 4a). The number of HBA and HBD and number of aromatic rings had the largest correlations, however the smaller number of compounds should be noted. The lack of correlation with BDDCS class 1 compounds is shown in Fig. 4b for comparison.

Uniformity of Predictions and Physicochemical Parameters

Next the variability relating to the accuracy of predictions was examined. Accurate predictions are typically defined as predictions that fall within two fold of observed values (16,26,27). Here, if a compound had predictions all falling either within two-fold or outside two-fold, it was categorized as “uniform”. If a compound had some predictions falling within two-fold, and some falling outside two-fold, it was categorized as “non-uniform”. The same properties were then examined to determine if any drive the difference between the two categories.

Returning to the 50 compiled compounds, there were 31 uniform compounds and 19 non-uniform compounds. Of the uniform predictions, 6 (19%) were accurate predictions, and 25 (81%) were inaccurate underpredictions. The most distinct difference between the uniform and non-uniform categories was seen with $CL_{int, in vivo}$. Compounds with uniform predictions typically had higher $CL_{int, in vivo}$ values (Fig. 5). Furthermore, 37% of non-uniform predictions had $CL_{int, in vivo}$ values <10 ml/min/kg compared to 10% of uniform predictions.

Accuracy of Predictions and Physicochemical Parameters

Finally, all 31 compounds with uniform predictions were further examined. It is expected that new understandings of mechanisms will help reduce the current IVIVE underprediction, but for now, it is important to know which new compounds may yield results that will be accurate, and which may not. Here only 6 compounds had accurate predictions, limiting the power of the evaluation. Despite this, there were accuracy distinctions when considering $\log D$, $CL_{int, in vivo}$ and f_{up} (Table IV). Of the accurate predictions, 83% had a $\log D$ of <1.0 compared to 28% of inaccurate predictions. 42% of compounds with $\log D < 1.0$ had accurate predictions and 5.0% of compounds with $\log D \geq 1.0$ had accurate predictions. For $CL_{int, in vivo}$, 31% of compounds with $CL_{int, in vivo} < 100$ ml/min/kg had accurate predictions compared to 6.7% with $CL_{int, in vivo} \geq 100$. Finally, for f_{up} , 11% of predictions with $f_{up} < 0.1$ were accurate compared to 33% of predictions with $f_{up} \geq 0.1$.

DISCUSSION

Variability in the *in vitro* data generated and used for IVIVE can significantly affect clearance predictions. This compilation found varying reported data for 50 compounds. Of

these, 33 had $n = 3$, and CV values for the same compound were as high as 99.8%. Trends were sought in hopes of determining in the future which new compounds may yield more reliable predictions than others. However, after confirming that variability was not dependent on n , no direct trends appeared with the physicochemical properties examined.

Upon more generally splitting the compounds into low and high CV groups though there appeared to be marked relative differences in the median values for f_{u_b} and f_{u_p} and the average number of rotatable bonds. After further examining the binding values though, an obvious trend did not appear. For f_{u_b} , 35% of the low CV group had high protein binding ($f_u > 0.05$) and 31% of the high CV group also had high binding. A similar result was seen with f_{u_p} where 47% of the low CV group had high protein binding and 38% of the high CV group did also. A difference did hold for rotatable bonds where in the lower CV half, 29% of compounds had ≥ 7 rotatable bonds compared to 6.3% of compounds with higher CV. It has previously been shown that decreasing rotatable bond count is paralleled by increasing permeation rate (28), and here this may lead to larger variability. Wood *et al.* (29) previously examined the importance of the unstirred water layer (UWL) on clearance predictions with hepatocytes. Given that the UWL has been shown to reduce the apparent permeability of highly permeable compounds, the authors showed that shaking of incubations can lead to 3 to 5-fold higher CL_{int} values (29). Perhaps the increase in variability noted with lower rotatable bond counts (and thus higher permeability) could be related to experimental differences for incubation shaking among laboratories and moving forward, this factor should be considered for new chemical entities.

