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RESEARCH PAPER

# Prediction of Human Metabolic Clearance from *In Vitro* Systems: Retrospective Analysis and Prospective View

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## ABSTRACT

***Purpose*** To provide a definitive assessment of prediction of *in vivo* $CL_{int}$ from human liver *in vitro* systems for assessment of typical underprediction.

***Methods*** A database of published predictions of clearance from human hepatocytes and liver microsomes was compiled, including only intravenous $CL_b$. The influence of liver model (well-stirred (WS) or parallel tube (PT)), plasma protein binding and clearance level on the relationship between *in vitro* and *in vivo* $CL_{int}$ was examined.

***Results*** Average prediction bias was about 5- and 4-fold for microsomes and hepatocytes, respectively. Reduced bias using the PT model, in preference to the popular WS model, was only marginal across a wide range of clearance with a consequential minor impact on prediction. Increasing underprediction with decreasing $fu_b$, or increasing $CL_{int}$, was found only for hepatocytes, suggesting fundamental *in vitro* artefacts rather than failure to model potentially unequilibrated binding during rapid extraction.

***Conclusions*** In contrast to microsomes, hepatocytes give a disproportionate prediction with increasing clearance suggesting limitations either at the active site, such as cofactor exhaustion, or with intracellular concentration equilibrium, such as rate-limiting cell permeability. A simple log linear empirical relationship can be used to correct hepatocyte predictions.

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## ABBREVIATIONS

| | |
|---|---|
| $CL_{int}$ | intrinsic clearance |
| $CL_b$ | blood clearance |
| WS | 'Well-stirred' (liver model) |
| PT | 'Parallel tube' (liver model) |
| $fu_b$ | fraction unbound in blood |
| CYP | Cytochrome P450 |

## INTRODUCTION

Human liver-derived *in vitro* systems provide currently indispensible tools for prediction of drug clearance which are vital to drug discovery and development. Although *in vitro–in vivo* prediction methodology has become established over the last 20 years, a tendency towards underprediction of unbound intrinsic clearance ($CL_{int}$) *in vivo* of a magnitude of several fold, using either human liver microsomes or hepatocytes, remains. This prediction bias is unresolved from imprecision (arising from human variability and experimental uncertainty), and the cumulative unknowns challenge the quantitative capability of these systems (1).

The relative convenience of using human liver microsomes may be outweighed by the incomplete set of clearance pathways offered, compared with hepatocytes (2–4), particularly given the widespread commercial availability of cryopreserved hepatocytes. However, it appears that cytochrome P450 (CYP) metabolising capacity of cryopreserved hepatocytes may differ from human liver microsomes—average CYP maximal activity has been found to be between 2- and 20-fold less than that of microsomes from the same commercial sources (1). This

apparent disadvantage of hepatocytes is not reflected in reported comparisons of intrinsic clearance between the systems (5–7), where prediction bias was found to be similar. Inter-individual variability is normally addressed by pooling of *in vitro* material from numerous liver donors or assay across several individual donors to give a range. However, representation of human variability could still be distorted because of the log-normal distribution of CYP activity between donors combined with the independence of expression between CYPs (1). The relative performance of human liver microsomes and hepatocytes for prediction of clearance remains unclear.

Failure to accurately predict clearance *in vivo* from either human liver *in vitro* system has necessitated critical consideration of the wider extrapolation methodology as a potential source of systematic underprediction. Several aspects of the methodology have been focused on in recent years. Scaling of *in vitro* intrinsic clearance measurements to the whole body has been exhaustively investigated; commonly used scaling factors, for either system, do not appear to be a source of bias or major imprecision (8). Physiological modelling of drug extraction by the liver relies on the simplistic Well-Stirred liver model, and this may be justified, as the accuracy of prediction is not improved upon by the use of either the Parallel Tube or Dispersion liver models, with the exception of only the most highly cleared drugs (9). Liver models incorporating modelling of active uptake processes have yet to be widely adopted, but active uptake may be relatively unimportant for the large number of highly permeable drugs investigated to date (10) and may not have had a major impact on predictions. Recently, however, significantly reduced prediction bias using the Parallel Tube model rather than the more commonly used Well-Stirred model has been reported (7,11), returning attention to drug dispersion in the liver. Another aspect of physiological modelling which has received attention recently is the appropriateness of the steady-state unbound fraction in blood ($fu_b$), which is commonly applied with liver models. It has been suggested that underprediction of clearance may be associated with unequilibrated uptake processes in liver, including binding to plasma protein, and hence the kinetics of metabolism, uptake and binding should be dynamically modelled (12). Possibly related to this is a trend of clearance dependence in prediction, evident in some reported predictions (5,7).

To provide a comprehensive basis for further investigation of the underprediction of clearance, we have compiled a database of predictions from various published studies involving human liver microsomes and/or hepatocytes. The assessment of prediction accuracy and precision was unified with respect to the use of unbound fraction *in vitro* and apparent intrinsic clearance *in vivo* (avoiding investigation that used the oral route). The impact of choice of liver model and extent of binding in blood on prediction has been examined, along with dependency on clearance. The main objective was to obtain a clearer profile of prediction bias for both human hepatic microsomes and hepatocytes which, together with potential indications of underlying causes of underprediction, would guide further investigation. The database presented also provides a valuable set of *in vivo* $CL_{int}$ values for 110 drugs (intravenously dosed) with corresponding *in vitro* predictions of $CL_{int}$ from cryopreserved hepatocytes (89 drugs) and hepatic microsomes (67 drugs). This compilation should prove to be valuable in the future assessment and comparison of novel *in vitro* systems that are currently under development, e.g. HepaRG, adenoviral transfected HepG2 cell systems (13,14).

## METHODS

### *In Vitro* and *In Vivo* Data Collation

*In vitro* intrinsic clearance ($CL_{int}$) determined either by drug depletion or metabolite formation kinetics reported for human cryopreserved hepatocytes (5–7,15,16) and human liver microsomes (7,16,17) was combined into a database for each system. *In vivo* plasma clearance ($CL_p$) and unbound fraction in plasma ($fu_p$) were collated from the above sources. Drugs were included only if the corresponding *in vivo* clearance was based on intravenous dose to avoid uncertainty arising from absorption processes. Drugs with *in vivo* clearance values greater than hepatic blood flow were excluded to avoid additional uncertainty from extra-hepatic effects.

### Determination of Unbound *In Vitro* Intrinsic Clearance

Where *in vitro* system unbound concentration was available (5,7,15), unbound *in vitro* $CL_{int}$ ($CL_{int,u}$) was determined using an estimated unbound fraction *in vitro* based on the appropriate lipophilicity relationship algorithm for either microsomes ($fu_{mic}$; (18)) or hepatocytes ($fu_{heps}$; (19)), below.

$$fu_{mic} = \frac{1}{1 + P \cdot 10^{0.072 \log P/D^2 + 0.067 \log P/D - 1.126}} \quad (1)$$

where $P$ is microsomal protein concentration, log $P/D$ is the log $P$ value for basic drugs and the log D value for acidic and neutral drugs

$$fu_{heps} = \frac{1}{1 + \frac{K_p \cdot V_R}{K_a \cdot P}\left(\frac{1 - fu_{mic}}{fu_{mic}}\right)} \quad (2)$$

where $K_p$ is the hepatocyte/medium concentration ratio, $K_a$ is the microsomal binding constant (125 for 1 mg of microsomal protein/ml and 1 million cells/ml) and $V_R$ is

the volume ratio of hepatocytes to medium (0.005 for 1 million cells/ml).