Interestingly BDDCS class 1 drugs had a larger CV range than BDDCS class 2 drugs and neutral drugs had more variation than acidic or basic. Although the number of drugs with main metabolizing enzyme information was more limited, CYP3A4 substrates had higher CV values, perhaps due to the potential of extrahepatic metabolism. When examining R^2 values with class 2 drugs and different properties, the number of HBA and HBD stood out, which has also been shown to be related to permeation rate (28,30). As more data are generated and shared, it would be useful to reevaluate these potential trends and their statistical significance with a larger sample size.

Some compounds had large CV values, however upon further examination, no matter which value was used, the predictions would have fallen outside of two-fold of the observed value and been considered inaccurate. For instance for triazolam that had a CV of 99.9%, data from three sources underpredicted by 3.8, 14, and 29 fold. For these cases, the compounds were deemed to have “uniform” predictions. The main difference noted between uniform and non-uniform compounds was that uniform compounds had higher $CL_{int, in vivo}$ values. The majority of the uniform compounds were uniformly inaccurate (80%), and all of these inaccurate compounds were underpredicted. This is not unexpected given the high inaccuracy previously reported (1,2) and emphasizes the need to find a mechanistic reason for the underprediction. It has been noted that compounds with high $CL_{int, in vivo}$ commonly have large error (2,31,32), which explains why these compounds would have uniform inaccurate predictions. More low clearance ($CL_{int, in vivo} < 10$ ml/min/kg) compounds fell in the non-uniform category, confirming the experimental challenges for low turnover compounds (5,33).

Finally, trends in accuracy for the 31 compounds with uniform predictions were examined. More or less confidence could theoretically be placed in a new compound's extrapolation results if any trends exist and accordingly more or less experiments may be needed. Of the 50 drugs examined, only 6 compounds had uniform accurate predictions, limiting the power of the evaluation. Of the accurate compounds, there were 5 accurate BDDCS class 1 and 0 accurate class 2 compounds (the 6th accurate compound was class 3) supporting the hypothesis that class 1 drugs would have more accurate predictions (1). Based on this dataset it appears that less lipophilic, lower intrinsic clearance, and lower protein binding compounds have more accurate predictions. The intrinsic clearance finding agrees with the idea of CL_{int} dependent underprediction mentioned earlier, and the protein binding finding agrees with previous studies concluding that highly bound compounds have more inaccuracy (34,35). It will be useful to reevaluate these trends as more uniform, accurate data are generated for compounds.

CONCLUSIONS

This investigation highlights the interlaboratory variability in generated CL_{int} values and the need for consistent and improved methodologies. Compounds with lower rotatable bond counts and therefore higher permeability had more variability, perhaps due to experimental differences in incubation shaking and the role of the unstirred water layer. Compounds with uniform predictions typically had higher $CL_{int, in vivo}$ values and uniform underpredictions, confirming a lack of mechanistic understanding with IVIVE; while compounds with non-uniform predictions typically had lower $CL_{int, in vivo}$ values, reaffirming the current experimental challenges for compounds falling within this clearance range. While only a limited number of uniform predictions were accurate, lipophilicity, intrinsic clearance, and protein binding may be determinants of accurate IVIVE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS AND DISCLOSURES

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

ABBREVIATIONS

BDDCS	Biopharmaceutics Drug Disposition Classification System
CL_{int}	Intrinsic clearance
CL_H	Hepatic clearance
CV	Coefficient of variation
fu	Fraction unbound
HBA	Number of hydrogen bond acceptors

HBD	Number of hydrogen bond donors
IVIVE	<i>In vitro</i> to <i>in vivo</i> extrapolation
MRT	Mean residence time
MW	Molecular weight
PSA	Polar surface area
VD_{ss}	Steady state volume of distribution