### Scaling of *In Vitro* Intrinsic Clearance to Whole Liver

*In vitro* intrinsic clearance ($CL_{int}$) values were scaled to the *in vivo* equivalent whole liver using average liver and body weight with either a microsomal recovery factor or hepatocellularity value (Eq. 3).

$$CL_{int} = \frac{CL_{int} \cdot SF \cdot HLW}{fu_{mic\ or\ heps}} \quad (3)$$

where *SF* is the either the microsomal average recovery factor of 40 mg microsomal protein/g liver or hepatocellularity of 120 million/g liver (20) and *HLW* is the human liver weight of 21.4 g liver/kg bodyweight (21).

### Determination of Apparent *In Vivo* Intrinsic Clearance

*In vivo* $CL_{int}$ was calculated from the *in vivo* $CL_p$, $fu_p$ and average hepatic blood flow ($Q_H$, 20.7 ml/min/kg (21)) using either the Well-Stirred (WS) or the Parallel Tube (PT) liver model (Eqs. 4 and 5). Blood clearance ($CL_b$) and unbound fraction in blood ($fu_b$) were determined using the blood/plasma concentration ratio ($R_b$), where available ($CL_p/R_b$ or $fu_p/R_b$)), or assuming $R_b = 1$ for basic and neutral drugs and 0.55 for acidic drugs.

$$In\ vivo\ CL_{int} = \frac{CL_b}{fu_b \bullet \left(1 - \frac{CL_b}{Q_H}\right)} \quad \text{(WS liver model)} \quad (4)$$

$$In\ vivo\ CL_{int} = \frac{Q_H}{fu_b} \bullet -\ln\left(\frac{Q_H - CL_b}{Q_H}\right) \text{(PT liver model)} \quad (5)$$

### Accuracy and Precision of Predictions

The accuracy of a set of individual drug *in vitro* $CL_{int}$/*in vivo* $CL_{int}$ predictions for each system was assessed using the average fold error (*afe*) metric (geometric mean error) determined using Eq. 6 (22).

$$afe = 10^{\left[\frac{1}{n}\sum \log \frac{predicted}{observed}\right]} \quad (\text{underprediction} = 1/afe) \quad (6)$$

The corresponding precision of the prediction sets was assessed using the root mean squared error (*rmse*) determined using Eq. 7 (23).

$$rmse = \sqrt{\frac{1}{n}\sum (predicted - observed)^2} \quad (7)$$

### Assessment of *in Vitro* Predictions of Intrinsic Clearance

For each *in vitro* system, accuracy and precision of prediction of $CL_{int}$ was determined and compared between liver model. For each liver model, these metrics were determined with or without exclusion of predictions involving *in vivo CL* greater than 80% $Q_H$ to examine sensitivity of the model to values at the nonlinear extreme. Using the WS liver model for each system, the predictions were segregated according to arbitrary ranges for both $fu_b$ (<0.05, 0.05–0.2, 0.2–1) and *in vivo* $CL_{int}$ (<10, 10–100, 100–1,000, >1,000 ml/min/kg) to assess potential dependencies in prediction with extent of binding in blood and clearance, respectively. Microsomes and hepatocytes were directly compared for those drugs used in both systems ($n$= 46), using the WS liver model. An empirical relation between the systems was obtained by least squares regression of the following log linear function using Microsoft Excel:

$$\log CL_{int,hepatocytes} = A \bullet \log CL_{int,microsomes} + B \quad (8)$$

The goodness of fit was assessed by examination of the residuals of the predicted values.

### Empirical Relation of Predicted *In Vitro* $Cl_{int}$ and *In Vivo*

To provide an empirical correction of *in vitro* $CL_{int}$ to *in vivo* $CL_{int}$,, where lack of proportionality was observed from the above analysis, the following log linear function was fitted by least squares regression using Microsoft Excel:

$$\log CL_{int,in\ vitro} = A \bullet \log CL_{int,in\ vivo} + B \quad (9)$$

To provide a simple (log) nonlinear comparison, the following quadratic function was also fitted:

$$CL_{int,in\ vitro} = A \bullet CL_{int,in\ vivo} - CL^2_{int,in\ vivo} \quad (10)$$

The goodness of fit was assessed and compared by examination of the residuals of the predicted values. These regression coefficients were then used to establish relationships between *in vivo* $CL_{int}$ and *in vitro* predictions.

## RESULTS

### Database of Human *In Vitro* and *In Vivo* Prediction of Intrinsic Clearance

Using the criteria described above, a net total of 89 and 67 drug predictions from hepatocytes and microsomes, respectively, was established (Table I). Individual predictions were based on an average of five source liver donors for hepatocytes and an

**Table I** Database of *In Vivo* and *In Vitro* Values for Published Studies Using Human Cryopreserved Hepatocytes and Human Liver Microsomes