REFERENCES

1. Bowman CM, Benet LZ. Hepatic clearance predictions from in vitro-in vivo extrapolation and the biopharmaceutics drug disposition classification system. *Drug Metab Dispos.* 2016;44:1731–5. [PubMed: 27519549]
2. 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;45:1178–88. [PubMed: 28887366]
3. Sohlenius-Sternbeck AK, Jones C, Ferguson D, Middleton BJ, Projean D, Floby E, et al. Practical use of the regression offset approach for the prediction of in vivo intrinsic clearance from hepatocytes. *Xenobiotica.* 2012;42:841–53. [PubMed: 22509824]
4. Nagilla R, Frank KA, Jolivet LJ, Ward KW. Investigation of the utility of published in vitro intrinsic clearance data for prediction of in vivo clearance. *J Pharmacol Toxicol Methods.* 2006;53:106–16. [PubMed: 16188462]
5. Stringer R, Nicklin PL, Houston JB. Reliability of human cryopreserved hepatocytes and liver microsomes as in vitro systems to predict metabolic clearance. *Xenobiotica.* 2008;38:1313–29. [PubMed: 18853387]
6. Hakooz N, Ito K, Rawden H, Gill H, Lemmers L, Boobis AR, et al. Determination of a human hepatic microsomal scaling factor for predicting in vivo drug clearance. *Pharm Res.* 2006;23:533–9. [PubMed: 16505976]
7. Barter ZE, Bayliss MK, Beaune PH, Boobis AR, Carlile DJ, Edwards RJ, et al. Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram liver. *Curr Drug Metab.* 2007;8:33–45. [PubMed: 17266522]
8. Hallifax D, Galetin A, Houston JB. Prediction of metabolic clearance using fresh human hepatocytes: comparison with cryopreserved hepatocytes and hepatic microsomes for five benzodiazepines. *Xenobiotica.* 2008;38:353–67. [PubMed: 18340561]
9. 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;39:656–62. [PubMed: 19622023]
10. Akabane T, Gerst N, Naritomi Y, Masters JN, Tamura K. A practical and direct comparison of intrinsic metabolic clearance of several non-CYP enzyme substrates in freshly isolated and cryopreserved hepatocytes. *Drug Metab Pharmacokinet.* 2012;27:181–91. [PubMed: 22027494]
11. Blanchard N, Alexandre E, Abadie C, Lavé T, Heyd B, Manton G, et al. Comparison of clearance predictions using primary cultures and suspensions of human hepatocytes. *Xenobiotica.* 2005;35:1–15. [PubMed: 15788364]
12. Hallifax D, Rawden HC, Hakooz N, Houston JB. Prediction of metabolic clearance using cryopreserved human hepatocytes: kinetic characteristics for five benzodiazepines. *Drug Metab Dispos.* 2005;33:1852–8. [PubMed: 16174807]
13. Jacobson L, Middleton B, Holmgren J, Eirefelt S, Fröjd M, Blomgren A, et al. An optimized automated assay for determination of metabolic stability using hepatocytes: assay validation, variance component analysis, and in vivo relevance. *Assay Drug Dev Technol.* 2007;5:403–15. [PubMed: 17638540]