| Substrate | *In vivo* | | | | *In vitro* | | | | | | Source | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL (ml/min/kg) | fub | CLint (ml/min/kg) liver model | | Hepatocytes | | | Microsomes | | | | | | | |
| | | | WS | PT | fu,inc | CLint,u (ml/min/kg) | n | fu,inc | CLint,u (ml/min/kg) | n | | | | | |
| | a | b | c | | | d | | | e | | a | b | c | d | e |
| Acebutolol | 4.10 | 0.96 | 5.33 | 4.76 | *a* | 5.1 | 4 | | | | 1 | 2 | | 1 | |
| Acetaminophen | 4.00 | 0.79 | 6.28 | 5.63 | *b* | 2.5 | 5 | | | | 3 | 3 | | 3 | |
| Alprazolam | 0.92 | 0.64 | 2.08 | 2.03 | *b* | 2.1 | 5 | *b* | 2.0 | 9 M | 4 | 4,5 | | 4 | 5 |
| Alprenolol | 12.5 | 0.27 | 117 | 71.0 | *a* | 64.5 | 6 | *c* | 48.5 | 16 | 2 | 2 | | 2 | 2 |
| Amitriptyline | 12.0 | 0.058 | 490 | 308 | | | | *b* | 13.0 | 6 | 4 | 4,5 | 5 | | 5 |
| Amobarbital | 0.36 | 0.26 | 1.40 | 1.39 | | | | *b* | 0.89 | 6 | 4 | 4,5 | 5 | | 5 |
| Antipyrine | 0.57 | 0.97 | 0.60 | 0.59 | *b* | 0.67 | 5 | *b* | 0.14 | ≥6 M | 4 | 4,5 | 5 | 4 | 5 |
| Bepridil | 5.30 | 0.005 | 1,583 | 1,361 | *a* | 337 | 6 M | *c* | 992 | 16 | 2 | 2 | | 2 | 2 |
| Betaxolol | 3.90 | 0.56 | 8.58 | 7.72 | *a* | 7.4 | 6 | | | | 1 | 2 | | 2 | |
| Bufuralol | 8.80 | 0.24 | 64.5 | 48.3 | *b* | 45.0 | 5 M+ | | | | 4 | 4 | | 4 | |
| Bupivacaine | 5.50 | 0.068 | 110 | 94.0 | *a* | 32.6 | 6 | *c* | 83.0 | 16 | 2 | 2 | | 2 | 2 |
| Buprenorphine | 17.6 | 0.040 | 2,938 | 983 | *a* | 40.0 | 6 | *c* | 449 | 16 | 2 | 2 | | 2 | 2 |
| Caffeine | 1.67 | 0.65 | 2.82 | 2.69 | *b* | 2.1 | 5 M | *b* | 0.43 | ≥6 M | 4 | 4,5 | 5 | 4 | 5 |
| Carbamazepine | 0.40 | 0.31 | 1.32 | 1.30 | *a* | 5.9 | 1 M | | | | 1 | 2 | | 1 | |
| Carvedilol | 8.70 | 0.030 | 500 | 376 | *b* | 282 | ≥3 M | | | | 3 | 3 | | 3 | |
| Chlorpheniramine | 1.30 | 0.30 | 4.62 | 4.48 | *a* | 9.4 | 4 | 1 | 2 | 1 | | | | | |
| Chlorpromazine | 8.60 | 0.053 | 287 | 217 | *a,b* | 182 | 6,5 | *c* | 208 | 16 | 2,4 | 2,4 | | 2,4 | 2 |
| Cimetidine | 3.20 | 0.90 | 4.21 | 3.86 | *b* | 3.4 | ≥3 M | | | | 3 | 3 | | 3 | |
| Clomipramine | 10.9 | 0.022 | 1,047 | 704 | *a* | 109 | 6 | *c* | 192 | 16 | 2 | 2 | | 2 | 2 |
| Clozapine | 5.16 | 0.051 | 160 | 131 | *a* | 20.8 | 1 | *b* | 4.4 | 6 | 1 | 2,5 | | 1 | 5 |
| Codeine | 9.50 | 0.93 | 18.9 | 13.7 | *b* | 35.0 | 5 | | | | 4 | 4 | | 4 | |
| Cyclosporin A | 4.70 | 0.040 | 152 | 133 | *b* | 13.5 | ≥3 | | | | 3 | 3 | | 3 | |
| Desipramine | 7.58 | 0.25 | 118 | 77.2 | *a,b* | 45.3 | 6,5 | *b* | 16.0 | 6 | 2,4 | 2,4 | | 2,4 | 5 |
| Dexamethasone | 3.91 | 0.34 | 14.0 | 12.6 | | | | *b* | 2.9 | 6 | 5 | 5 | | 5 | 5 |
| Diazepam | 0.51 | 0.036 | 15.3 | 15.1 | *b* | 6.6 | 5 | *b* | 10.0 | ≥6 | 4 | 4,5 | 5 | 4 | 5 |
| Diclofenac | 4.00 | 0.014 | 418 | 373 | *a,b* | 86.8 | 6,5 M+ | *c* | 108 | 16 | 2,4 | 2,4 | | 2,4 | 2 |
| Diflunisal | 0.18 | 0.005 | 34.3 | 34.1 | *b* | 9.9 | >3 | | | | 3 | 3 | | 3 | |
| Diphenhydramine | 9.60 | 0.19 | 94.2 | 67.9 | *a* | 16.0 | 1 | | | | 1 | 2 | | 1 | |
| Diltiazem | 12.0 | 0.20 | 143 | 89.7 | *a* | 16.0 | 6 | *c* | 40.6 | 16 | 2 | 2 | | 2 | 2 |
| Dofetillide | 1.50 | 0.36 | 4.50 | 4.33 | | | | *b* | 0.40 | ≥6 M | 5 | 5 | 5 | | 5 |
| Domperidone | 9.10 | 0.06 | 275 | 203 | *a* | 88.1 | 6 | *c* | 520 | 16 | 2 | 2 | | 2 | 2 |
| Etodolac | 1.31 | 0.020 | 69.9 | 67.7 | *b* | 81.2 | ≥3 | | | | 3 | 3 | | 3 | |
| Felodipine | 7.54 | 0.003 | 4,300 | 3,399 | | | | *b* | 98.0 | ≥6 | 5 | 5 | 5 | | 5 |
| Fenoprofen | 0.60 | 0.018 | 34.3 | 33.8 | *a* | 27.4 | 6 | *c* | 13.5 | 16 | 2 | 2 | | 2 | 2 |
| FK1052 | 12.7 | 0.021 | 1,600 | 959 | | | | *b* | 40.0 | 15 | 5 | 5 | 5 | | 5 |
| FK480 | 2.49 | 0.008 | 340 | 319 | | | | *b* | 51.0 | 15 | 5 | 5 | 5 | | 5 |
| Flumazenil | 17.0 | 0.52 | 183 | 68.5 | *a* | 16.3 | 6 | | | | 2 | 2 | | 2 | |
| Flunitrazepam | 3.04 | 0.28 | 12.7 | 11.7 | *b* | 4.5 | 5 | *b* | 5.0 | 9 M | 4 | 4,5 | 5 | 4 | 5 |
| Fluoxetine | 7.80 | 0.06 | 228 | 178 | *a* | 5.3 | 1 | | | | 1 | 2 | | 1 | |
| Fluphenazine | 9.90 | 0.012 | 1,581 | 1,122 | *a* | 69.9 | 6 | *c* | 302 | 16 | 2 | 2 | | 2 | 2 |
| Furosemide | 1.70 | 0.022 | 84.9 | 81.3 | *b* | 0.91 | 5 | | | | 4 | 4 | | 4 | |
| Gemfibrosil | 1.70 | 0.036 | 68.4 | 65.5 | *a,b* | 24.9 | 6,5 | *c* | 30.1 | 16 | 2,4 | 2,4 | | 2,4 | 2 |
| Glimepiride | 0.69 | 0.14 | 5.10 | 5.01 | *a* | 9.4 | 6 | *c* | 35.4 | 16 | 2 | 2 | | 2 | 2 |
| Glipizide | 0.96 | 0.020 | 50.3 | 49.1 | *b* | 7.1 | ≥3 | | | | 3 | 3 | | 3 | |
| Glyburide | 1.30 | 0.004 | 385 | 373 | *a* | 17.2 | 6 | *c* | 57.9 | 16 | 2 | 2 | | 2 | 2 |
| Granisetron | 11.0 | 0.70 | 33.5 | 22.4 | *b* | 29.7 | ≥3 | | | | 3 | 3 | | 3 | |