14. 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;30:1446–54. [PubMed: 12433818]
15. Lu C, Li P, Gallegos R, Uttamsingh V, Xia CQ, Miwa GT, et al. 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;34:1600–5. [PubMed: 16790553]
16. 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;32:1247–53. [PubMed: 15286053]
17. 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;31:580–8. [PubMed: 12695346]
18. 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;33:1304–11. [PubMed: 15932954]
19. Soars MG, Burchell B, Riley RJ. In vitro analysis of human drug glucuronidation and prediction of in vivo metabolic clearance. *J Pharmacol Exp Ther.* 2002;301:382–90. [PubMed: 11907196]
20. Sohlenius-Sternbeck A-K, Afzelius L, Prusis P, Neelissen J, Hoogstraate J, Johansson J, et al. Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. *Xenobiotica.* 2010;40: 637–49. [PubMed: 20624033]
21. Gaulton A, Hersey A, Nowotka M, Bento AP, Chambers J, Mendez D, et al. The ChEMBL database in 2017. *Nucleic Acids Res.* 2017;45:D945–54. [PubMed: 27899562]
22. Obach RS, Lombardo F, Waters NJ. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. *Drug Metab Dispos.* 2008;36:1385–405. [PubMed: 18426954]
23. Benet LZ, Broccatelli F, Oprea TI. BDDCS applied to over 900 drugs. *AAPS J.* 2011;13:519–47. [PubMed: 21818695]
24. Hosey CM, Chan R, Benet LZ. BDDCS predictions, self-correcting aspects of BDDCS assignments, BDDCS assignment corrections, and classification for more than 175 additional drugs. *AAPS J.* 2016;18:251–60. [PubMed: 26589308]
25. El-Kattan AF, Varma MV, Steyn SJ, Scott DO, Maurer TS, Bergman A. Projecting ADME behavior and drug-drug interactions in early discovery and development: application of the extended clearance classification system. *Pharm Res.* 2016;33:3021–33. [PubMed: 27620173]
26. Houston JB, Carlile DJ. Prediction of hepatic clearance from microsomes, hepatocytes, and liver slices. *Drug Metab Rev.* 1997;29: 891–922. [PubMed: 9421679]
27. Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, et al. The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J Pharmacol Exp Ther.* 1997;283:46–58. [PubMed: 9336307]
28. Verber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem.* 2002;45:2615–23. [PubMed: 12036371]
29. 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;46:268–78. [PubMed: 29233818]
30. Clark DE. Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. *J Pharm Sci.* 1999;88:807–14. [PubMed: 10430547]
31. Hallifax D, Foster JA, Houston JB. Prediction of human metabolic clearance from in vitro systems: retrospective analysis and prospective view. *Pharm Res.* 2010;27:2150–61. [PubMed: 20661765]
32. 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: 124–36. [PubMed: 21058916]

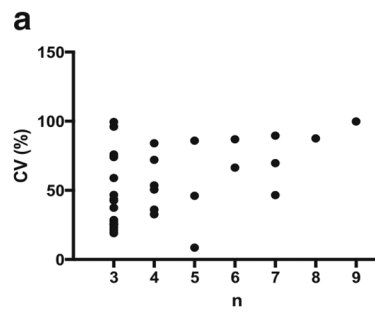
33. Di L, Obach RS. Addressing the challenges of low clearance in drug research. *AAPS J.* 2015;17:352–7. [PubMed: 25567366]
34. Baker M, Parton T. Kinetic determinants of hepatic clearance: plasma protein binding and hepatic uptake. *Xenobiotica.* 2007;37:1110–34. [PubMed: 17968739]
35. Soars MG, McGinnity DF, Grime K, Riley RJ. The pivotal role of hepatocytes in drug discovery. *Chem Biol Interact.* 2007;168:2–15. [PubMed: 17208208]

Author Manuscript

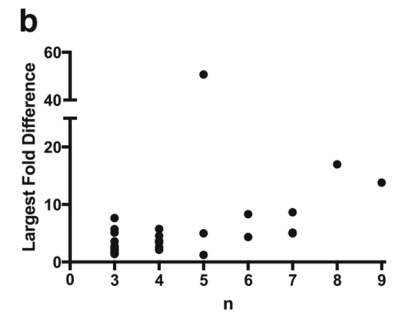
Author Manuscript

Author Manuscript

Author Manuscript



n	3	4	5	6	7	8	9
# values	17	6	3	2	3	1	1
Mean CV	45.3	54.8	46.8	76.7	68.6	87.6	99.8
SD	26.4	20.1	38.7	14.5	21.5	-	-



n	3	4	5	6	7	8	9
# values	17	6	3	2	3	1	1
Mean Largest Dif.	2.8	3.7	19	6.3	6.3	17	14
SD	1.8	1.3	28	2.8	2.1	-	-

Fig. 1.
The dependence of CV (**a**) and the largest fold difference (**b**) on n.

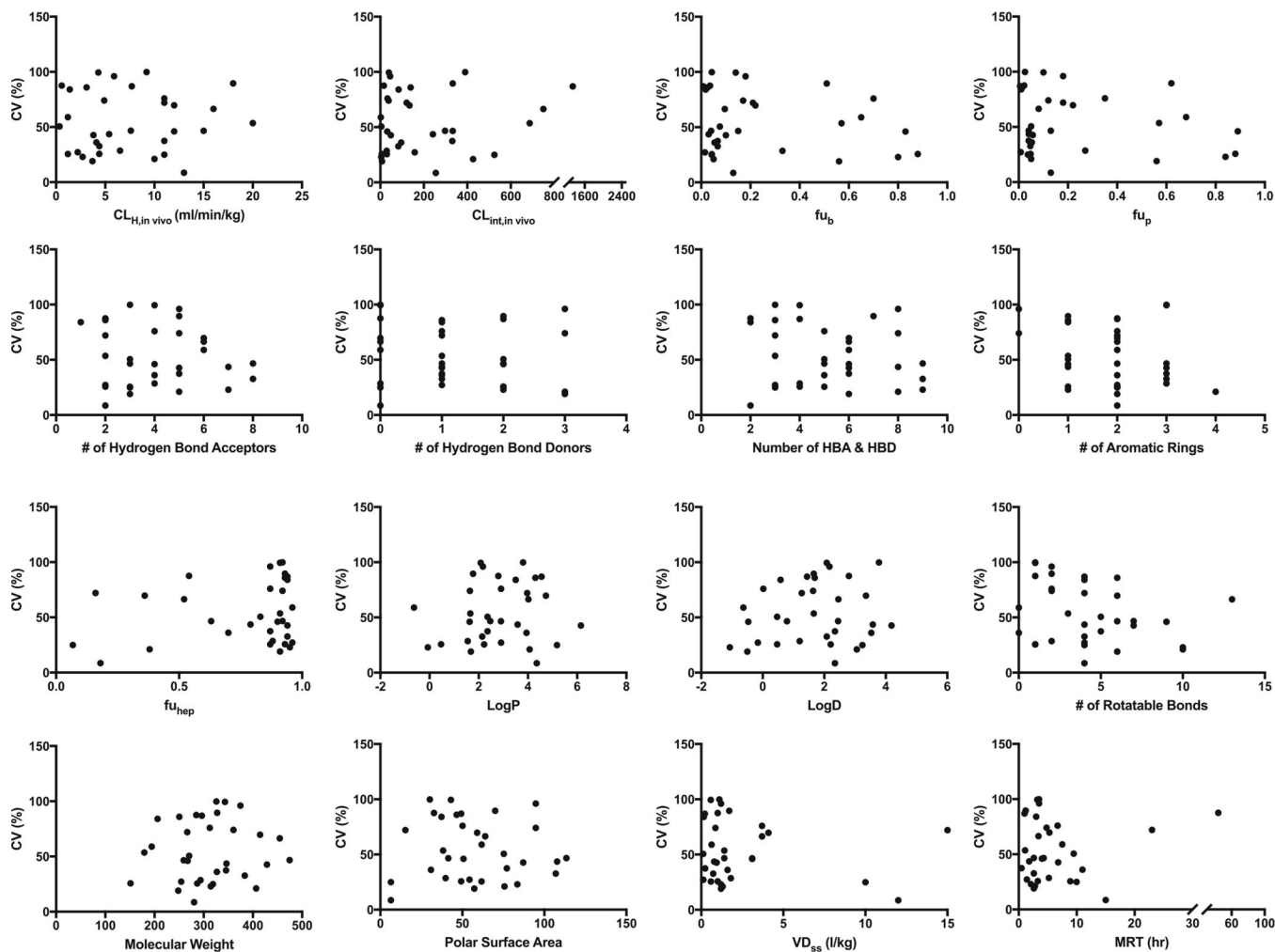


Fig. 2. Trends between various physicochemical and pharmacokinetic properties and CV.