**Table 1** (continued)

| Substrate | *In vivo* | | | | *In vitro* | | | | | | Source | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL (ml/min/kg) | fub | CLint (ml/min/kg) liver model | | Hepatocytes | | | Microsomes | | | | | | | |
| | | | WS | PT | fu,inc | CLint,u (ml/min/kg) | n | fu,inc | CLint,u (ml/min/kg) | n | | | | | |
| | a | b | c | | | d | | | e | | a | b | c | d | e |
| Ibuprofen | 0.81 | 0.015 | 59.1 | 58.0 | *a,b* | 32.6 | 6,5 | *b* | 8.2 | ≥6 M+ | 2,4 | 2,4 | 5 | 2,4 | 5 |
| Imipramine | 13.5 | 0.13 | 318 | 175 | *a,b* | 42.8 | 6,5 | *b* | 18.0 | ≥6 M+ | 2,4 | 2,4 | 5 | 2,4 | 5 |
| Indinavir | 14.7 | 0.39 | 130 | 65.7 | | | | *b* | 16.0 | 6 M | 5 | 5 | 5 | | 5 |
| Indomethacin | 2.24 | 0.020 | 126 | 119 | *b* | 27.1 | ≥3 | | | | 3 | 3 | | 3 | |
| Irbesartan | 3.85 | 0.040 | 118 | 106 | *b* | 58.8 | ≥3 | | | | 3 | 3 | | 3 | |
| Ketamine | 16.5 | 0.59 | 138 | 56.0 | *a* | 40.5 | 6 | *c* | 28.6 | 16 | 2 | 2 | | 2 | 2 |
| Ketoprofen | 1.20 | 0.017 | 77.5 | 75.2 | *a,b* | 11.0 | 6,5 | | | | 2,4 | 2,4 | | 2,4 | |
| Labetalol | 18.1 | 0.32 | 450 | 134 | *a* | 16.4 | 6 | *c* | 18.4 | 16 | 2 | 2 | | 2 | 2 |
| Levoprotilene | 14.6 | 0.19 | 261 | 133 | *a* | 8.1 | 6 | | | | 2 | 2 | | 2 | |
| Lidocaine | 11.5 | 0.33 | 82.1 | 51.5 | *b* | 15.3 | 5 | *b* | 3.1 | ≥6 M | 2,4 | 2,4 | 5 | 4 | 5 |
| Lorazepam | 1.20 | 0.090 | 14.2 | 13.7 | *b* | 1.0 | 5 | | | | 4 | 4 | | 4 | |
| Lorcainide | 18.9 | 0.30 | 710 | 167 | | | | *b* | 48.0 | 6 | 5 | 5 | 5 | | 5 |
| Methohexital | 15.9 | 0.39 | 180 | 78.9 | | | | *b* | 47.0 | 6 | 5 | 5 | 5 | | 5 |
| Methoxsalen | 17.9 | 0.13 | 1,000 | 310 | | | | *b* | 38.0 | 6 | 5 | 5 | 5 | | 5 |
| Methylprednisolone | 6.70 | 0.22 | 45.0 | 36.8 | *b* | 33.0 | 5 | | | | 4 | 4 | | 4 | |
| Metoclopramide | 6.20 | 0.76 | 11.6 | 9.70 | *a* | 10.0 | 6 | | | | 2 | 2 | | 2 | |
| Metoprolol | 13.3 | 0.80 | 62.2 | 29.2 | *a,b* | 5.3 | 6,5 | *b* | 18.0 | ≥6 M | 2,4 | 2,4 | 5 | 2,4 | 5 |
| Mexiletine | 6.76 | 0.39 | 26.0 | 21.2 | | | | *b* | 0.77 | 5 M | 5 | 5 | 5 | | 5 |
| Mianserin | 18.8 | 0.14 | 1,463 | 353 | *a* | 22.3 | 6 | *c* | 34.6 | 16 | 2 | 2 | | 2 | 2 |
| Midazolam | 6.50 | 0.072 | 134 | 110 | *a,b* | 138 | 6,5 | *c* | 708 | 16 | 2,4 | 2,4 | | 2,4 | 2 |
| Montelukast | 1.27 | 0.001 | 1,503 | 1,456 | *b* | 96.3 | ≥3 | | | | 3 | 3 | | 3 | |
| Morphine | 18.0 | 0.77 | 179 | 54.8 | *a* | 64.6 | 2 M | | | | 1 | 2 | | 1 | |
| Nadolol | 2.90 | 0.97 | 3.48 | 3.22 | *a* | 7.7 | 6 M | | | | 2 | 2 | | 2 | |
| Naproxen | 0.11 | 0.018 | 5.86 | 5.84 | *b* | 1.4 | 5 | | | | 4 | 4 | | 4 | |
| Nicardipine | 17.8 | 0.068 | 1,900 | 603 | | | | *b* | 1,200 | 15 | 5 | 5 | 5 | | 5 |
| Nifedipine | 8.10 | 0.068 | 196 | 152 | *b* | 146 | 5 M | | | | 4 | 4 | | 4 | |
| Nivaldipine | 18.0 | 0.016 | 8,400 | 2,569 | | | | *b* | 1,200 | 15 | 5 | 5 | 5 | | 5 |
| Omeprazole | 13.0 | 0.068 | 520 | 304 | | | | *b* | 67.0 | 15 | 5 | 5 | 5 | | 5 |
| Ondansetron | 6.06 | 0.27 | 31.8 | 26.58 | *a* | 3.9 | 5 | *b* | 1.7 | ≥6 M | 1 | 2,5 | 5 | 1 | 5 |
| Oxaprozin | 0.07 | 0.001 | 100 | 100 | *b* | 24.4 | ≥3 | | | | 3 | 3 | | 3 | |
| Oxazepam | 1.60 | 0.045 | 38.5 | 37.0 | *b* | 6.9 | 5 | | | | 4 | 4 | | 4 | |
| Oxprenolol | 5.70 | 0.30 | 26.2 | 22.2 | *a* | 14.9 | 6 | M | | | 2 | 2 | | 2 | |
| Phenacetin | 19.6 | 0.60 | 615 | 101 | *a* | 36.2 | 6 | *c* | 42.3 | 16 | 2 | 2 | | 2 | 2 |
| Phenytoin | 0.47 | 0.12 | 4.00 | 3.95 | | | | *b* | 0.16 | ≥6 M | 5 | 5 | 5 | | 5 |
| Pindolol | 4.20 | 0.55 | 9.58 | 8.53 | *a* | 7.8 | 4 | | | | 1 | 2 | | 1 | |
| Prazosin | 2.85 | 0.085 | 39.7 | 36.9 | *a* | 6.2 | 7 | | | | 1,2 | 2 | | 1 | |
| Prednisolone | 2.40 | 0.10 | 27.1 | 25.5 | *b* | 30.0 | 5 | | | | 4 | 4 | | 4 | |
| Prednisone | 1.91 | 0.10 | 21.0 | 20.0 | | | | *b* | 2.6 | 6 | 5 | 5 | 5 | | 5 |
| Prochlorperazine | 16.4 | 0.003 | 29,240 | 12,048 | *a* | 45.6 | 6 | *c* | 199 | 16 | 2 | 2 | | 2 | 2 |
| Promazine | 14.3 | 0.029 | 1,595 | 838 | *a* | 64.6 | 6 | *c* | 62.8 | 16 | 2 | 2 | | 2 | 2 |
| Promethazine | 12.3 | 0.023 | 1,318 | 812 | *a* | 101 | 6 | *c* | 76.3 | 16 | 2 | 2 | | 2 | 2 |
| Propafenone | 10.0 | 0.059 | 328 | 232 | *a* | 76.4 | 6 | *c* | 133 | 16 | 2 | 2 | | 2 | 2 |
| Propranolol | 13.2 | 0.14 | 267 | 154 | *a,b* | 29.2 | 6,5 | *c* | 7.8 | 16 | 2,4 | 2,4 | | 2,4 | 2 |
| Quinidine | 4.02 | 0.15 | 34.2 | 30.2 | *b* | 18.0 | 5 M+ | *b* | 3.2 | 6 | 4 | 4,5 | 5 | 4 | 5 |
| Ranitidine | 2.90 | 0.77 | 4.38 | 4.06 | *b* | 3.0 | ≥3 | | | | 3 | 3 | | 3 | |
| Risperidone | 5.40 | 0.17 | 43.0 | 36.8 | | | | *c* | 43.3 | 16 | 2 | 2 | | | 2 |