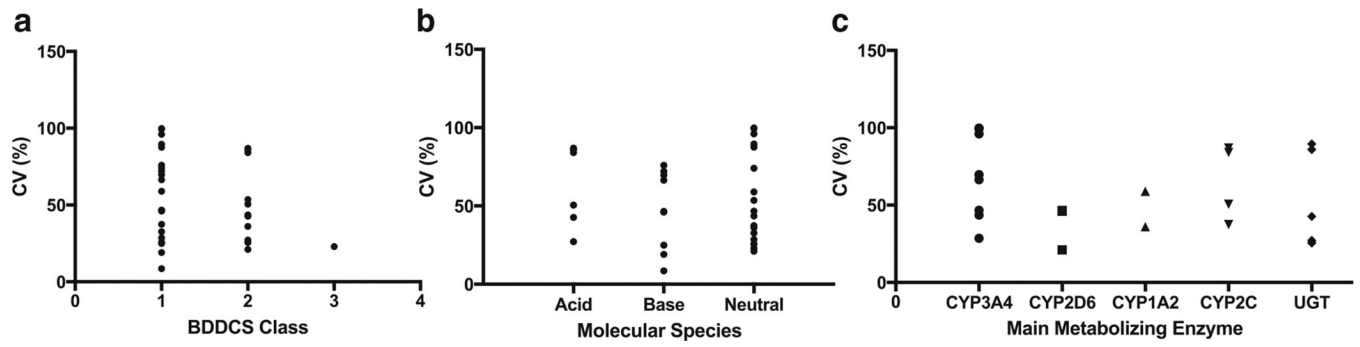


Fig. 3. Trends between CV and BDDCS class (a), molecular species (b), and main metabolizing enzyme (c).