**Table I** (continued)

| Substrate | *In vivo* | | | | *In vitro* | | | | | | Source | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL (ml/min/kg) | fub | CLint (ml/min/kg) liver model | | Hepatocytes | | | Microsomes | | | | | | | |
| | | | WS | PT | fu,inc | CLint,u (ml/min/kg) | n | fu,inc | CLint,u (ml/min/kg) | n | | | | | |
| | a | b | c | | | d | | | e | | a | b | c | d | e |
| Ritonavir | 1.20 | 0.015 | 86.1 | 83.5 | *b* | 30.5 | ≥3 | | | | 3 | 3 | | 3 | |
| Scopolamine | 11.0 | 0.88 | 26.7 | 17.8 | *a* | 19.6 | 1 | | | | 1 | 2 | | 1 | |
| Sildenafil | 6.00 | 0.094 | 89.8 | 75.3 | *a* | 24.4 | 6 M | *c* | 121 | 16 | 2 | 2 | | 2 | 2 |
| S-Warfarin | 0.06 | 0.018 | 3.31 | 3.30 | *b* | 1.9 | 5 M+ | | | | 4 | 4 | | 4 | |
| Temazepam | 1.30 | 0.027 | 51.4 | 49.7 | *a* | 5.7 | 2 | *b* | | | 1 | 2 | 5 | | 1 |
| Tenidap | 0.01 | 0.001 | 8.30 | 8.30 | | | | *b* | 7.9 | 6 | 5 | 5 | 5 | | 5 |
| Tenoxicam | 0.05 | 0.013 | 3.33 | 3.33 | *b* | 8.8 | 5 | *b* | 1.6 | 6 | 3 | 3,5 | 5 | 3 | 5 |
| Theophylline | 1.29 | 0.53 | 2.61 | 2.51 | *b* | 2.6 | 5 | *b* | 0.03 | 7 M | 4 | 4,4 | 5 | 4 | 5 |
| Timolol | 11.0 | 0.48 | 49.3 | 33.0 | *b* | 4.4 | 5 | | | | 4 | 4 | | 4 | |
| Tiprolidine | 8.00 | 0.100 | 130 | 101 | *b* | 39.6 | ≥3 M | | | | 3 | 3 | | 3 | |
| Tolbutamide | 0.36 | 0.16 | 2.82 | 2.80 | *b* | 0.38 | 5 M+ | | 1.2 | ≥6 M | 4 | 4,5 | 5 | 4 | 5 |
| Trazodone | 1.80 | 0.061 | 32.3 | 30.9 | *a* | 17.4 | 6 | *c* | 65.4 | 16 | 2 | 2 | | 2 | 2 |
| Triazolam | 3.70 | 0.17 | 30.6 | 27.3 | *a,b* | 12.3 | 6,5 | *c* | 43.5 | 16 | 2,4 | 2,4 | | 2,4 | 2 |
| Trimipramine | 15.9 | 0.051 | 1,344 | 593 | *a* | 138 | 6 | *c* | 205 | 16 | 2 | 2 | | 2 | 2 |
| Verapamil | 13.3 | 0.12 | 310 | 177 | *a* | 33.4 | 6 M | *c* | 193 | 16 | 2 | 2 | | 2 | 2 |
| Warfarin | 0.02 | 0.005 | 4.50 | 4.50 | | | | *b* | 0.49 | ≥6 M | 5 | 5 | 5 | | 5 |
| YW796 | 6.19 | 0.63 | 14.0 | 11.7 | | | | *b* | 15.0 | 12 M | 5 | 5 | 5 | | 5 |
| Zolpidem | 4.30 | 0.17 | 31.9 | 28.4 | *a* | 8.0 | 6 | *c* | 23.1 | 16 | 2 | 2 | | 2 | 2 |

*a* Calculated from logPD (Stringer *et al*. (7)) and Eq. 2 (Kilford *et al*. (19))

*b* Value incorporated in source calculation

*c* Calculated from logPD (Stringer *et al*. (7)) and Eq. 1 (Hallifax and Houston (18))

*In vitro* CLint assay by substrate depletion except M (metabolite formation); both methods M+

Mean value given where two or more sources are indicated

*WS* Well-stirred liver model (Eq. 4), except where source (c) given

*PT* Parallel tube liver model (Eq. 5)

*n* Number of liver source donors

1 McGinnity *et al*. (15)

2 Stringer *et al*. (7)

3 Riley *et al*. (5)

4 Brown *et al*. (6)

5 Ito and Houston (9)

average of 11 donors for microsomes. *In vivo* hepatic metabolic clearance following single intravenous dose (from several studies, in most cases) was obtained for each drug.

## Liver Model Dependency in Prediction of Intrinsic Clearance and Effect of Exclusion of High Clearance Substrates

For each system, the total set of predictions was assessed for accuracy and precision using both the WS and PT liver models. In addition, to examine the impact of model sensitivity to $CL_b$, an upper limit of 80% $Q_H$ was used to exclude the most rapidly cleared drugs. All predictions are shown graphically in Fig. 1. Bias (*afe*) was 5.2 and 3.9 for microsomes and hepatocytes, respectively, using the WS model and 3.5 and 2.9, respectively, using the PT model. Excluding drugs with $CL_b$, >80% $Q_H$, *afe* was 4.7, 3.4 and 3.6, 3.0, respectively, indicating minimal impact of proximity to $Q_H$.

Overall, bias was marginally less from the PT liver model than from the WS model. Precision (*rmse*) was similar

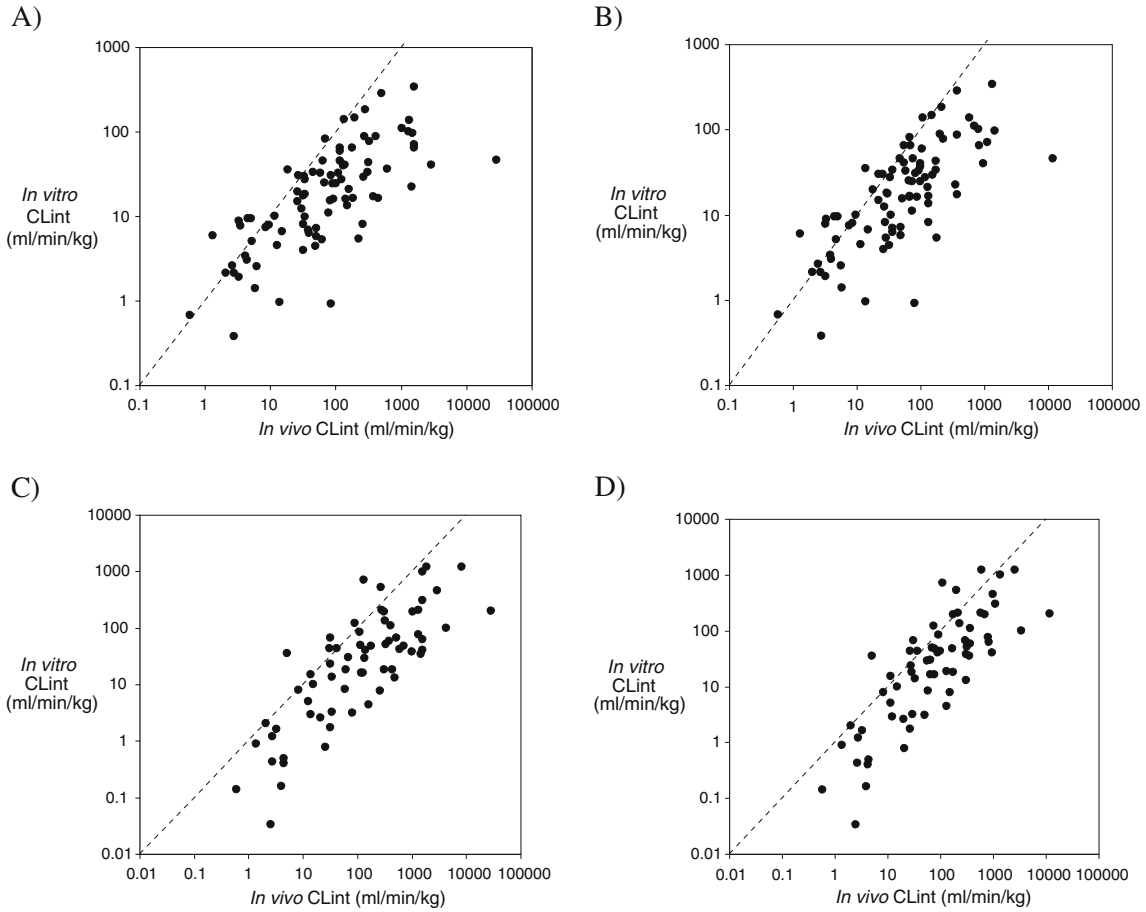


**Fig. 1** Prediction of $CL_{int}$ from the database for human hepatocytes (**A** and **B**) and liver microsomes (**C** and **D**) using the WS (**A** and **C**) or PT (**B** and **D**) liver model.

(within 20% difference) between *in vitro* systems; *rmse* was 3740/3850 and 3140/3230 including/excluding drugs with $CL_b$, >80% $Q_H$, for microsomes and hepatocytes, respectively, using the WS model. However, precision was considerably greater (>2-fold difference in *rmse*) using the PT model (*rmse* was 1540/1620 and 1300/1350 including/excluding drugs with $CL_b$, >80% $Q_H$, for microsomes and hepatocytes, respectively).

**Table II** Accuracy (*afe*) of Prediction of $CL_{int}$ from the Database for Human Hepatocytes and Liver Microsomes Using the WS or PT Liver Model, According to Level of $fu_b$

| *In vitro* system (human) | Accuracy (*afe*) of prediction of intrinsic clearance | | | | | |
|---|---|---|---|---|---|---|
| | Liver model | | | | | |
| | Well-stirred | | | Parallel tube | | |
| | *fu* b range | | | | | |
| | 0.2–1 | 0.05–0.2 | <0.05 | 0.2–1 | 0.05–0.2 | <0.05 |
| Liver microsomes (n) | 6.7 (22) | 3.4 (25) | 6.9 (20) | 4.2 (22) | 2.3 (25) | 5.0 (20) |
| Cryopreserved hepatocytes (n) | 2.1 (33) | 4.7 (26) | 6.4 (30) | 2.2 (33) | 4.2 (26) | 5.0 (30) |

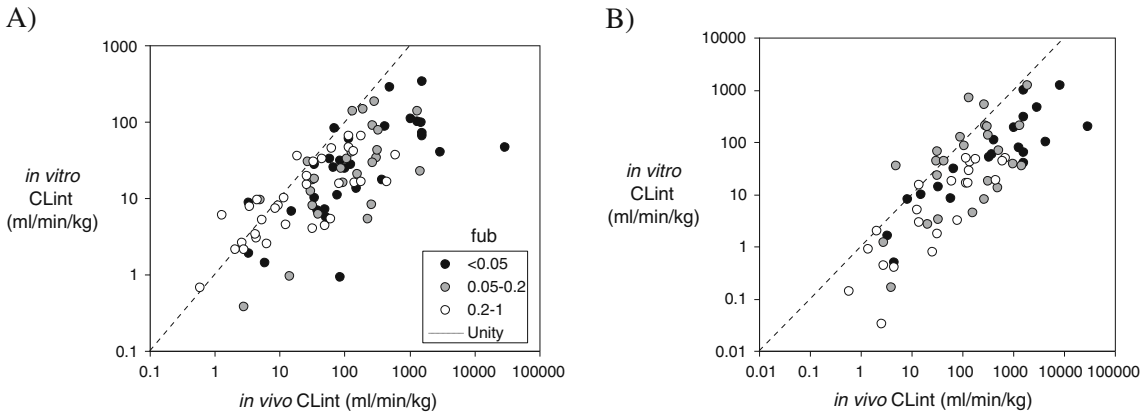


**Fig. 2** Prediction of $CL_{int}$ from the database for human hepatocytes (**A**) and liver microsomes (**B**), according to range of $fu_b$, using the WS liver model.

## Blood-Binding Dependency in Prediction of Intrinsic Clearance

The database of predictions of $CL_{int}$ from hepatocytes and microsomes was segregated according to the level of $fu_b$ to assess if prediction was dependent on the extent of binding in blood. There was little variation in *afe* between low and high binding for microsomes using either the WS or PT liver model (although *afe* was less in the mid-range); however, for hepatocytes, underprediction increased with decreasing $fu_b$ with both models (Table II), indicating a difference in prediction between the systems associated with binding in blood. The distribution of binding level with $CL_{int}$ is shown if Fig. 2.

## Comparison of Prediction of Intrinsic Clearance Between Hepatocytes and Microsomes

Predictions of $CL_{int}$ for substrates common to both the hepatocyte and microsome databases ($n=46$) were directly compared to assess the relationship between systems according to clearance level. A trend of decreasing relative $CL_{int}$ from hepatocytes with increasing $CL_{int}$ was clearly evident, further indicating a fundamental system difference (Fig. 3). The relationship between the systems was adequately described (via least squares regression analysis) by the log linear function: $\log CL_{int,\ hepatocytes} = 0.53 \log CL_{int,\ microsomes} + 0.59 (r^2 = 0.68)$. This is supported by low standard errors (SE) of the slope (0.008) and intercept (0.014).

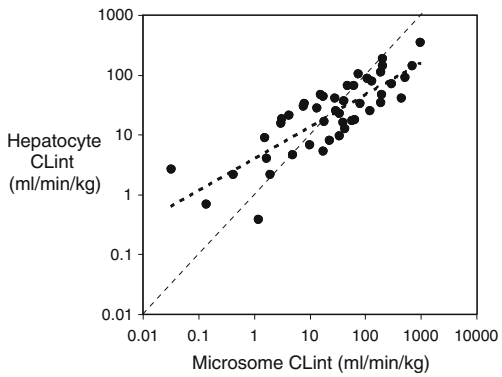


**Fig. 3** Comparison of prediction of $CL_{int}$ from the database between human hepatocytes and liver microsomes, using the WS liver model. Dashed lines represent unity and power function fit.

## Clearance Dependency in Prediction of Intrinsic Clearance

The log ratio of predicted to observed $CL_{int}$ indicated a clear distinction in trend between the systems: prediction from microsomes appeared to be constant across the range of *in vivo* $CL_{int}$, whereas prediction from hepatocytes appeared to be dependent on *in vivo* $CL_{int}$ (Fig. 4). To quantify the dependency of predictions according to estimated *in vivo* $CL_{int}$, the database predictions were segregated according to four arbitrary levels of *in vivo* $CL_{int}$. For hepatocytes, there was a clear shift from an *in vitro–in vivo* correspondence for drugs at *in vivo* $CL_{int} < 10$ ml/min/kg to an underprediction of about 3-fold at 10–100 ml/min/kg increasing to about 30-fold at >1,000 ml/min/kg (Fig. 5a); *afe* increased disproportionally over this range, approximately equally for either liver model (Table III). In contrast, for microsomes, underprediction was relatively constant, in the range 3–6-fold, from <10 to 1,000 ml/min/kg (Fig. 5b; Table III).