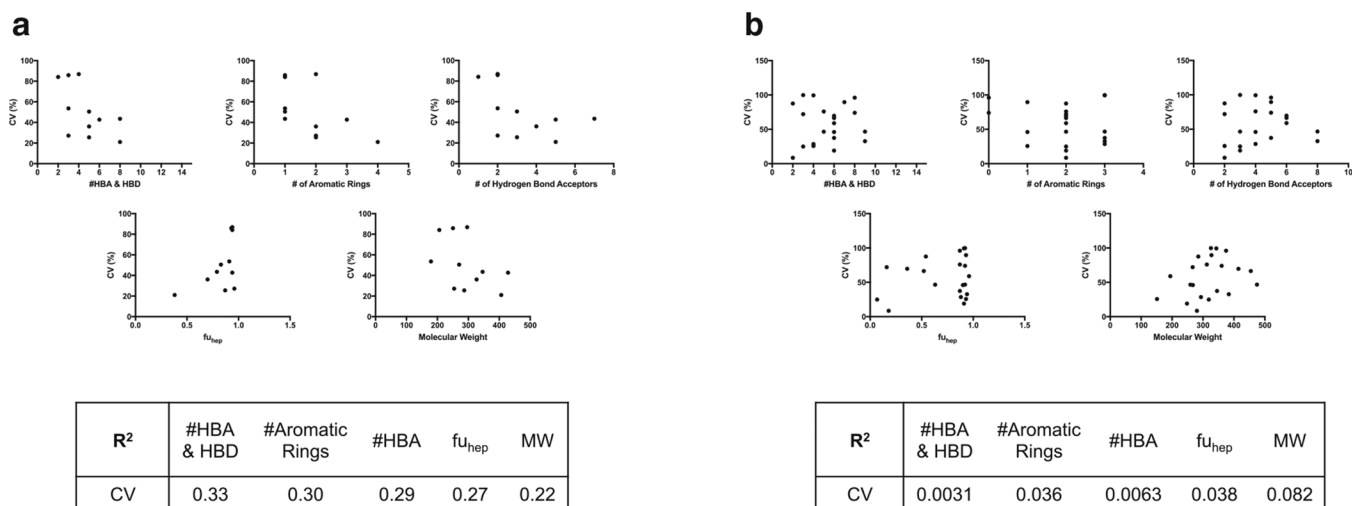


Fig. 4. The highest correlations of CV with physicochemical properties for BDDCS class 2 compounds (**a**) and the lack of correlation for BDDCS class 1 compounds (**b**).