The relationship between *in vivo* and *in vitro* $CL_{int}$ was adequately described by a log linear function (Eq. 9) for both systems ($r^2 = 0.54$ and 0.64, respectively; $p < 0.01$, by *t*-test), as indicated by an unbiased distribution of residuals. The data were also described by a quadratic function (Eq. 10), but as the distribution of residuals was very similar to that of the power function, the latter, as the simpler model, was considered the most justifiable representation. There was a considerable difference in parameter values between the

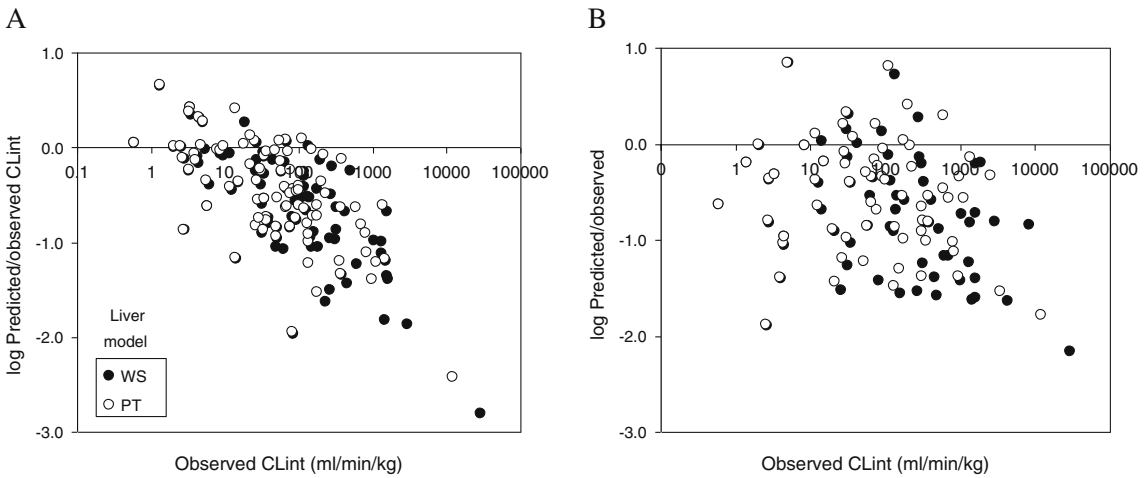


**Fig. 4** Ratio (log) of predicted to observed $CL_{int}$, as a function of observed $CL_{int}$, for human hepatocytes (**A**) and liver microsomes (**B**).

systems: the slope of fitted predictions from hepatocytes diverged significantly from unity (Eq. 11, Fig. 6a), whereas the slope for predictions from microsomes was only marginally divergent from unity (Eq. 12, Fig. 6b). This is supported by low SE of the slope (hepatocytes, 0.005; microsomes, 0.009) and intercept (hepatocytes, 0.011; microsomes, 0.020).

$$\log CL_{\text{int, }\textit{hepatocytes}} = 0.512 \log CL_{\text{int, }\textit{in vivo}} + 0.293 \quad (11)$$

$$\log CL_{\text{int, }\textit{microsomes}} = 0.787 \log CL_{\text{int, }\textit{in vivo}} - 0.287 \quad (12)$$

## DISCUSSION

An extensive database of published predictions of clearance from human hepatocytes and liver microsomes was compiled for analysis of some potential causes of the prevailing uncertainties of negative bias and imprecision in current methodology. Focus was maintained on systemic hepatic extraction by exclusive use of predictions involving intravenous $CL_b$. The influence of two alternative liver models (WS and PT), plasma protein binding and clearance level was examined.

Variation in drug metabolising capacity between hepatocytes or microsomes from different donors, possibly reflecting the source phenotypes, will manifest as part of the imprecision in a database such as this. The relatively small number of source individuals (average 6) for hepatocytes compared with microsomes (average 11) contributing to individual drug predictions might also affect the measured system average prediction (bias). Methodological differences between laboratories may also contribute to overall imprecision as well as bias. However, these potential effects are minimized by the relatively large number of predictions in each system dataset enabling trends with *in vivo* and between the systems to be resolved.

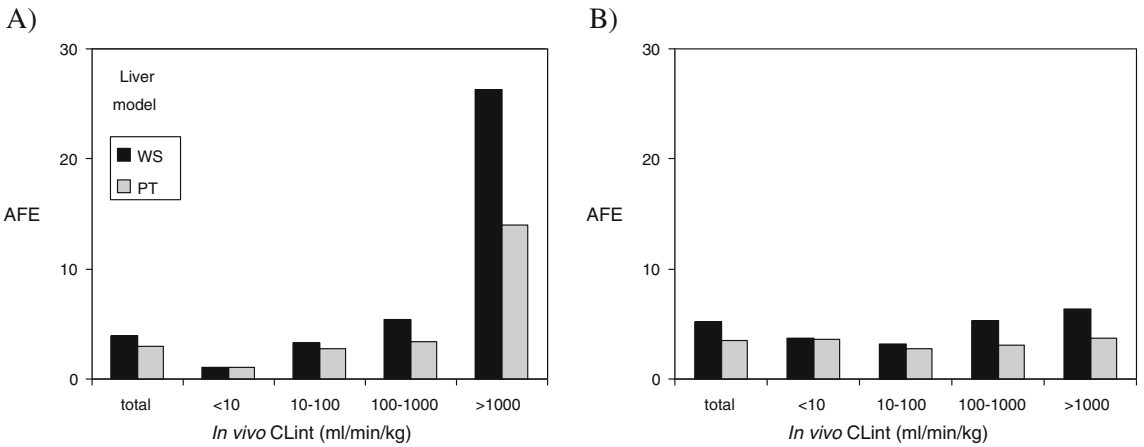


**Fig. 5** Bias (*afe*) of predicted $CL_{int}$ with level of *in vivo* $CL_{int}$ from the database for human hepatocytes (**A**) and liver microsomes (**B**) using the WS and PT liver models.

**Table III** Accuracy (*afe*) of Prediction of $CL_{int}$ from the Database for Human Hepatocytes and Liver Microsomes Using the WS or PT Liver Model, According to Level of *In Vivo* $CL_{int}$

| *In vitro* system (human) | Accuracy (*afe*) of prediction of intrinsic clearance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver model | | | | | | | |
| | Well-stirred | | | | Parallel tube | | | |
| | *In vivo* CL int (ml/min/kg) | | | | | | | |
| | <10 | 10–100 | 100–1,000 | >1,000 | <10 | 10–100 | 100–1,000 | >1,000 |
| Liver microsomes (n) | 3.7 (12) | 3.2 (18) | 5.3 (23) | 6.3 (14) | 3.7 (12) | 2.7 (18) | 3.0 (23) | 3.7 (14) |
| Cryopreserved hepatocytes (n) | 1.1 (18) | 3.3 (33) | 5.4 (28) | 26 (10) | 1.0 (18) | 2.8 (33) | 3.4 (28) | 14 (10) |

Reduced bias in prediction for high clearance drugs has been reported for the PT liver model, which, accordingly, has been advocated in preference to the widely used WS liver model (7,11). The sensitivity of either liver model to the value of $CL_b$ increases with convergence on $Q_H$, but is relatively greater for the WS liver model. The effect is proportional with respect to $fu_b$ and hence independent of this parameter, which can have a major influence on apparent $CL_{int}$ *in vivo*. Consequently, for the WS liver model, an uncertainty of 10% in $CL_b$ at 80% $Q_H$ leads to an uncertainty in estimated $CL_{int}$ of about 2-fold. This should not be a cause of prediction bias, as any error in $CL_b$ is expected to be random. The relatively greater precision with the PT model observed in the database analysis reported here appears to reflect this assumption. This study also shows that the PT model offers a marginal reduction of bias, compared with the WS model, and that this appears to be dependent on clearance, hence the previous assertions that the PT model is more appropriate for high clearance drugs. However, the overall reduction in bias using the PT model compared with the WS model is marginal (30%), including clearance up to 1,000 ml/min/kg (which includes the vast majority of drugs). Considering the level of underprediction by either *in vitro* system, the difference between liver models cannot be considered to have a major impact on prediction.

A high degree of binding to plasma protein ($fu_p < 0.1$) is common among drugs, and therefore the $fu_b$ term frequently has a large influence on the estimation of $CL_{int}$ from $CL_b$. The established WS and PT liver models accommodate only a steady state estimate of binding ($fu$), although there may exist unequilibrated levels of free drug in blood during extraction of rapidly cleared drugs in the liver. Consequently, dynamic modelling of hepatic extraction and blood binding has been advocated to provide a rationale to explain the underprediction of clearance observed using current methodology (12). Any tendency of the WS or PT liver model to overestimate unbound $CL_{int}$ from $CL_b$ (due to false assumption of equilibration of binding in blood) might be expected to correlate with $fu_b$ because this would tend to

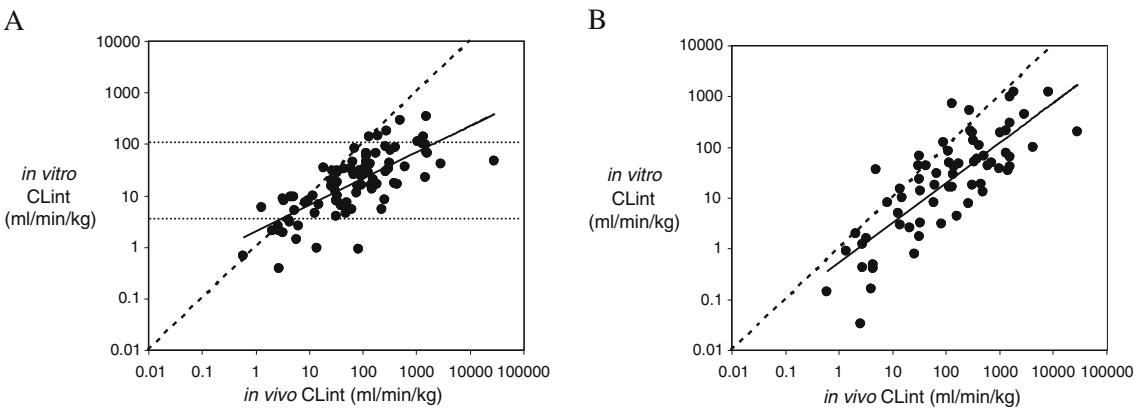


**Fig. 6** Relation of predicted $CL_{int}$ *in vitro* and $CL_{int}$ *in vivo* for hepatocytes (**A**) and microsomes (**B**). Dashed lines represent unity, fitted power functions and (**A**) upper and lower limits of bias correction for hepatocytes.

(inversely) reflect high association/low dissociation binding rate constants. There was evidence of a dependency of bias on $fu_b$ with hepatocytes in this study, but this was not reflected with microsomes and therefore does not support the concept of hepatic modelling inadequacies as a source of underprediction of $CL_{int}$.

Differences in dependency of prediction bias on $fu_b$ (and presumably, by correlation, with clearance) between hepatocytes and microsomes indicate fundamental system differences. When the systems were directly compared—using only predictions involving drugs common to both systems—a nonlinear relationship was observed. This difference is supported by the greater dependency of prediction bias for hepatocytes on *in vivo* $CL_{int}$, compared with microsomes (for the entire databases). Accuracy of predictions from microsomes may be considered effectively independent of *in vivo* $CL_{int}$, so, by comparison, hepatocytes appear to respond nonlinearly to a 'subcellular' $CL_{int}$; the cause of this can only be speculative but may involve endogenous cofactor depletion, enzymatic activity loss (24) or permeability limitation (25).

Currently, as there is no mechanistic basis for incorporating the extent of underprediction into prediction modelling, bias in $CL_{int}$ may be minimized empirically. As indicated above, this study provides a large dataset on which to base an empirical correction which incorporates the widest range of drugs and liver donors. For hepatocytes, using Eq. 11 and assuming the WS model for extrapolation to *in vivo CL*, average predicted *in vivo* $CL_{int}$ can be expressed as

$$\log CL_{int,\ in\ vivo} = \frac{\log CL_{int,\ hepatocytes} - 0.293}{0.512} = 1.95 \log CL_{int,\ hepatocytes} - 0.572 \quad (13)$$

Applying this equation to the hepatocyte database, *afe* of prediction of *in vivo* $CL_{int}$ was 1.0 (*rmse*=4,270); hence, bias was eliminated with only a marginal effect on precision. It may be recognized that this relationship for hepatocytes differs from what would be determined from datasets from individual laboratories; a different slope and intercept might appear to apply to a particular laboratory. However, it is not feasible to distinguish any such apparent methodological effects from differences in drugs (clearance range) and source liver activity. Nevertheless, correction of clearance-dependent bias for hepatocytes is an important step in reduction of uncertainty, and use of a 'global' correction may be realistic. A suggested working range for (unbound) hepatocyte $CL_{int}$ is approximately 5–100 ml/min/kg, which would be suitable for the vast majority of established drugs; it has been shown that the practicable lower limit for this system with the substrate depletion method is between 1 and 10 ml/min/kg (7). For microsomes, correction of bias in a similar range of $CL_{int}$ may be achieved using Eq. 12; however, given the proximity of the prediction slope to unity for this system, an adequate approach would be multiplication of the (unbound) microsomal $CL_{int}$ by 5 (*afe* of whole dataset). Correction of the microsome database this way eliminated bias (*afe* $CL_{int}$= 1.0; *rmse*=3,610).

$$CL_{int,\ in\ vivo} = 5 \bullet CL_{int,\ microsomes} \quad (14)$$

There are issues with both hepatocytes and microsomes concerning variability between donors and variable quality of tissue available. Hence, there is a need for novel cellular systems that are devoid of this limitation and display the advantages of both systems. Cell lines which are genetically engineered to provide activities comparable to what is believed to be occurring *in vivo* should be the aspiration (14,26). The large database of *in vivo* numbers enclosed here indicates the range that is required and provides the necessary *in vivo* correlates to allow a top-down assessment of any novel *in vitro* system for clearance prediction. Such considerations are particularly timely, as the quality of human tissue available will inevitably continue to decline with the increase in transplantation success and associated procedures. There have been several reports of genetically engineered cell lines being successful in predicting P450 induction (27,28). Although human hepatocytes are regarded as the gold standard for induction, variability both in basal level and response presents a major challenge, and the advantages of a more stable system providing adequate response are obvious. Such systems would address the ethical concerns associated with the use of human tissue and in addition address the scientific needs of having a robust *in vitro* system where the capacity is comparable to that observed under the *in vivo* situation.

Despite the high degree of uncertainty inherent in predictions of clearance from human liver-derived *in vitro* systems, this study has further distinguished the relative quantitative prediction capabilities of microsomes and hepatocytes, most notably in terms of bias. Bias is an important component of prediction uncertainty, and this report has shown that both clearance-dependent (hepatocytes) and clearance-independent (microsomes) bias can be corrected empirically. For a large set of drugs undergoing early evaluation, average prediction should be improved, although for individual cases, caution is required because a high level of imprecision in prediction remains.

## ACKNOWLEDGEMENTS

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