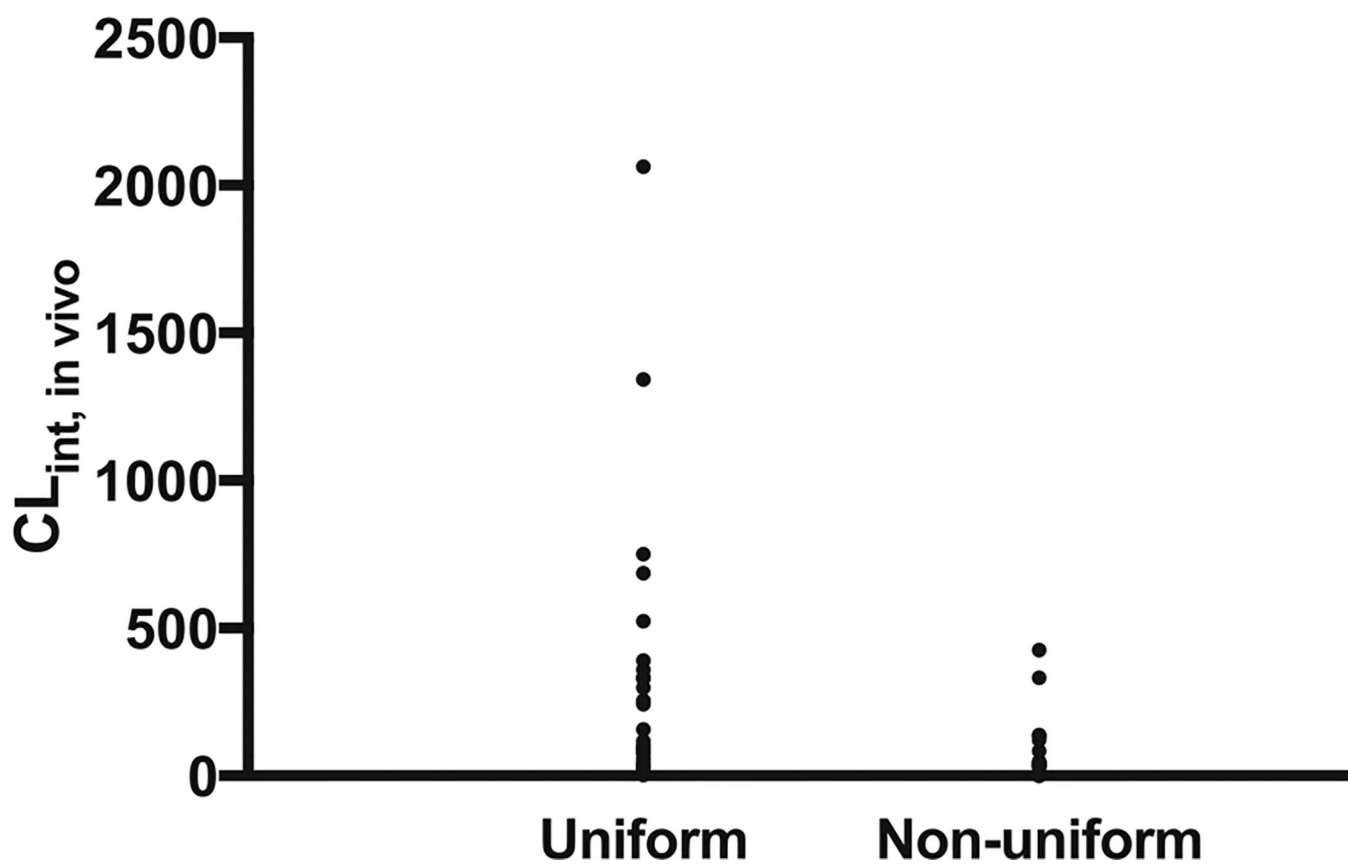


Fig. 5.
Relationship between compounds with uniform vs. non-uniform predictions and $CL_{int, in vivo}$.

Table I

Human Hepatocyte Data Examined for this Evaluation

Source	Human Hepatocytes	Donors
Akabane <i>et al.</i> (10)	Cryopreserved	Individual, 9–11 donors
Blanchard <i>et al.</i> (11)	Cryopreserved	Individual, 2 donors
Floby <i>et al.</i> (9)	Fresh	Individual, 7 donors
Hallifax <i>et al.</i> (8)	Fresh	Individual, 5 donors
Hallifax <i>et al.</i> (12)	Cryopreserved	Individual, 5 donors
Jacobson <i>et al.</i> (13)	Cryopreserved	Pooled, 2 donors
Lau <i>et al.</i> (14)	Cryopreserved	Pooled, 5+ donors
Lu <i>et al.</i> (15)	Cryopreserved	Pooled, 4 donors
McGinnity <i>et al.</i> (16)	Fresh	Individual, 1–90 donors
Naritomi <i>et al.</i> (17)	Cryopreserved	Individual, 5–7 donors
Riley <i>et al.</i> (18)	Not Reported	Individual, 3+ donors
Soars <i>et al.</i> (19)	Cryopreserved	Individual, 3 donors
Sohlenius-Sternbeck <i>et al.</i> (20)	Cryopreserved	Pooled, 2–5 donors
Sohlenius-Sternbeck <i>et al.</i> (3)	Cryopreserved	Pooled, 5 donors

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table IIHighest Correlations, R^2 , of CV with Parameters

	#Rot Bonds	#Aromatic Rings	$f_{u_{hep}}$	f_{u_p}	#HBD & HBA
CV	0.071	0.059	0.037	0.031	0.027

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table III

Median parameter values for compounds with lower vs. higher CV values with (n)

CV	f _h	f _p	# Rot Bonds	HBA & HBD	LogD	CL _{int, in vivo}	LogP	PSA	HBA	VD _{SS}	CL _{int, in vivo}	MRT	f _{hep}	MW	# Aromatic Rings	HBD
<50%	0.067(17)	0.053(17)	4.0(17)	6.0(17)	2.2(17)	5.4(17)	2.5(17)	57(17)	4.0(17)	1.2(17)	96(17)	3.3 (17)	0.88(17)	314(17)	2.0(17)	1.0(17)
>50%	0.16(16)	0.11 (16)	2.5(16)	4.5(16)	1.7(16)	9.8(16)	2.9(16)	50(16)	3.5(16)	1.1 (15)	103(16)	3.5(15)	0.91 (16)	304(16)	2.0(16)	1.0(16)

Table IV

Properties of Compounds with Accurate, Uniform Predictions

Parameter	#Accurate	#Inaccurate	%Accurate
LogD			
<1.0	5	7	41.7%
1.0		18	5.26%
CL _{int, in vivo}			
<100	5		31.3%
100		14	6.67%
f _{u,p}			
<0.1	2	17	10.5%
0.1	4	8	33.3%